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PRE-CLINICAL EVALUATION OF HYDRO-ALCOHOLIC EXTRACT OF *MARSILEA QUADRIFOLIA* LEAVES FOR ANTHELMINTIC ACTIVITY

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ABSTRACT

Marsilea quadrifolia is a well-known medicinal plant, traditionally used in various ailments. In this study, we aimed to evaluate the anthelmintic activity of the hydro-alcoholic extract of *Marsilea quadrifolia* leaves. The hydro-alcoholic extract of *Marsilea quadrifolia* leaves was prepared using cold percolation methods, and the phytochemical analysis was performed. The anthelmintic activity of the extract was evaluated using the Limit test, Main test, and comparative study. The results showed that the hydro-alcoholic extract of *Marsilea quadrifolia* leaves exhibited significant anthelmintic activity. The time taken for paralysis and death of the *Tubifex tubifex* was noted to find out the minimum concentration of dose that was responsible for the death of all animals within 5 hours and the potency of the test substance as compared to the control standard Albendazole, and herbal standard. The phytochemical analysis of the extract revealed the presence of various bioactive compounds such as alkaloids, flavonoids, tannins, and saponins, which might be responsible for the observed anthelmintic activity. Therefore, the hydro-alcoholic extract of *Marsilea quadrifolia* leaves can be considered as a potential natural anthelmintic agent.

Keywords: *Marsilea quadrifolia*, hydro-alcoholic extract, phytochemicals, anthelmintic activity

INTRODUCTION:

Helminthiasis, one of the most prevalent illnesses in humans and animals, can be treated with anthelmintic or anthelminthic medications or substances that kill or trigger the ejection of such parasitic intestinal worms. A region of the body is infected by parasitic worms like pinworms, roundworms, or tapeworms in helminthiasis, a microparasites disease that affects both humans and animals. Due to poor management procedures, this illness is very common, especially in third-world nations. Helminthiasis can have immunomodulatory effects on the host, which could have an impact on any co-infecting infections. Many medicinal plants have been utilized to treat parasite illnesses in both humans and animals **[1]**. A large percentage of the world's population is susceptible to helminthic infections, which are among the most prevalent illnesses in humans. They pose a serious hazard to public health in underdeveloped nations and increase the risk of pneumonia, eosinophilia, anemia, and malnutrition. Although the majority of worm illnesses are often restricted to tropical regions, some of them can originate in temperate temperatures and can affect tourists who have travelled there. The helminths that infect the intestine are cestodes, such as tapeworms (*Taenia solium*), nematodes, such as hookworms (*Ancylostoma duodenale*), roundworms (*Ascaris lumbricoides*), and trematodes or flukes (*Schistosoma mansoni* and *Schistosoma haematobium*) **[2]**.

Since the beginning of time, humans have searched for natural remedies for their illnesses. Everything was based on experience because there was not enough information available at the time regarding the causes of the ailments or the specific plants that could be used as a remedy. As the rationale for using particular medicinal plants to cure particular ailments came to light over time, the use of medicinal plants gradually renounced the empiric framework and was instead built on explicative facts. There is substantial evidence, including written records, preserved monuments, and even the original plant remedies, that shows the relationship between man and his hunt for pharmaceuticals in nature extends back a long time. Due to the many years of fighting diseases, humans learned to look for pharmaceuticals in the barks, seeds, fruit bodies, and other parts of plants. This led to the development of an awareness of the use of medicinal plants **[3-6]**. According to estimates from the World Health Organization, traditional medicine serves as the primary source of care for approximately 80% of the population. More than 3.3 billion people in less developed countries regularly use medicinal plants since they represent the "backbone" of traditional medicine **[7]**.

Nearly 5000 years ago, in India, China, and Egypt, as well as at least 2500 years ago in Greece and Central Asia, there were recorded profiles of medicinal herbs **[8]**.

The leaves *Marsilea quadrifolia* contain a broad range of chemical compounds, including phenolic compounds, tannins, saponins, flavonoids, steroids, terpenoids, alkaloids, carbohydrates, and more. This drug's significant medical application has been examined. This plant extract has a variety of therapeutic effects, including cytotoxicity, anti-inflammatory, diuretic, anti-venom, hypoglycaemia, and antibacterial effects **[9]**. In this research *Marsilea quadrifolia* leaves was chosen to find out the anthelmintic activity on *Tubifex tubifex.*

MATERIALS AND METHOD:

Animals:

Aquarium worms of the species *Tubifex tubifex* were bought from the local market and local area of Netaji Subhas Chandra Bose Institute of Pharmacy (NSCBIP) for research purposes. The worms were between 1 to 1.5 centimetres long.

Plant Extraction:

The leaves of *Marsilea quadrifolia* were collected from local market of NSCBIP then washed gently with distilled water and dried under the shed.

Leaves crushed by hand. 100 gm of crushed leaves were placed in the percolator with 300 ml of 70% hydro-alcoholic mixture for 72 hours **[10]**. Then the extract was collected, dried and stored.

Preliminary phytochemical investigations of extract:

Hydro-alcoholic extracts of *Marsilea quadrifolia* were analysed for various phytochemical constituents like Alkaloids, Carbohydrates, Tannins, Saponins, Flavonoids and proteins **[11]**.

Anthelmintic activity: [12-14]

The Limit test and Main test were carried out to evaluate the anthelmintic potentiality of the *Marsilea quadrifolia* leaves hydro-alcoholic extract. The dose needed to paralyse and killed all animal within five hours was ascertained as well as the maximum paralysis and the time till death were documented. Pre-complete paralysis was considered to have begun when the worms ceased their typical motions or movements. To confirm the full paralysis time, external stimuli were used to make an aquatic worm move. When *Tubifex tubifex* lost all of their motion, their body colour disappeared, and they demonstrated no movement when submerged in heated water (50^0c). This was the death time, and it was noted at that moment.

To determine the potency of our test sample in comparison to the control, commercially available standard drug and standard herbal drug, comparative research was conducted.

Limit test:

For one animal, in a petri dish 20 ml of distilled water and the test dose of 2000mg/kg was taken. The main test was conducted if the animal expired.

If the animal lived, a total of 4 other animals given the same dose following the previous one, for a total of 5 animals, will be evaluated. If three animals perished through the limit test, the main test is then carried out. If three or more than three animals remained alive, then it was assumed that the (Lethal Dose 100) LD100 was exceeded 2000 mg/kg in five hours. If one animal surprisingly perished late in the trial for remaining alive animals, in order to find out if any other animals perished during the same observation period (up to 5 hours), further dosing was delayed and the remaining animals were examined.

A limit test of 5000 mg/kg body weight was performed on one animal for 5 hours in accordance with research guidelines. The main test was conducted if the animal expired. If the animal survived, two more animals received doses. If both subjects lived, the experiment was terminated.

Main test:

As per OECD guidelines, 175 mg/kg, 550 mg/kg, 2000 mg/kg, and 5000 mg/kg doses were administered to or mixed each of the four petri dishes used for the main test, which contained 20 ml of distilled water. To determine the minimal LD100 dose within 5 hours, 6 *Tubifex tubifex* were chosen then introduced in each petri dish. To analyse the anthelmintic activity, time for paralysis and death for each group of animals were recorded. If more dilution or dose division was required for the studies, it was created and used.

Comparative Study:

A comparative study was carried out between our minimal LD100 dose of *Marsilea quadrifolia* leaves hydro-alcoholic extract as test, the untreated control (distilled water), the standard medicine Albendazole ('NO WORM' product of Alkem Laboratories Ltd.) and the herbal standard medicine ('KRIMI MUDGAR RAS' product of Dabur India Limited) at the same dose of test substance.

In a similar manner to the main test, four petri dishes were used, each having 20 ml of distilled water, and the test substance and standard as well as the herbal standard medicine were mixed or added in each petri dish. Six *Tubifex tubifex* were then placed in each petri dish for a total of 5 hours, and their paralysis and death times were recorded for the purposes of the comparison.

Statistical Analysis:

The observations data are displayed as mean± S.E.M The mean and S.E.M were statistically analysed using an ANOVA and Dunnett's test, with $P<0.001$ vs control being considered a significant value.

RESULT AND DISCUSSION:

Extract preparation:

Marsilea quadrifolia leaves were dried. The yield of plant extract was 3.39% after a 72-hour cold percolation extraction process using 70% ethanol.

Preliminary phytochemical investigation: The hydro-alcoholic extract obtained from the leaves of *Marsilea quadrifolia* (*M. quadrifolia*) was subjected to preliminary phytochemical investigation and the following observations were found **(Table 1)**.

Anthelmintic activity: (Table 2) (Figure 1, 2)

The hydroalcoholic extract of *M. quadrifolia* leaves showed significant anthelmintic activity within 5 hours, at the minimum concentration of 3000 mg/kg, the extract exhibited 100% paralysis and death of aquarium worms (*Tubifex tubifex*) within 221.87±2.44 minutes and 269.05±1.49 minutes, respectively. To find out exact minimum LD 100% dose within 5 hours, dose divisions were processed and applied but no significant result was found within 5 hours except 3000 mg/kg **(Table 3)**.

Table 1: Result of preliminary phytochemical investigation

Sl. No.	Phyto constituents	Result
1.	Alkaloids	+
2.	Saponin glycoside	+
3.	Flavonoid	+
4.	Tannin	+
5.	Steroid	+
6.	Glycoside	+
7.	Carbohydrates	+
8.	Amino acid	+

Table 2: Screening of anthelminthic activity

Groups of Treatment dose	Paralysis Time (Minute)	Death Time (Minute)
Control	-	-
MQ 5000mg/kg	62.01±1.72***	78.95±1.68***
MQ 4000 mg/kg	134.09±1.33***	163.38±2.18***
MQ 3000 mg/kg	221.87±2.44***	269.05±1.49***
MQ 2800 mg/kg	-	-
MQ 2000 mg/kg	-	-
MQ 550 mg/kg	-	-
MQ 175 mg/kg	-	-

Results are expressed as mean±SEM,n=6, ***p<0.0001 as compare to control

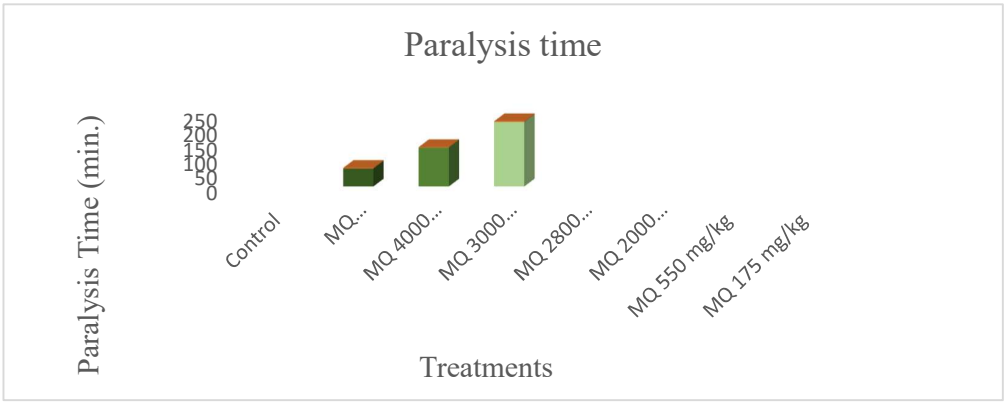

Figure 1: Impact of *Marsilea quadrifolia* leaves hydro-alcoholic extract on paralysis time of *Tubifex tubifex*

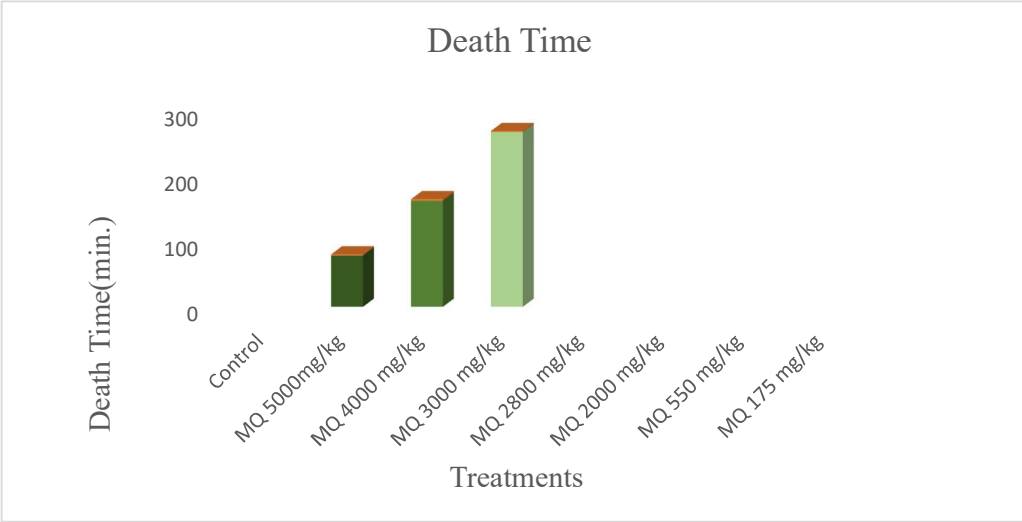

Figure 2: Impact of *Marsilea quadrifolia* leaves hydro-alcoholic extract on death time of *Tubifex tubifex*

Table 3: Result of comparative study

Groups of Treatment dose	Paralysis Time (Minute)	Death Time (Minute)
Control	-	-
M. quadrifolia 3000 mg/kg	221.87±2.44***	269.05±1.49***
Albendazole 3000 mg/kg	21.88±0.92***	45.33±1.6***
Krimi Mudgar Ras 3000 mg/kg	59.93±2.13***	143.9±2.41***

Results are expressed as mean±SEM,n=6, ***p<0.0001 as compare to control

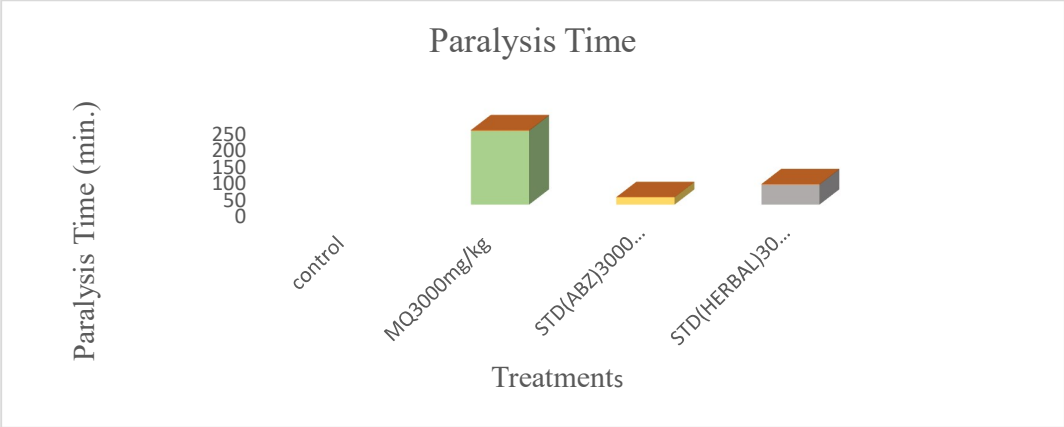

Figure 3: Result of comparative study on Paralysis time

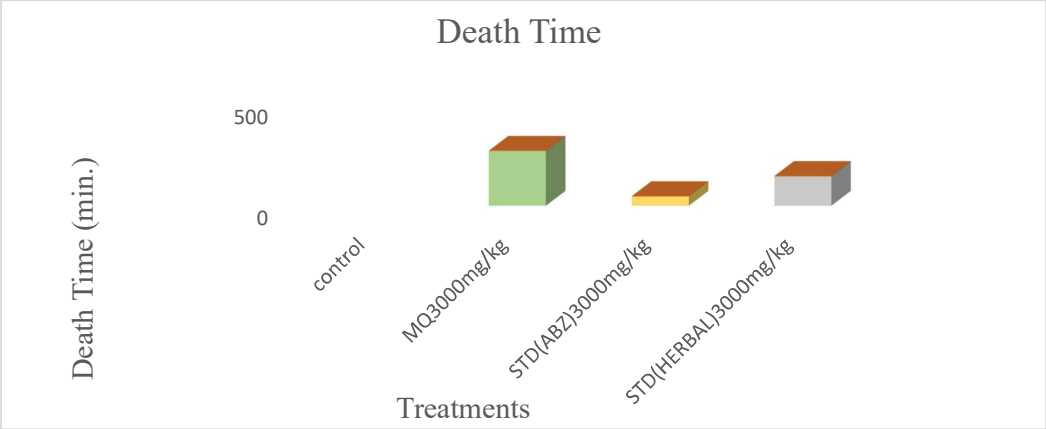

Figure 4: Result of comparative study on Death time

In the comparative study control (distilled water) did not show any effects, the standard drug, Albendazole, showed significant anthelmintic activity with 100% paralysis and death of aquarium worms (*Tubifex tubifex*) within 21.88±0.92 minutes and 45.33±1.6 minutes, and the herbal standard (Krimi Mudgar Ras) also showed paralysis and death within 59.93±2.13 and 143.9±2.41 minutes respectively. So, through the comparative study the test substance showed significant anthelmintic activity.

The results of the present study indicate that the hydroalcoholic extract of *M. quadrifolia* leaves possesses significant anthelmintic activity, which could be attributed to the presence of phytochemicals such as flavonoids, tannins, and alkaloids. These phytochemicals have been reported to possess anthelmintic activity **[15]**. The aquatic worm model used in this study is a widely accepted model for screening the anthelmintic activity of plant extracts, and the results obtained from this model could be useful for the development of novel anthelmintic agents. However, further studies are required to isolate and characterize the active compounds responsible for the anthelmintic activity of *M. quadrifolia* extract and to evaluate its efficacy and safety in animal models and human subjects.

CONCLUSION:

The present study evaluated the anthelmintic activity of hydroalcoholic extract of *Marsilea quadrifolia* leaves. The findings suggest that the extract possesses significant anthelmintic activity, as evidenced by the paralysis and mortality of the test worms. The results indicate that the hydroalcoholic extract of *Marsilea quadrifolia* leaves has the potential to be used as a natural anthelmintic agent. Further studies are needed to elucidate the mechanism of action and safety of the extract before it can be recommended for clinical use. Overall, this study highlights the importance of exploring natural products as potential sources of anthelmintic agents, especially in the face of increasing drug resistance and the need for alternative therapies.

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