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***Research Article** (Open Access)*

ANTHELMINTIC PROPERTIES OF LEAVES EXTRACT OF SHAHATUT, *MORUS ALBA*

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ABSTRACT
Shahatut is a medicinal plant and is typically found in Asia's subtropical regions. The purpose of this study is to investigate the anthelmintic properties of aqueous, hydro- alcoholic, alcoholic extracts (prepared by maceration) and methanolic extracts (prepared by soxhlation) of leaves of *Morus alba.* All of the extracts were screened for phytoconstituents using preliminary phytochemical screening. Albendazole was used as a standard medication to test the anthelmintic activity of the red wiggler, *Eisenia fetida*. All the extracts were endowed with anthelmintic properties, leading to the paralysis and death of *Eisenia.* Methanol extract has highly substantial anthelmintic action, producing earthworm paralysis and death at a concentration of 30 mg/ml and is more efficient than albendazole suspension. The paralysis and death time of methanolic extract were 17 ± 2.52 minutes and 42 ± 4.81 minutes, respectively, while that of standard medication (albendazole) was analysed as 28 ± 1.74 minutes and 56 ± 2.01 minutes. Thus, the anthelmintic potency of *Morus alba* leaf extract has been proven for the first time on *Eisenia fetida*.

***Keywords*:** *Morus alba, Eisenia fetida, Anthelmintic activity, Albendazole, Earthworm*

INTRODUCTION
Helminth infestations are one of the most frequent illnesses in humans, affecting a huge percentage of the global population. They are a significant hazard to public health in under-developed nations. They harm the host by making the availability of food scanty, hemorrhage, organ injuries, impediment of intestinal or lymphatic and by toxin secretion. Helminthiasis is rarely fatal, but is a major cause of morbidity (Bundy 1994). An anthelminthic drug must have a broad stimulatory effect.
Mulberry is a medicinally important plant belonging to genus Morus and is found in many countries, including India. White mulberry is act as main source of food for silkworms and is (Aditya 2012). Its root bark is very important and has property like anthelmintic, etc. (Singh and Ghosh 1992). The anthelmintic activity of leaves of medicinal plants like *Tamarindus indica* has been reported by researchers (Sampat *et al.,* 2009). The earthworm is chosen in the majority of anthelminthic examinations because it is morphologically and physiologically similar to the human intestinal roundworm, Kuldeep (2021). The present study was performed to determine the anthelmintic properties of *Morus alba* leaf extract.

MATERIALS AND METHODS
Plant material collection
The leaves of *Morus alba* were collected from the university campus of MDU, Rohtak, Haryana. After collection, the leaves were cleaned and dried in the sunlight for 2 days. The leaves were crushed and used for further extraction.
Worms collection
Eisenia fetida was collected from BhooJeevan Organics, Najafgarh, New Delhi, India. Worms were acclimatised for 7 days at room temperature. To remove any faecal debris, the worms were rinsed with normal saline. Earthworms of 8–12 cm were used for the experiment protocol.

Extract preparation

The preparation of extracts was done as per the method specified by Kumanan *et al.,* (2010).

Aqueous extract of *Morus alba* (AQMA)– The crushed leaves of *Morus alba* (100 g) were soaked in 500 mL of distilled water for 3 days.

Hydro-alcoholic extract of *Morus alba* (HAMA)– The crushed leaves of *Morus alba* (100 g) were soaked in 500 mL of hydro-alcoholic solution (water and ethanol in 1:1) for 3 days.

Alcoholic extract of *Morus alba* (ALMA)– The crushed leaves of *Morus alba* (100 g) were soaked in 500 mL of ethanol for 3 days.

After soaking leaves in AQMA, HAMA and ALMA, the concentrated extract was dried using a rotary evaporator at 60–70^{o}C and then the semisolid extract percentage was calculated.

Methanolic extract of *Morus alba* (MEMA)–soxhlation method

The crushed leaves of *Morus alba* (100 g) were placed in a thimble and extracted with 250 mL of methanol repeatedly for 8 hours using the Soxhlet apparatus. The concentrated extract was dried at 60–70 ^{o}C using a rotary evaporator. The percentage yield of semisolid extract was calculated.

The percentage yield of leaf extract is calculated as:

$$\text{Percentage yield} = \frac{\text{weight of residue of extract after solvent removal (in grams)}}{\text{weight of leaves taken (im grams)}} \times 100$$

The semisolid extracts were refrigerated until used.

Phytochemical qualitative analysis:

All extracts were subjected to phytochemical analysis in accordance with the standard method as per Sandhar *et al.,* 2011.

Carbohydrate analysis

Benedict's test was used for carbohydrate examination. Each extract was dissolved in 5 mL of distilled water and filtered. The presence of carbohydrates was determined by testing the filtrates. For this, Benedict's reagent was added to the filtrates and then gently heated. The presence of reducing sugars is indicated by an orange-red precipitate.

Saponin examination

A froth test was followed by saponin analysis.

Examination of phenols

The presence of phenols was checked by the ferric chloride test.

Tannin analysis

The Gelatin test was used to determine the presence of tannins.

Examination of flavonoids

The presence of flavonoids was confirmed by the alkaline reagent test.

Examination of alkaloids

Wagner's test was performed to check for the presence of alkaloids in the extract.

Phytosterol investigation

Salkowski's test was followed to determine the presence of phytosterols in the extarct.

Protein and amino acid analysis

The presence of proteins was confirmed by the Xanthoproteic Test.

Analysis of glycosides

Extracts were treated with dilute hydrochloric acid before testing for glycosides. Glycoside analysis was performed by Borntrager's Test. For this, the extracts were submerged in boiling water for around 5 minutes. After cooling, the mixture was extracted with equal parts of benzene. After separating the benzene layer, the mixture was treated with ammonia solution. The presence of glycosides is confirmed by the appearance of a rose-pink colour.

Anthelminthic activity evaluation

In *Eisenia fetida*, extracts of *Morus alba* were tested for anthelmintic activity (Ghosh *et al.,* 2003). The extracts were prepared as test samples at various concentrations, including 10, 20, and 30 mg/ml. Normal

saline was used as a control and albendazole was used as a standard. *Eisenia fetida* were segregated into 14 groups, with three earthworms each. *Eisenia fetida* were introduced in 9 cm petri dishes with various amounts of extract solution as well as a control and a standard (albendazole) medication.
The group arrangements of *Eisenia* are as follows:
Group 1: Normal saline water (control)
Group 2: Albendazole solution at a concentration of 20 mg/mL (standard)
Group 3: Aqueous extract of *Morus alba* (AQMA)
Group 4: Hydro-alcoholic extract of *Morus alba* (HAMA)
Group 5: Alcoholic extract of *Morus alba* (ALMA)
Group 6: Methanolic extract of *Morus alba* (MEMA)
(Group 3 to Group 6 were prepared at three concentrations of 10 mg/mL, 20 mg/mL and 30 mg/mL each)
Statistical analysis: All values are represented as Mean ± SEM; where n=3 in each group. Values are determined using ONE way ANOVA. $P<0.05$ was regarded significant when comparing the standard and treated groups.

RESULTS AND DISCUSSION

The colour, consistency, and percentage yield of AQMA, HAMA, ALMA, and MEMA are expressed in the Table 1.

Phytochemical analysis

The presence of numerous secondary metabolites was investigated in all of the plant extracts. According to the phytochemical screening, the aqueous extract of *Morus alba* showed the presence of carbohydrates, flavonoids, alkaloids, phytosterols, and glycosides. The hydro-alcoholic of *Morus alba* extract showed positive tests for saponins, carbohydrates, flavonoids, alkaloids, and glycosides. The alcoholic extract of *Morus alba* does not showed positive results for tannins and phytosterols; the rest of the tests are positive. The methanolic extract of *Morus alba* that was prepared by the soxhlation method shows the presence of all secondary metabolites responsible for anthelminthic activity (Table 2).

Anthelminthic activity

When tested on the Indian earthworm *Eisenia fetida*, the leaves of *Morus alba* demonstrated crucial anthelminthic action, Fig1. The earthworm is completely unaffected by the normal saline (control) regime. All of the extracts, however, paralyse and kill the earthworm. The MEMA possessed highly substantial anthelminthic activity when compared to the standard drug (albendazole) at 20 mg/mL and 30 mg/mL concentrations. The paralysis time and death time (Table 3) at 20 mg/mL of methanolic extract of *Morus alba* were noted as (21±4.24) and (49±3.76), while that of the standard drug was recorded as (28±1.74) and (56±2.01), respectively. The HAMA and ALMA also displayed potential anthelminthic activity. However, it is less than the standard medication of albendazole. The AQMA demonstrated minor anthelminthic activity at different dosages when compared to other leaf extracts.

Table 1: Colour, consistency and % yield of *Morus alba* extracts

Extract	Colour observed in day light	Consistency	% Yield (w/w)
AQMA	Greenish-brown	Semisolid	4.8
HAMA	Dark green	Semisolid	6.6
ALMA	Dark green	Semisolid	6.2
MEMA	Dark green	Semisolid	8.4

Table 2: Qualitative phytochemical analysis of different extracts of *Morus alba*

Tests	AQMA	HAMA	ALMA	MEMA
Carbohydrates	+	+	+	+
Saponins	-	+	+	+
Phenols	-	-	+	+
Tannins	-	-	-	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Phytosterols	+	-	-	+
Proteins	-	-	+	+
Glycosides	+	+	+	+

+ show the presence, - show the absence

Table 3. Paralysis time and death time of *Eisenia fetida* in different extracts of *Morus alba.*

Extract	Concentration (mg/mL)	*Eisenia fetida*	
		Paralysis (mins.)	Death (mins.)
Control (normal saline)	-	-	-
Standard (Albendazole)	20	28 ± 1.74	56 ± 2.01
AQMA	10	160 ± 2.33	198 ± 6.32
	20	132 ± 5.72	170 ± 5.34
	30	104 ± 2.96	142 ± 3.06
HAMA	10	110 ± 5.64	145 ± 5.28
	20	91 ± 3.26	136 ± 2.49
	30	50 ± 4.35	88 ± 3.43
ALMA	10	62 ± 3.46	101 ± 3.57
	20	48 ± 4.91	83 ± 7.21
	30	30 ± 3.82	57 ± 1.74
MEMA	10	32 ± 5.23	65 ± 3.82
	20	21 ± 4.24	49 ± 3.76
	30	17 ± 2.52	42 ± 4.81

The above values are represented as Mean ± SEM; where n=3 for each set. Values are determined using ONE-way ANOVA. P<0.05 was regarded as significant when comparing the standard and treated sets.

Helminth infection is caused by the warm, humid climate of tropical areas as well as a lack of sanitation. Numerous secondary metabolites found in medicinal plants have anthelmintic properties. *The Morus alba* plant has been previously reported to have anthelmintic properties. The results obtained in this experiment are in agreement with the experimental results of *Morus alba* using *Pheretima posthuma*, proving the anthelmintic property (Aditya *et al.,* 2012). However, the anthelmintic effect of *Morus alba* on the experimental organism *Eisenia fetida* has not been explored previously. The results of this study show that the sequential extracts of *Morus alba* have significant anthelmintic properties in a dose-dependent fashion for the criteria evaluated, namely paralysis and death. Despite the fact that all of the extracts were endowed with anthelmintic properties, this experiment reveals that the methanol extract is more efficient than the others. This is in agreement with the results of *Luffa cylindrical* on *Pheretima posthuma* (Sangh *et al.*, 2012).

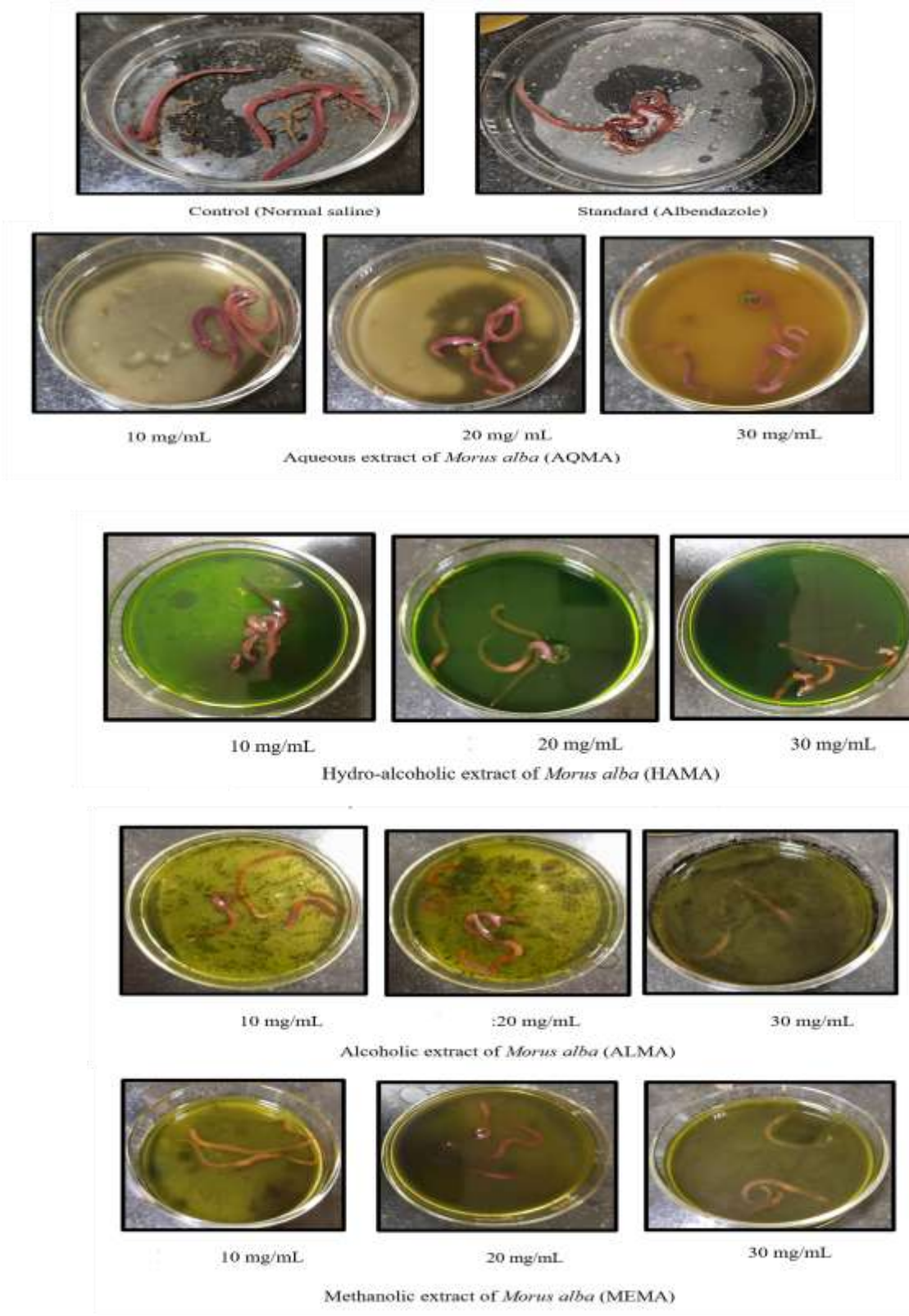

Figure 1: Anthelminthic activity of Earthworm under different extracts

The maximum anthelminthic activity was seen at the highest concentration of methanolic extract, 30 mg/mL, which takes 17 ± 2.52 minutes to cause paralysis of earthworms and 42 ± 4.81 minutes to cause the death of earthworms. The action of ethanolic extract was similar to that of albendazole (standard drug). Also, normal saline had no effect on the mortality of worms.

The potential of the extract is inversely proportional to the time taken for paralysis (vermifugal) and death (vermicidal). This result is in agreement with the results of *Psidium guajava* on *Pheretima posthuma* (Swetha *et al.*, 2018) *Clitoriaternatea* on *Eisenia fetida* (Sandhar *et al.*, 2010).

Earthworms have no way of storing energy, so to satisfy their metabolic needs they need to eat food consistently. Anthelminthics primarily kill worms by depriving them of food till they die or by paralysing them. Most adult parasites can be killed by interrupting their feeding for 24 hours or less. Parasites will also perish if they become paralysed and lose their capacity to sustain their placement in the gut for an extended period of time. Phytochemical screening of all extracts of *Morus alba* shows positive results for different secondary metabolites like saponins, carbohydrates, phenols, flavonoids, etc. Chemically synthesized phenolic anthelminthics uncouple the oxidative phosphorylation in helminth parasites, preventing them from producing energy, Athnasiadau (2001). Tannins may responsible for uncoupling oxidative phosphorylation in which energy generation is disturbed (Thompson 1993). They may also interfere with the glycoproteins present on the surface of the cell. They can also work by attaching to proteins in the gastrointestinal tract of the host or to the cuticle of the parasite and causing the death of worms (Sandhar *et al.*, 2010).

Flavonoid chemicals, such as apigenin, can suppress larval growth and arachidonic acid metabolism, which can lead to neuron degeneration and therefore cause the death of the worm (Sutthaya and Wannee 2017). Besides this, tannins, alkaloids, phenols, and flavonoids increase insulin release while decreasing glucose absorption in the intestine. According to the literature, all of these phytochemicals have alpha-amylase inhibitory action (Junita *et al.*, 2020).

CONCLUSION

In light of the findings, the *Morus alba* leaf extracts have shown considerable anthelmintic action as a vermifuges and vermicidals on *Eisenia fetida* at all tested concentrations when compared to control; with the maximum activity displayed by the higher concentration (30 mg/mL), confirming the ethno-medicinal claim. It can be concluded that the *Morus alba* leaf extracts have considerable ($P<0.05$) anthelmintic properties. As a result, we can consider this plant as a potential alternative source of anthelmintic medications as well as a potential source of new active leads for anthelmintic drugs.

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REFERENCES

Aditya RSJ, Ramesh CK, Riaz M & Prabhakar BT (2012). Anthelmintic and Antimicrobial Activities in Some Species of Mulberry. *International Journal of Pharmacy and Pharmaceutical Sciences,* **4**(5) 335 – 338

Athnasiadau S, kyriazakis I, Jackson F & Coop RL (2001). Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. *Veterinary Parasitology,* **l**(99) 205-219.

Bundy DA (1994). Immunoepidemiology of intestinal helmintic infection I: The global burden of intestinal nematode disease. *Transactions of the Royal Society of Tropical Medicine & Hygiene,* **8** 259–61.

Ghosh T, Maity TK, Bose A & Dash GK (2003). Anthelmintic activity of Bacopa monnieri. *Indian Journal of Natural Products,* **21**(2) 16-19.
Junita R, Khaga RS & Yuba RP (2020). Antioxidant and alpha amylase inhibitory activity of Nepalese medicinal plants from Gorkha district. *Journal of Pharmacognosy and Phytotherapy,* **12(**2) 28-35
Kuldeep K, Ajeet PS & Amar P (2021). Investigation of Anthelmintic effect of neolamarckia cadamba fruit extracts in ascariasis. *International Journal of Current Pharmaceutical Research,* **13(**5) 70-75
Kumanan R, Sridhar C, Jayaveera KN, Sudha, Rubesh KS & Duganath N (2010). Comparative Study of Anthelmintic Activity of Different Leaf Extracts of *Tecoma stans* (L.) on Adult Indian Earthworms. *International Journal of Pharmaceutical and Clinical Research,* **2**(2) 63-65
Sampat VM, Mute VM, Patel KA, Sanghavi K, Mirchandani D & Babaria PC (2009). Anthelmintic effect of *Tamarindus indica* Linn leaves juice extract on *Pheretima posthuma*. *International Journal of Pharmaceutical Research and Development*, **7,** 1–7.
Sandhar HK, Kumar B, Prasher S, Tiwari P, Salhan M & Sharma P (2011). A Review of Phytochemistry and Pharmacology of Flavonoids. *International Pharmaceutical Science,* **1**(1) 25-41
Sangh P, Saurabh K, Amit K, Neeraj K, Sharma K & Jha K (2012). In-Vitro Anthelmintic Activity of Luffa cylindrica Leaves in Indian Adult Earthworm. *Journal of Pharmacognosy and Phytochemistry,* **1**(2) 27 – 30
Singh KP & Ghosh PL (1992). Mulberry cultivation under agro forestry and land management. *Indian silk.* **31** 16.
Sutthaya P & Wannee J (2017). An update review on the Anthelmintic activity of bitter gourd *Momordica charantia*. *Pharmacognosy Review*, **11**(21) 31-34.
Swetha NR, Susheel RY, Geetha VP, Nikhil P, Mani SGG, Vasudha B & Narender B (2018). Assessment of Anthelmintic Activity and Phytochemical Analysis of *Psidium guajava* Leaves: An In-Vitro Design. *Journal of Pharmaceutical and Scientific Innovation*, **7**(3), 82 – 87
Thompson DP & Geary TG (1995). The structure and function of helminth surfaces in Biochemistry and Molecular Biology of parasites. (J.J.Marr, ed.), 1st Edn. Academic press, New York, 203-232.