Original Research Article

'Evaluation of the *in vitro* anthelmintic activity of *Melia azedarach* and *Swertia chirata* aqueous extracts against *Haemonchus contortus*"

ABSTRACT

Aim: This research intended to assess the in vitro anthelmintic properties of Ma haneemleaves (Melia azedarach) and whole plant of Chirata (Swertia chirata).

Study Design: *In vitro* study was conducted aganist *Haemonchus contortus* eggs and adult stage by egg hatch assay (EHA) and adult motility inhibition test (AMIT).

Place and duration of study: This study was conducted in the departement of Veterinary Parasitology ,WBUAFS,Kolkata-37 between November,2023 to August, 2024

Methodology: The aqeous extract of Mahaneem and Chirata whole plants were prepared by decoction method, dried and desolved in 2% Dimethyl Sulfoxide to make desirable concentration of extract solutions. Adult *H.contortus* were collected from freshly slaughter abomasum of sheep for separation of eggs and active motile adult was used for AMIT. Each EHA and AMIT was performed in triplicate.

Results: The result revealed that both Chirata and Mahaneem extracts shows anthelmintic activity against Haemonchus contortus. Chirata extract had better efficacy against both eggs and adult stage of *Haemonchus contortus* compare to Mahaneem leave extract. In EHA, Chirata extract efficacy at the dose of 50 mg/ml and 25 mg/ml was more statistically significant ($p<0.05$) than Mahaneem extract. In AMIT, also Chirata shows significantly more efficacy at 50mg/ml and 25 mg/ml than Mahaneem,but at the dose rate of 10 mg Mahaneem shows better efficacy (38.88 ± 2.22) than Chirata (31.11 ± 2.22) extract. At 50 mg/ml concentration after 10 hours of experiment Chirata shows higest efficacy (82.22 ± 2.22) compare to Mahaneem (57.77 ± 2.22).

Conclusion: Both Chirata and Mahaneem aqueous extract can potentially be effective against *Haemonchus contortus* and other GINs in small rumunats when administer properly with selected doses.

Keywords: Chirata, Mahaneem, Anthelmintic, *Haemonchus contortus*, EHA, AMIT

1. INTRODUCTION

The herb *Swertia chirata,* widely referred to as 'Chirata,' is highly esteemed for its various medicinal properties since the time of the 'Atharvaveda (Joshi and Dhawan, 2005) and it has been asserted to be a highly effective medication for recurrent fever, dermatological issues, gastrointestinal parasites,managing the intestines and also function as an anti-inflammatory and blood sugar-lowering agent (Kumar et al.,2010).*Melia azedarach* (Family Meliaceae), usually referred to as Mahaneem is primarily located in the forests of the North-West Himalaya region in India, Pakistan, China, and various tropical and subtropical nations (Nakatani et al.,1998).Haemonchosis can result in large economic losses by causing appetite depression, damages to gastric function, and alterations in total protein content, energy, and mineral metabolism of livestock (Zarlenga et al., 2016) and the control has relied on the use of synthetic anthelmintics leads to growing anthelmintic resistance against commonly available drugs (McRay et al.,2015). In addition, there has been an increasing concern over chemical residues in edible animal products associated with the use of anthelmintic drugs in livestock (Waller, 1997;Spellberg et al.,2016).In this scenario, It is an importants to search alternative of anthelmintic to control the parasitic problem in the animal husbandry sector.Studies to find alternative strategies for the control of nematodes have focused on various options, one of the strategy is to explore the anthelmintic properties of plants containing bio active compounds such as secondary metabolites. In this context, the investigation and evaluation of different plants for new anthelmintic substances (Villegas et al.,2011) is very much necessary. The herbal deworming method could be a alternative treatment for the efficient management of parasitic infestation and additionally to fight against parasitic resistance toward the chemical anti-parasitic agents (Jain and Sahni, 2009). Anthelmintics derived from indigenous plants have pragmatic features for marginal farmers who cannot afford the commercial product, and for large commercial farmers who are shifting to organic farming (Gradé et al., 2008; Taylor et al., 2001). Furthermore, herbal anthelmintics as a natural

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product are implicated to least likely bioaccumulate in the tissues of animal and the environment (Taylor et al.,2011). They are like wise considered eco-friendly and biodegradable (Hammond et al.,1997; McCorkle et al.,1995). Above all, multiple bioactive compounds present in herbal anthelmintics may translate to multiple mechanisms in killing the parasites, which then limit the likelihood of developing anthelmintic resistance (Chagas, 2015). Since ancient times, people have been exploring the nature particularly medicinal plants in search of new drugs. Medicinal plants are used by 80% of the world population for their essential health needs and this efficacy depends upon the current knowledge about taxonomic features of plant species, plant parts and biological property of medicinal plants which in turn depends upon the occurrence of primary and secondary metabolites (Vinoth et al.,2011). Screening for effective anthelmintic compounds remains a major obstacle in the drug development process and screening in the natural hosts is typically very expensive, requiring appropriate facilities and can raise concerns about animal welfare (Kumarasingha et al.,2014). So, *in vitro* examination can be an good option for preliminary study of different herbal anthelmintic activity

2. MATERIALS AND METHODS

2.1. Plant Materials Collection: Two ethnomedicinal plants Mahaneem or the beed tree leaves (*Melia azedarach*) about 500 gm and Chirata or bitter stick whole plant *(Swertia chirata*) about 500 gm were collected from in and around the WBUAFS,Kolkata and Mohanpur campus (22°36′23″N, 88°23′14″E / 22.6065264°N, 88.3872166°E/22.6065264; 88.3872166.)for in vitro anthelmintic activity screening on different parasitic stags of *Haemonchus contortus*.Then all materials was washed 3 times with clean water and dry at room temperature (27^0C temperature) for 7 to 15 days depend on materials avoiding direct sunlight and to avoid dust and rat contamination materials was put in the incubation.Then all the material was powdered separately by motor grinder and measure desirable amount for the preparation of extract. Plants was identified as per available literature on identification (Bentley and Trimen, 1880; Joshi and Dhawan, 2005; Lu et al.,2021).

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2.2. Aqueous Extract Preparation (Decoction):

About 100 gm finely powder was kept in 500 ml distilled water for overnight and next day boil for 30 minutes. Resulting after boiling decoction solution was cooled and filtered with Whatman's filter paper no-1 to collect the acquous solution.Then extraction solution was evaporated and concentrated by lyophiliser (Simeco,India) and further dry by keeping at 40^0C in hot air oven. Dry weight measure and dissolved in 2 % dimethyl sulfoxide (DMSO) as per required concentration and store at 4^0c for future uses.

2.3. Recovery of Adult *Haemonchus contortus* Worms and Eggs

Adult female worms were collected from the abomasum of slaughtered sheep collected from the local abattoir (New Market,Kolkata) and adult *Haemonchus contortus* were identified as per morphological characteristic (Solusby,1982). Collected worms was washed three times in normal saline to make the worm free from abomasal contents and feed debris. Then collected active motile alive adult worms were used for adult motile inhibition test (AMIT) and others adult female *Haemonchus contortus* were triturated in a clean pestle and mortar to recover the eggs for egg hatch assay. Recovery of eggs from eggs contain triturated materials and test procedure was performed as per method described Cole *et al*.,(1992). The eggs were recovered with saturated saline solution by flotation and washed repeatedly in distilled water to get final aqueous egg suspension from triturated materials. Eggs were separated, quantified and used within 90 minutes for the test.

2.4. Egg Hatch Assay (EHA)

The guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) was followed for conducting EHA (Coles et al.,1992). Approximately 50 numbers of *Haemonchus contortus* egg suspension in 100 µl of distilled water was incubated with different concentrations (1, 2.5, 5,10, 25, 50 mg/mL) of each plant extract in 2% DMSO in a 96 flat-bottomed micro titre plate to obtain a final tested concentration of 1 to 50 mg/ml. Albendazole served as a positive control and was dissolved in 2% DMSO in de-ionized water to obtain a concentrations of 50 µg/ml and 20 µg/ml), while 2% DMSO and PBS served as the negative control. The setup was incubated in triplicate for each ex-tract at 27^0C for 48 hours. At the end of 48 hours, a drop of Lugol's iodine solution was added to each well and the number of larvae vs unhatched eggs (including larvated ones) was counted with an inverted microscope to calculate egg hatch inhibition (Fig.1,2,3,4,5). All experiments were under-taken

in triplicate on three separate occasions

The percentage inhibition of egg hatching was calculated using the formula by Cala et al., (2012)

$$\text{Inhibition of egg hatching (\%)} = \frac{(\text{Eggs}+\text{L})-\text{L}}{(\text{Eggs}+\text{L})} \text{ X100}$$

L = Number of larvae in a particular well.

2.5. **Adult Worm Motility Inhibition (%WMI) Assay:**

The AWMI test was performed in 50 mm diameter glass Petri dish according to Sharma et al.,(1971). Adult *Haemonchus contortus* worms (Fig.6) were recovered from the sheep abomasums at laboratory collected and brought from local slaughter house (New Market, Kolkata). Then washed the worms thrice with PBS (pH ,7.2) to make debries free and in each petri dish 10 (Ten) numbers actively motile worms were taken to make the test in triplicates to each plant extracts (50, 25, 10 and 5 mg/mL) in different concentration in separate petri dishes at temperature (28 ±1°C). Positive (albendazole @ 0.20mg /mL) and negative controls (worms with PBS) were included in the assay. The inhibition of motility of the worms exposed to the above concentrations was used as an indicator for anthelmintic activity. The motility of worms was observed by examination under a dissecting microscope at magnification x20 at intervals of 4 h till the worms in negative control lost their active motility, for 10 h of assay. Finally, the extracts and albendazole were washed away and the worms were re-suspended in lukewarm fresh PBS for 30 minutes to observe and test the revival of motility. All the motile (alive) and immotile (dead) worms in three replicates of each concentration and control were counted. Death of the worms was ascertained by the absence of motility for observation period of 5–6 seconds. Worm motility inhibition (%WMI) percentage was calculated as per Rabel *et al.*,(1994).

Dose-dependent % AWMI

$$= \frac{\text{No.of motile worms in negative control} - \text{No.of motile worms in treatment}}{\text{No.of motile worms in negative control}} \text{ X100}$$

2.6. Statistical Analyses

Comparison of mean percentages of egg hatch inhibition and larval paralysis at different concentration with the control was performed by one-way ANOVA by using SPSS IBM Statistics® v. 20 and probability values P≤ 0.05 were deemed as significant. The Duncan multiple range test was used to identify variations between treatment means when the treatments effect was significant.

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3. RESULTS

In this present study two ethnomedicinal plants parts Mahaneem leaves (*Melia azedarach*) and Chirata or bitter stick leaves and stems (*Swertia chirata*) aquous extract were prepare for *in vitro* anthelmintic activity study at different concentration. *In vitro* study were conducted against *Haemonchus contortus* eggs by egg hatch assay (EHA) and against adult stage of *Haemonchus contortus* by adult motility inhibition test (AMIT).

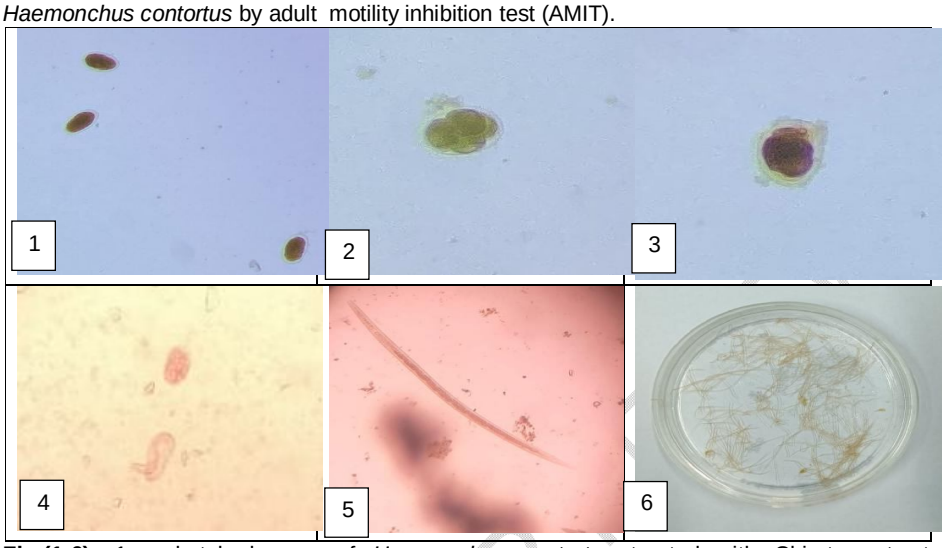

Fig.(1-6): 1. unhatched eggs of *Haemonchus contortus* treated with Chirata extract (50mg/ml); 2. Morula stage of *Haemonchus contortus* egg treated with Mahaneem extract (25 mg/ml); 3. unhatched larve stage 1 (L1) of *Haemonchus contortus* with uneven shaped egg shell; 4. Newly hatched L1 and unhatched egg of *Haemonchus contortus* in Chirata extract (10 mg/ml);5. Larvae stage 2 (L2) of *Haemonchus contortus* in Mahaneem extract (5mg/ml) after 48 hours 6. Adult active motile *Haemonchus contortus* collected for experiment.

The result revealed that Chirata leave extract have better efficacy against both eggs and adult compare to Mahaneem leave extract. In EHA, chirata extract efficacy @ 50 mg ,25 mg and 10 mg/ml were 85.599 ± 2.389, 60.180 ± 0.878, 39.954 ± 0.690 respectively more compare to Mahaneem at same concentration with 77.442 ± 0.963, 54.178 ± 0.848,35.898 ± 1.328 respectively (Table 1 and Fig.7). At the dose rate of 50 mg and 25 mg concentration, Chirata extract more statistically significant ($p<0.05$) than Mahaneem extract. But at 5 mg/ml to 1 mg/ml concentration Mahaneem shown more efficacy than Chirata and statistically significant only observed at 5 mg/ml concentration.

Table 1. Mean efficacy (percentage±S.E) of aquoues extract of *Melia azedarach* and *Stewaria chirata* on *Haemonchuscontortus* egg hatching inhibition.

Concentration (mg/ml)	Extract		
	Mahaneem leaves	Chirata whole plant	p value
50 mg	77.442±0.963	85.599 ± 2.389	p<0.05
25mg	54.178± 0.848	60.180 ± 0.878	p<0.05
10 mg	35.898 ± 1.328	39.954 ± 0.690	p>0.05
5 mg	24.134 ± 0.773	18.295 ± 1.511	p<0.05
2.5 mg	8.437 ± 0.543	7.610 ± 0.561	p>0.05
1 mg	5.015 ± 0.198	4.967 ± 0.151	p>0.05
2 % DMSO	2.44 ± 0.08	3.12 ± 1.07	p>0.05
Albendazole-			
50 µg/ml	100 ± 0.00	100 ± 0.00	p>0.05
20 µg/ml	93.09 ± 1.18	93.40 ± 0.97	

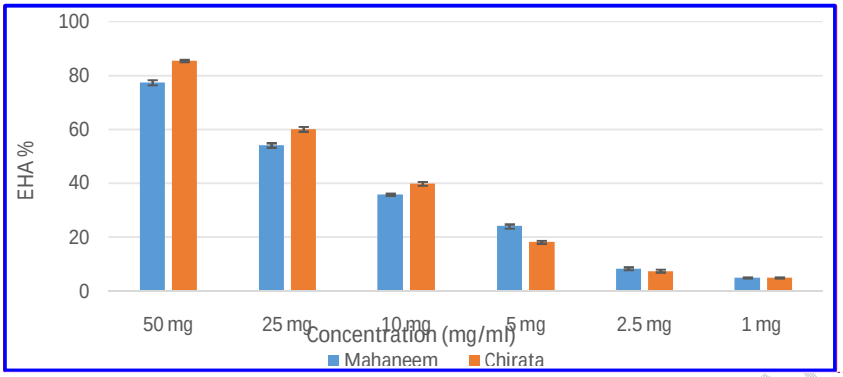

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Fig.7: Inhibition of egg hatching (%) in EHA with different concentration of Mahaneem and Chirata extracts

In AMIT, Chirata shows statistical significantly ($p<0.05$) more efficacy at the 50 mg/ml and 25 mg/ml than Mahaneem (Table 2 and Fig.8) , but at the concentration of 10 mg/ml Mahaneem shows better efficacy (38.88 ± 2.22) than Chirata (31.11 ± 2.22). At the 50 mg/ml concentration, after 10 hours experiment for AMIT, Chirata extract shows highest efficacy with 82.22 ± 2.22 percent compare to Mahaneem with 57.77 ± 2.22 percent.

Table 2. Mean efficacy (percentage±S.E.) of aqueous extract of *Melia azedarach* and *Stewaria chirata* on adult *Haemonchus contortus* motility inhibition.

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Extract name	Concentration (mg/ml)	Time post exposure			
		1 hour	3 hours	6 hours	10 hours
Chirata Whole plant	50	24.44±2.22	31.11±2.22	51.11 ± 2.22	82.22±2.22
	25	15.55±2.22	22.2 ±2.22	35.55 ± 2.22	51.11±2.22
	10	8.88 ± 2.22	11.11±2.22	22.22 ± 2.22	31.11±2.22
	5	6.66 ±0.00	8.88 ±2.22	15.55 ± 2.22	22.22±2.22
Mahan-eem leave	50	15.55±2.22	22.22±2.22	37.77 ± 2.22	57.77±2.22
	25	11.11±2.22	15.55±2.22	31.11± 2.22	37.77±2.22
	10	8.88 ± 2.22	13.33±0.00	22.22± 2.22	38.88±2.22
	5	4.44 ± 2.22	8.88 ±2.22	11.11± 2.22	15.55±2.22
PBS		0.00 ± 0.00	0.00±0.00	0.00 ± 0.00	0.00 ±0.00
2 % DMSO		0.00 ± 0.00	0.00 ±0.00	0.00 ± 0.00	0.00 ±0.00
Albendazole (0.20 mg/ml)		100 ± 0.00	100 ±0.00	100 ± 0.00	100 ±0.00

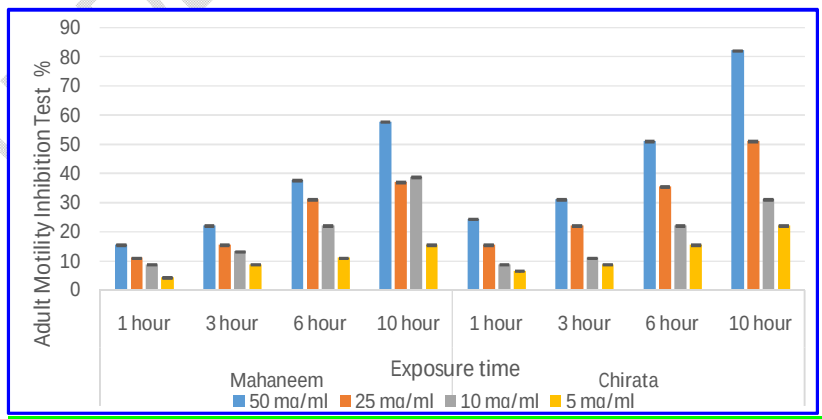

Fig.8. Adult Motility Inhibition of *Haemonchus contortus* with Mahaneem and Chirata extract

4. DISCUSSION

Chemical analysis of the extracts from the *Melia azedarach* revealed the presence of tannins, phenolic compounds, flavonoids, alkaloids, saponins and steroids (Dantas et al., 2000; Maciel et al., 2006; Sharma and Paul., 2013). Tannins are compounds noted for having anthelmintic properties. Tannins in the extracts could be the active component affecting the eggs and larvae of *Haemonchus contortus* (Athanasiadou et al., 2001) and they may operate through binding to free proteins, which lowers nutrient a viability and leads to larval mortality by starvation, or attaching to the larval cuticle, abundant in glycoproteins, resulting in death. Alkaloid can influence the central nervous system, resulting in paralysis of the parasite and subsequently death (Roy et al.,2010), whereas saponin alters the permeability of the parasite's cell membrane, leading to vacuolization, tegument disintegration and finally death (Melzig et al., 2001). Flavonoid (isoflavones) blocks the glycolysis enzyme, disrupts calcium balance, hinders Nitrous Oxide function and leads to the parasite's evertual death (Stepek et al., 2006), while exhibiting low toxicity in mammalian animal hosts. Akhtar and Riffat (1984) assessed the effectiveness of *Melia azedarach* in com bating gastrointestinal nematodes in goats. They have indicated a 99.4=12 decrease in EPG in animals treated with *Melia azedarach* fruit powder at a dosage of 30 mg/kg. Falbo et al. (2008) studied gastrointestinal nematodes in sheep, achieving an efficiency of 33.2%. Squires et al.(2010) indicated that in small ruminants, the rumen might act as a storage site, delaying the movement of the anthelmintic treatment and thereby extending *Haemonchus contortus*'s exposure to the active ingredient. The aqueous and hydroalcoholic extracts from the *Melia azedarach* leaves inhibited 99.4% and 100% of egg hatching, and fully stopped larval development at a concentration of 12.5 mg/ml respectively (Kamaraj et al., 2010).

Khanal et al. (2014) noted that *Swertia chirata* is also effective in fighting intestinal worms. The aqueous and hydroalcoholic extracts from the leaves blocked 99.4% and 100% of egg hatching, and completely prevented larval development at a concentration of 12.5 mg/ml respectively (Kamaraj et al., 2010). Iqbal et al., (2006) stated that in vitro investigation into the anthelmintic characteristics of *Swertia chirata* showed that at a concentration of 25 mg/ml, the crude aqueous extract from the entire *Swertia chirata* plant displayed an anthelmintic effect on live *Haemonchus contortus.* PaezLeon et al., (2022) observed an 85.88% reduction in the hatching of *Haemonchus contortus* eggs at a concentration of 20 mg/ml after 48 hours of exposure. In a study, goats was given crude powdered and aqueous extracts of *Swertia chirata* at a dosage of 500 mg/kg body weight,orally for seven continuous days and results showed that *Swertia chirata* demonstrated considerable anthelmintic effectiveness against gastrointestinal nematodes namely *Bunostonairm spp.,Trichostrongyles spp., Oesophagostomum spp.,* and *Haemonchus spp.* account for about 70 to 90 percent (Jain and Sahni, 2009).

5. CONCLUSION:

It concluded that both Mahaneem (*Melia azedarach*) and Chirata (*Swertia chirata)* have the anthelmintic activity against Haemonchus contortus at different concentration and both can potentially be effective against Haemonchus contortus and other GINs in small rumunats when administere properly. However,these plant's anthelmintic activity on gastrointestinal nematodes in small ruminants remains to be clarified by *in vivo* experiments.

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