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Aman Saroj
P.G Research Scholar, Faculty of Pharmacy, P.K University, Shivpuri, Madhya Pradesh, India

Sunil Kumar
Associate Professor, Faculty of Pharmacy, P.K University, Shivpuri, Madhya Pradesh, India

Sonam Baghel
Assistant Professor, Faculty of Pharmacy, P.K University, Shivpuri, Madhya Pradesh, India

Jitendra Malik
Professor, Faculty of Pharmacy, P.K University, Shivpuri, Madhya Pradesh, India

Gyan Singh
Associate Professor, Faculty of Pharmacy, P.K University, Shivpuri, Madhya Pradesh, India

Vinay Kumar Siroliya
Assistant Professor, Faculty of Pharmacy, P.K University, Shivpuri, Madhya Pradesh, India

Anadi Tiwari
Assistant Professor, Faculty of Pharmacy, P.K University, Shivpuri, Madhya Pradesh, India

Corresponding Author:
Aman Saroj
P.G Research Scholar, Faculty of Pharmacy, P.K University, Shivpuri, Madhya Pradesh, India

To evaluate the pharmacological activity of *Nymphaea alba* using *in-vitro anthelmintic* model

Aman Saroj, Sunil Kumar, Sonam Baghel, Jitendra Malik, Gyan Singh, Vinay Kumar Siroliya and Anadi Tiwari

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Abstract
This research explored the anthelmintic potential of an ethanolic extract derived from *Nymphaea alba* flowers against earthworms, roundworms, and tapeworms. The outcomes indicate a concentration-dependent anthelmintic effect of the extract. Introduction: *Nymphaea alba*, commonly referred to as the white-water lily, has a rich history of medicinal utilization across various traditional healing systems. This study aimed to scrutinize the potential anthelmintic properties of its flowers. Materials and Methods: The ethanolic extract of *Nymphaea alba* flowers was meticulously prepared. Anthelmintic activity was evaluated using earthworms, roundworms, and tapeworms sourced from pigs. Various concentrations (25, 50, and 100 mg/ml) of the extract were tested, and the paralysis and death times of the worms were meticulously recorded. Results: The extract demonstrated concentration-dependent anthelmintic activity against all three worm species. Higher concentrations led to accelerated paralysis and death times. Albendazole and piperazine citrate served as positive controls, exhibiting a more robust anthelmintic effect compared to the extract. Conclusion: The findings suggest that the ethanolic extract of *Nymphaea alba* flowers holds promise as an anthelmintic agent. However, further investigation is warranted to elucidate the specific active constituents and their efficacy *in vivo* models.

Keywords: *Nymphaea alba*, ethanolic extract, anthelmintic activity, earthworms, roundworms, tapeworms

Introduction
Traditional medicine, particularly through the use of medicinal herbs, is widely used by a significant portion of the global population. Ayurveda, a medical system dating back to 2000 BC, is practiced in India and has been studied and documented for its therapeutic potential. Herbal therapy offers a milder approach to treating various disorders, particularly those that are chronic or difficult to manage through traditional means [1]. Over the past few years, there has been a significant increase in the field of herbal medicine due to the perception that herbal treatments come from natural sources and may have less severe adverse effects than more traditional ones. Herbal medicine faces a significant obstacle in the form of standardization, as the efficacy and safety of herbal treatments can be negatively impacted by factors such as variations in plant quality, incorrect identification, and inappropriate preparation. It is essential to give careful consideration to the possibility of having interactions with traditional treatments. India, known as the "botanical garden of the world," is home to an extensive variety of plants used for therapeutic purposes and plays an important role in the investigation of herbal treatments [2, 3]. Helminthic infections, caused by parasitic worms, are a widespread public health concern, particularly in developing countries. These infections contribute to malnutrition, anemia, eosinophilia, and pneumonia. Common intestinal helminths include cestodes, nematodes, and trematodes. Parasitic worm infections such as lymphatic filariasis, onchocerciasis, and schistosomiasis can cause severe health problems and have significant economic and social consequences in endemic areas. Helminthiasis, a parasitic worm infection caused by helminths, is classified as a neglected tropical disease due to its widespread prevalence and limited resources dedicated to its control. Over a billion people globally are infected with at least one helminth species, contributing to severe health problems, chronic illness, decreased productivity, and hindering socioeconomic development.

Helminths can also modulate the immune system, potentially impacting the body's response to other pathogens. The etiology of helminthiasis is complex, with unsanitary conditions being a major contributor. Humans can become infected by ingesting contaminated food or water containing eggs or larvae of the parasite, or by forming cysts encapsulated by tissue.

Helminthiasis is a serious health issue caused by helminth infections, which can cause direct damage to internal organs and pressure exerted by growing parasites. Large nematodes like Ascaris and tapeworms like Taenia and Diphyllobothrium can obstruct the intestines, while schistosome eggs can form granulomas that block blood flow through the liver, leading to pathological changes. Additionally, cysts formed by the tapeworm Echinococcus multilocular is can develop in various organs like the liver, brain, and lungs, causing abnormal enlargement, organ damage due to pressure, and even tissue death (Necrosis) [1-4].

Indirect damage from the host immune response is also a significant challenge in helminthiasis control. Hypersensitivity reactions lead to the formation of granulomas around deposited parasite eggs, blocking liver sinusoids and causing pathological changes in the liver. This hypersensitivity-related inflammation likely also contributes to lymphatic blockage observed in filarial infections. The clinical presentation of helminth infections can vary depending on several factors, including the specific helminth species, the intensity of the infection, and the age of the infected individual. Examples include Taenia Solium, Schistosoma, Echinococcus granuloses, and hookworm and Schistosomiasis. Pregnant women are particularly at risk, potentially causing neonatal prematurity and increasing maternal complications and mortality [4, 5]. Children, particularly those of school age or younger, are more susceptible to intestinal worm and schistosome infections compared to other age groups. These infections can have a significant impact on a child's development, leading to growth retardation, decreased physical fitness, and impaired memory and cognitive function.

Treatment strategies for helminthiasis include medications known as anthelmintics, which work in various ways to eliminate the worms, such as paralysis, damage to the worm's outer cuticle, and interference with the worm's metabolism. However, the effectiveness of an anthelminthic drug can vary depending on the specific helminth species [7-12].

Helminthic infections are a significant burden on human health and economy, surpassing the global burden of malaria and tuberculosis. These parasitic worms not only impact human health but also cause substantial economic losses in livestock and crop industries worldwide.

Roundworms, such as Ascaris lumbricoides (Common roundworm) and Ancylostoma duodenale (Hookworm), can migrate through the body and cause health problems like migraines, eosinophilia (Increased white blood cells), and respiratory issues due to transmission through skin contact with contaminated soil. Common intestinal helminth infections, like those caused by Ascaris and Trichuris trichiura (Whipworm), often cluster within households due to shared environments. Eggs deposited in the perianal area can reinfect the same person (Autoinfection) or contaminate surfaces like carpets and curtains. Accidental ingestion of these eggs can lead to further infection. Additionally, inhalation of a small number of eggs can occur. Once ingested, the larvae can damage the lungs, liver, and central nervous system.

Flukes, like Schistosoma species (Blood flukes), are transmitted through direct contact with contaminated freshwater. These parasites penetrate the skin, enter the bloodstream, and migrate to the liver and intestines where they mature. Eggs are then released in feces and urine, perpetuating the life cycle. Tapeworms, such as Taenia Solium (pork tapeworm), can be transmitted through humans as intermediate hosts. Ingestion of undercooked pork containing cysts can lead to the development of adult tapeworms within the intestines, causing mild abdominal discomfort. In severe cases, pork tapeworm or fluke infections can invade the central nervous system, leading to a condition called neurocysticercosis, which can be treated with medications like albendazole and praziquantel. Helminth infections can lead to various health complications, including iron-deficiency anemia, seizures, portal hypertension (High blood pressure in the portal vein), and chronic diarrhea [8-15]. These complications contribute to high rates of morbidity (Illness) and mortality (Death). School-aged children and women of childbearing age are particularly vulnerable to helminth infections, which can exacerbate malnutrition, anemia, and cognitive development issues. In conclusion, helminthic infections pose a significant threat to human health and economy, particularly in tropical regions. The transmission and clinical impact of these parasitic worms are complex and multifaceted, with potential for severe health consequences for individuals and populations [9, 10].

Methodolgogy

The study focuses on the extraction and characterization of plant extracts from *Nymphaea alba*, a plant known for its antioxidant and anti-inflammatory properties. The plant material was collected from a local area in Varanasi and dried under the sun. The dried flower was then powdered and stored in plastic bags. The extraction procedure involved defatting the plant material, extracting it with petroleum ether by maceration, and then evaporated above boiling points. The percentage yields of each extract were calculated using a formula: weight of extract = - x 100. Phytochemical tests were conducted to detect alkaloids, carbohydrates, glycosides, saponins, phenols, flavonoids, proteins, and diterpenes. Alkaloids were detected using Wagner's reagent, Hager's reagent, Fehling's test, Legal's test, Froth test, ferric chloride test, sodium hydroxide test, lead acetate test, xanthoproteic test, and copper acetate test.The total flavonoids content was determined using the aluminium chloride method. Standard quercetin was prepared in methanol, and various aliquots of 5-25μg/ml were prepared in methanol. Extracts were prepared by dissolving 10 mg of dried extract in methanol and filtering. Three ml (1mg/ml) of this extract was used for the estimation of flavonoids. *In vitro* antioxidant activity was measured using different models, DPPH method, hydrogen peroxide method, and *in vitro* anthelmintic effect of ethanolic extract. The study also investigated the *in vitro* antioxidant activity of the ethanolic extract. The results showed that the extract had a significant antioxidant activity, with a 50% reduction in the formation of free radicals. The extract also showed a significant anti-inflammatory effect. In conclusion, the study provides valuable insights into the extraction and characterization of plant extracts from *Nymphaea alba*, highlighting their potential as an antioxidant and anti-inflammatory agent [2-5].

The study aimed to estimate the total alkaloids content, total phenol content, and antioxidant activity of the ethanolic extract of *Nymphaea alba* flower using different models. The plant extracts were dissolved in methanol, added 2 N HCl, and filtered. The mixture was then shaken with chloroform and collected in a 10-ml volumetric flask. A set of reference standard solutions of atropine were prepared and the absorbance for test and standard solutions was determined against the reagent blank at 470 nm using an UV/Visible spectrophotometer. The total phenol content was determined by the modified Folin-Circlet method. Two ml of the dried extract was dissolved in 10 ml methanol and filtered. The extract and each standard were mixed with 1 ml of Folin-Ciocalteu reagent and 1 ml of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10 minutes for color development. The absorbance was measured at 765 nm using a spectrophotometer. The antioxidant activity of the ethanolic extract of *Nymphaea alba* flower was measured using the DPPH method. The stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. The decrease in absorbance in the presence of sample extract at different concentrations (10-100 µg/ml) was noted after 15 minutes. The DPPH scavenging activity was measured by the spectrophotometer. The hydrogen peroxide (H2O2) scavenging activity of the plant extract was determined by the method of Ruch. The ethanolic extract (4 ml) prepared in distilled water at various concentrations was mixed with 0.6 ml of 4 mm H2O2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 minutes. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using the following equation. *In-vitro* anthelmintic studies of the ethanolic extract of *Nymphaea alba* were conducted on adult Indian earthworm, roundworm, and tapeworm. Five groups of approximately similar size earthworm, roundworm, and tapeworm were released in 50ml of desired formulation. Each worm was given 1 percent gum acacia in normal saline, albendazole (25, 50, 100 mg/ml), herbal extracts in various concentrations (25, 50, 100 mg/ml), and other formulations [5, 8]. When the worms did not resuscitate in normal saline solution, the time taken for paralysis was recorded. The worms died when their motility ceased and the loss of their body color was recorded.

Result and Discussion

Preliminary phytochemical investigation of the extract

Physical characteristics of extract

Finally extraction of defatted flowers was done with ethanol solvent and % yield was found to be 6.12% w/w and their characteristics are reported in table 1.

Table 1: Physical characteristics of extract

Extract	Consistency	Colour	Odour	% Yield
Ethanolic	Solid	Dark Brown	Pungent	6.12

Fig 1: Extract of flowers of *Nymphaea alba*

Qualitative chemical test: Results obtained from qualitative chemical tests are tabulated in Table 2.

Table 2: Qualitative chemical tests of extract of *Nymphaea alba*

S. No.	Bioactive constituents	Ethanolic extract
1	Alkaloids	+ve
2	Carbohydrates	-ve
3	Glycosides	-ve
4	Saponins	+ve
5	Phenols	+ve
6	Flavonoids	+ve
7	Proteins	-ve
8	Diterpenes	-ve

+ ve – Present, - ve – Absent

Fig 2: Photograph during phytochemical screening

Results of quantitative study

Estimation of total flavonoids content (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y=0.032X + 0.018, R2=0.998, where Xis the quercetin equivalent (QE) and Y is the absorbance.

Calibration curve of Quercetin

Table 3: Preparation of Calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Absorbance
1	5	0.194
2	10	0.3486
3	15	0.513
4	20	0.656
5	25	0.812

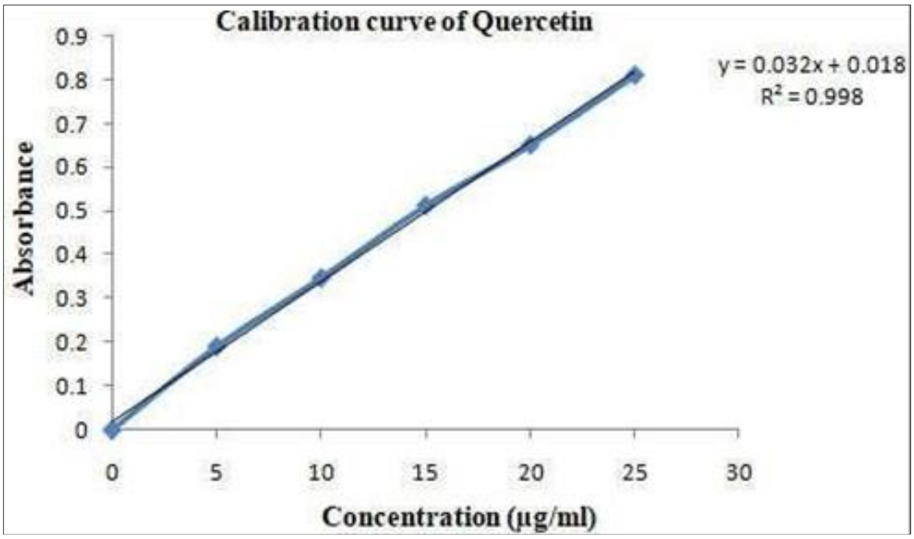


Fig 3: Graph of calibration curve of Quercetin

Estimation of total alkaloid content (TAC)

Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: Y=0.007X+ 0.024, R2=0.995, where X is the Atropine equivalent (AE) and Y is the absorbance.

Calibration curve of Atropine

Table 4: Preparation of calibration curve of Atropine

S. No.	Concentration (µg/ml)	Mean Absorbance
1	40	0.322
2	60	0.455
3	80	0.604
4	100	0.722
5	120	0.846

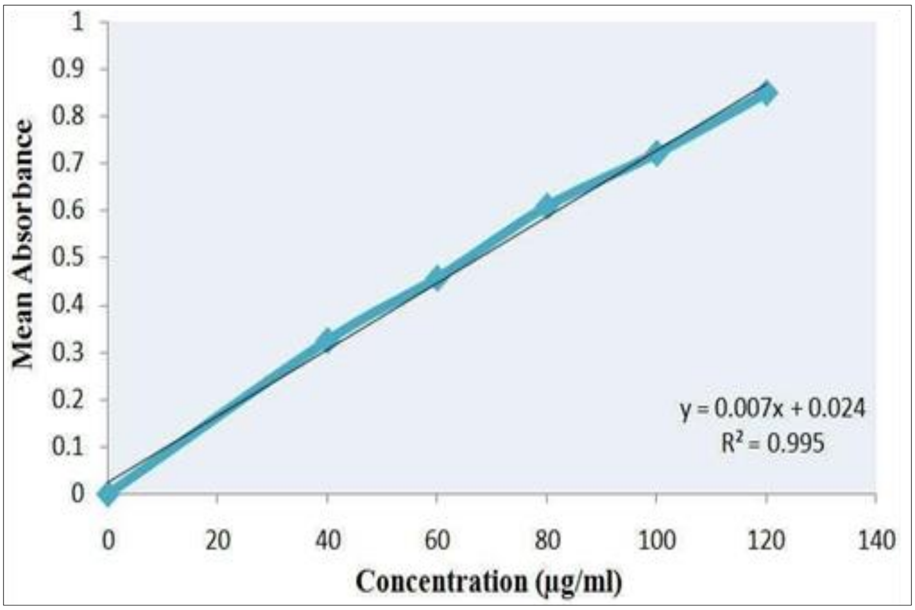


Fig 4: Graph of calibration curve of Atropine

Table 5: Hydroachoholic extract of *Nymphaea alba* against earthworm

S. No	Drug Name	Paralysis Time (In Minutes)	Death Time (In Minutes)
1	Standard Albendazole 15 mg /ml	15.6±0.34	28±0.68
2	Standard Piperazine 15mg/ml	36.0±0.61	54.0±0.73
3	Hydroachoholic extract of *Nymphaea alba* 15mg/ml	47.1±0.85	76.0±1.495
4	Hydroachoholic extract of *Nymphaeaalba* 30mg/ml	35.2±0.83	62.2±0.60
5	Hydroachoholic extract of *Nymphaea alba* 60mg/ml	20.0±0.62	29.0±1.06

Mean±SEM

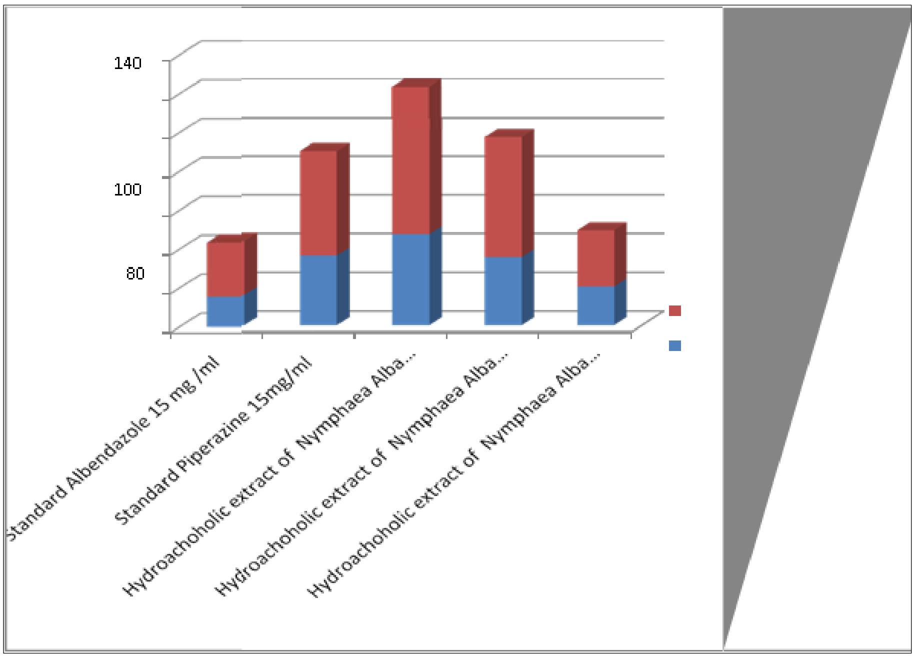
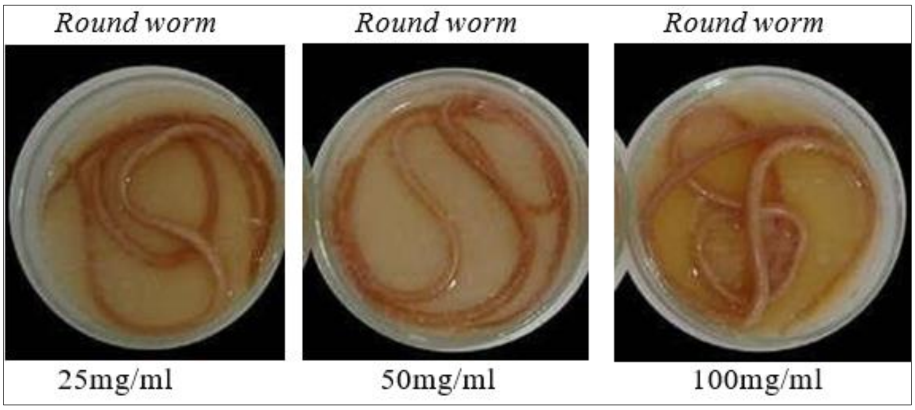


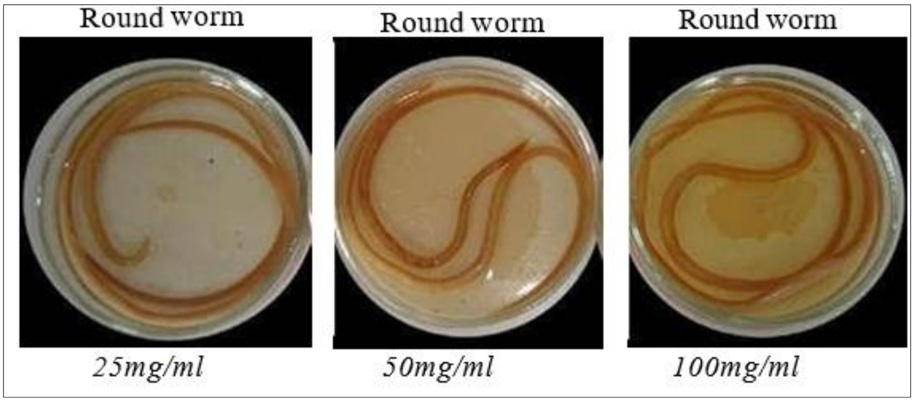
Fig 5: Hydroachoholic extract of *Nymphaea alba* against earthworm

Hydroachoholic extract of *Nymphaea alba* against round worm

Albendazole



Piperazine Citrate



Hydroachoholic extract of *Nymphaea alba*

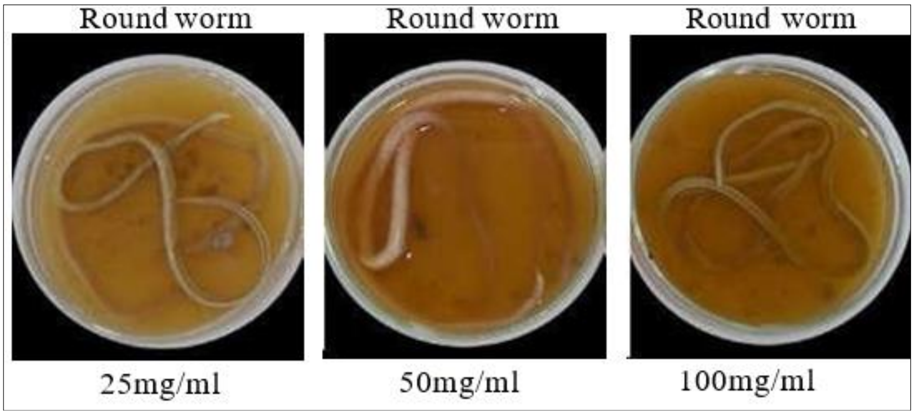


Table 6: Hydroachoholic extract of *Nymphaea alba* against round worm

S. No.	Drug Name	Paralysis Time (In Minutes)	Death Time (In Minutes)
1	**Standard Albendazole**		
	25mg/ml	57.6±0.76	191.8±0.60
	50mg/ml	36.7±0.49	84.8±0.60
	100mg/ml	25.0±0.72	56.2±0.46
2	**Standard Piperazine**		
	25mg/ml	123.5±0.42	143.6±0.51
	50mg/ml	91.0±0.46	113.6±0.53
	100mg/ml	76.3±0.22	99.5±0.42
3	**Hydroachoholic extract of *Nymphaea alba***		
	25mg/ml	67.3±0.66	124.5±0.42
	50mg/ml	44.5±0.95	97.3±0.666
	100mg/ml	31.2±0.60	78.8±0.9452

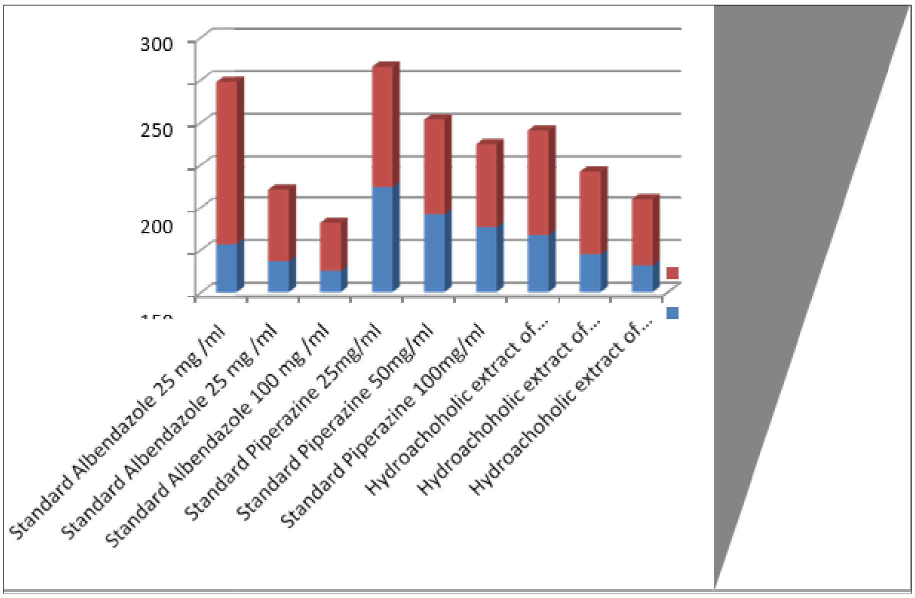
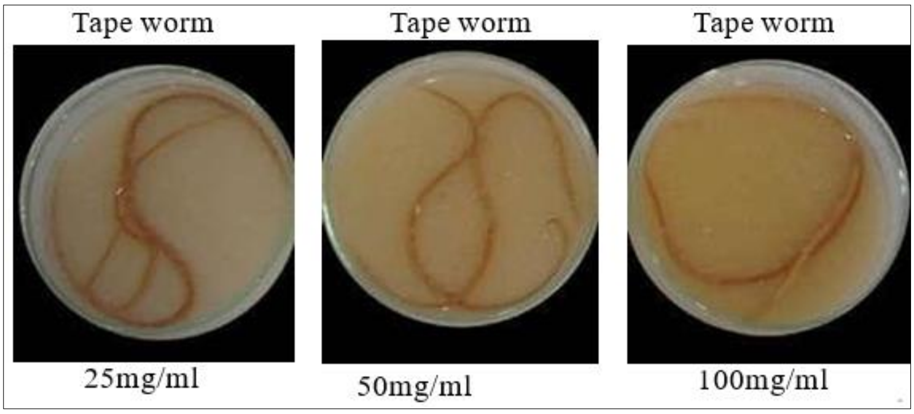


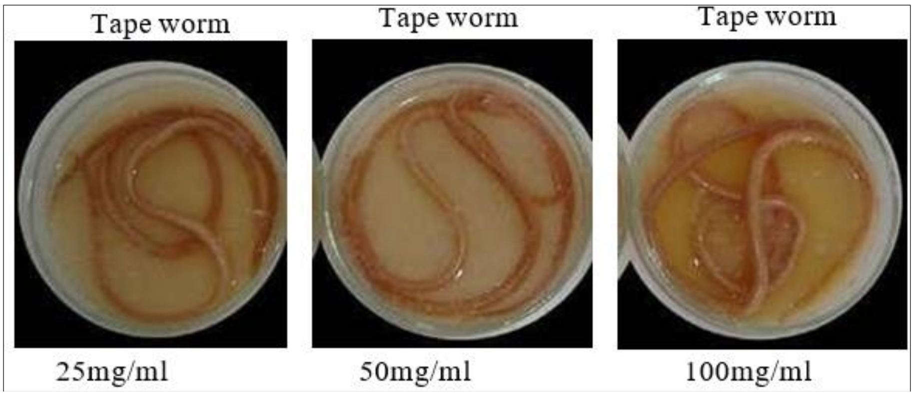
Fig 6: Hydroachoholic extract of *Nymphaea alba* against round worm

Hydroachoholic extract of *Nymphaea alba* against tape worm

Albendazole



Piperazine Citrate



Hydroachoholic extract of *Nymphaea alba*

Table 7: Hydroachoholic extract of *Nymphaea alba* against tape worm

S. No	Drug Name	Paralysis Time (In Minutes)	Death Time (In Minutes)
1	**Standard Albendazole**		
	25mg/ml	79.6±1.23	228.3±1.05
	50mg/ml	69.8±1.30	188.6±1.28
	100mg/ml	58.2±1.01	173.8±0.93
2	**Standard Piperazine**		
	25mg/ml	163.8±0.96	284.3±1.02
	50mg/ml	138.3±0.71	250.0±0.93
	100mg/ml	101.6±0.98	225.3±0.52
3	**Hydroachoholic extract of *Nymphaea alba***		
	25mg/ml	91.6±0.75	249.2±0.70
	50mg/ml	77.0±0.73	215.6±0.88
	100mg/ml	68.3 0.13	172.6±0.66

Summary and Conclusion

This study investigated the potential of a white water lily (*Nymphaea alba*) flower extract as a future weapon against parasitic worms. The extract, made with a combination of water and alcohol (hydroalcoholic extract), was tested in a petri dish setting (*in vitro*) against earthworms, roundworms, and tapeworms at various concentrations (25, 50, and 100 mg/ml).The researchers used a technique developed by Mathew *et al*. to assess the extract's anthelmintic (Worm-killing) properties. Earthworms from India were chosen because of their similarities to human intestinal roundworm parasites in terms of body structure and function. Roundworms and tapeworms were also obtained from pigs in India for the testing. The researchers tracked how long it took for individual worms to become paralyzed and die after exposure to the extract. Worms were considered paralyzed if they didn't regain movement when placed in a normal saline solution. Death was determined by a return of movement followed by a loss of body color. A preliminary study on white water lily flower extract shows potential for future anthelmintic medications. Future research could explore the extract's anthelmintic activity, conduct animal studies, conduct clinical trials, and standardize the extract preparation method. This research could lead to gentler, more natural treatments for parasitic worm infections, potentially improving public health. Further research and animal testing are necessary before incorporating the extract into medicine.

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