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## In-Vitro Anthelmintic Activity of *Mollugo Nudicaulis* Lam, *Syzygium Cumini* Linn And *Hibiscus Vitifolius* Linn On *Pheretima* Posthuma

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### ABSTRACT

The present paper investigated the *In-vitro* anthelmintic activity of ethanolic extracts of *Mollugo nudicaulis* Lam, *Syzygium cumini* Linn and *Hibiscus vitifolius* Linn to determine the possible effects by using *Pheretima Posthuma* (Indian adult earthworm). Two concentrations of each extracts (50,100mg/ml) were used in this activity and the same concentration of Albendazole as a standard drug and distilled water with 1%w/v carboxyl methyl cellulose as control. Determination of paralysis time (vermifuge) and death time (vermicidal) of the worms were noted. It was found that the ethanolic extracts of three plants exhibited significant anthelmintic activity at a concentration of 100mg/ml of MNE (highest activity) followed by SCE and HVE (least activity). The above traditional plants confirms the presence of ethanobotanical uses. The plants may be further explored for isolation of active constituents to bring a new active lead for suitable anthelmintic activity.

**Keywords:** *Mollugo nudicaulis* Lam, *Syzygium cumini* Linn, *Hibiscus vitifolius* Linn, Anthelmintic activity, Albendazole, Vermifuge, Vermicidal.

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## INTRODUCTION

Anthemintics or antihelminthics are drugs that treat to expel or killing the parasitic worms from the intestine.<sup>1</sup> Helminths is a disease which is a part of body that affected by pinworm, tapeworm, whipworm, hookworm, threadworm and roundworm. Helminthic infections are most worldwide disease which cause of chronic ill health amongst the children<sup>2</sup>. In world, more than half of the population suffers from worm infections and also affect domestic animals and live stocks<sup>3</sup>. As per WHO, only synthetic drugs are frequently used in the treatment of helminth infections<sup>4</sup>. But these synthetic drugs have a lot of side effects<sup>5</sup>. To overcome these side effects, has been made to study the anti-parasitic activity. Traditional system of medicine mostly medicinal plants to cure diseases in man and animals. Likewise, *Mollugo nudicaulis* Lam (Molluginaceae)<sup>6</sup> is traditionally used to treat cough and cold, bleeding nose, gonorrhoea, eye disease, skin disease and Vermifuge<sup>7</sup>; *Syzygium cumini* Linn (Myrtaceae)<sup>8</sup> is traditionally used to treat diarrhoea, pharyngitis, diuretic, antibacterial, skin disease, wounds and antidiabetics<sup>9</sup>; *Hibiscus vitifolius* Linn (Malvaceae)<sup>10</sup> is traditionally used to cure diarrhoea, normal renal function, ophthalmic infection and edema<sup>11</sup>. Anthelmintic activity was evaluated for the selected three medicinal plants against adult Indian earthworms, *Pheretima Posthuma* due to its anatomical and physiological resemblance with intestinal round worm parasites of human beings<sup>12-15</sup>. Based on the literature survey it has been found that the selected plant species (whole plants of *Mollugo nudicaulis* Lam, *Hibiscus vitifolius* Linn and seeds of *Syzygium cumini* Linn) possesses various pharmacological activities. Till date no one reported the anthelmintic activity of ethanolic extract of these plants with respective parts (whole plants and seeds). So, the present research was designed to estimate the *in-vitro* anthelmintic activity and to bring the novel anthelmintic herbal drugs.

## MATERIALS AND METHOD

### **Plant Material:**

Fresh and healthy whole plants of *Mollugo nudicaulis* Lam, *Hibiscus vitifolius* Linn and seeds of *Syzygium cumini* Linn were collected, identified and authenticated by Prof. P. Jayaraman, director, National Institute of herbal science in Chennai (PARC/2011/958), Dr.G.V.S. Murthy, T.N.A.U in Coimbatore (BSI/SRC/5/23/2011-12/Tech/1057) and Dr.M.Palanisamy, T.N.A.U in Coimbatore (BSIS/RC/5/23/2017/Tech/586) respectively. The voucher specimen kept in our department.

### **Drugs and Chemicals:**

Suspension of Albendazole (Caplin point Laboratory, Chennai), Carboxyl Methyl Cellulose –CMC (A.B Enterprises, Mumbai) and 95%w/v Ethanol (Rankem Laboratory, India) were used for experimental protocol. All the above chemicals used are analytical grade.

**Preparation of Plant Extract<sup>16</sup>:**

The three plants materials were washed with fresh water and dried in room temperature, grinded, stored separately and used for extraction. Taken 50gm of coarse powder of *Mollugo nudicaulis* Lam (whole plant), *Syzygium cumini* Linn (*seeds*) and *Hibiscus vitifolius* Linn (whole plant) were packed separately in Soxhlet extractor with 500ml of 95%w/v ethanol. They were extracted for 72hr and temperature was maintained throughout the process.

Then the above extracts were evaporated and concentrated at room temperature. All the extracts were properly stored for the experiment.

**Preliminary Phyto-chemical Investigation<sup>17</sup>:**

All the ethanolic extracts of *Mollugo nudicaulis* Lam (MNE), *Syzygium cumini* Linn (SCE) and *Hibiscus vitifolius* Linn (HVE) were subjected to preliminary phyto-chemical analysis.

**Selection of Experimental Model:**

*Pheretima Posthuma* (Indian adult earthworm) <sup>18</sup> was used for this experiment which was in the length of 10-12cm and width of 0.3-0.5cm. Earthworms were collected from Organic Fertilizer, Salem. These were washed with normal distilled water to remove the extraneous matter<sup>19</sup>.

**Experimental Design:**

The worms of equal size were divided into nine groups containing six worms in each group. All the three extracts of MNE, SCE and HVE prepared in the concentration of 50mg/ml and 100mg/ml and the same concentration of standard Albendazole suspension were freshly prepared with 1%w/v CMC. Alone 1%w/v CMC in distilled water was taken as control. 10ml of each extracts and standard were poured in each group of petridish and time was noted. Observations were made for paralysis and death<sup>20</sup>.

Paralysis time was recorded when no movement could be observed except when the worms were shaken vigorously. Death time was noted when worms neither moved while shaken nor when dipped in warm water followed by white secretions and fading away of their body colour<sup>21</sup>.

**RESULTS AND DISCUSSION:**

The obtained percentage yield of whole plant of *Mollugo nudicaulis* Lam (50gm), seeds of *Syzygium cumini* Linn (50gm) and whole plant of *Hibiscus vitifolius* Linn (50gm) coarse powder was 25.23%w/w, 14.40%w/w and 9.22%w/w respectively.

By the preliminary phyto chemical investigation studies, MNE shows the presence of flavonoid , phenol, saponin, steroid, terpenoid, amino acid , carbohydrate. SCE shows the presence alkaloid, glycoside, phenol, saponin, tannin, terpenoid, amino acid, carbohydrate. HVE shows the presence

of alkaloid, glycoside, flavonoid, phenol, saponin, steroid, tannin, amino acid, carbohydrate. The results of phytoconstituents are reported in the Table: 1.

The Anthelmintic activity observations were noted, which is shown in the Table: 2 and Graph: 1&2, the standard Albendazole drug at concentration of 50mg/ml and 100mg/ml caused the paralysis in 32minutes 11seconds and 20minutes 23seconds respectively. MNE at concentration of 50mg/ml and 100mg/ml caused the paralysis in 75minutes 46seconds and 40 minutes 59 seconds respectively. The worms failed to move and muscle relaxation and flaccid paralysis were noted early in MNE (100mg/ml) then followed by SCE (100mg/ml), HVE (100mg/ml) when compare to 50mg/ml of all the three extracts. When compared to standard Albendazole (100mg/ml), the MNE, SCE and HVE extracts were less potent to cause paralysis.

The fading away of body, colors were noted. Haemorrhagic and Necrotic spots were observed externally on worms earlier in Albendazole (100mg/ml) then followed by MNE, SCE and HVE (100mg/ml), when compare to 50mg/ml of their respective three extracts and its shown by Figure: 1 to 5 . The time taken for death of worms is less when compared to Albendazole (100mg/ml), then followed by MNE, SCE and HVE(100mg/ml) than 50mg/ml of standard and all the three extracts. Potency of the extract was inversely proportional to the time for paralysis (vermifuge) and death (vermicidal) of the worms. From the results, it is clear that MNE (100mg/ml) and SCE (100mg/ml) extracts were showed potent anthelmintic activity when compared to Albendazole (100mg/ml). The HVE (100mg/ml) extract showed a dose dependent anthelmintic activity. The time taken for death worms were least in HVE (100mg/ml) extract compared to Albendazole.

**Table 1: Preliminary Phyto-chemical screening of MNE, SCE and HVE.**

S No	Phyto-constituents	MNE	SCE	HVE
1	Alkaloids	-	+	+
2	Glycosides	-	+	+
3	Saponins	+	+	+
4	Flavonoids	+	-	+
5	Tannins	-	+	+
6	Steroids	+	-	+
7	Terpinoids	+	+	-
8	Amino acids	+	+	+
9	Carbohydrates	+	+	+
10	Phenols	+	+	+

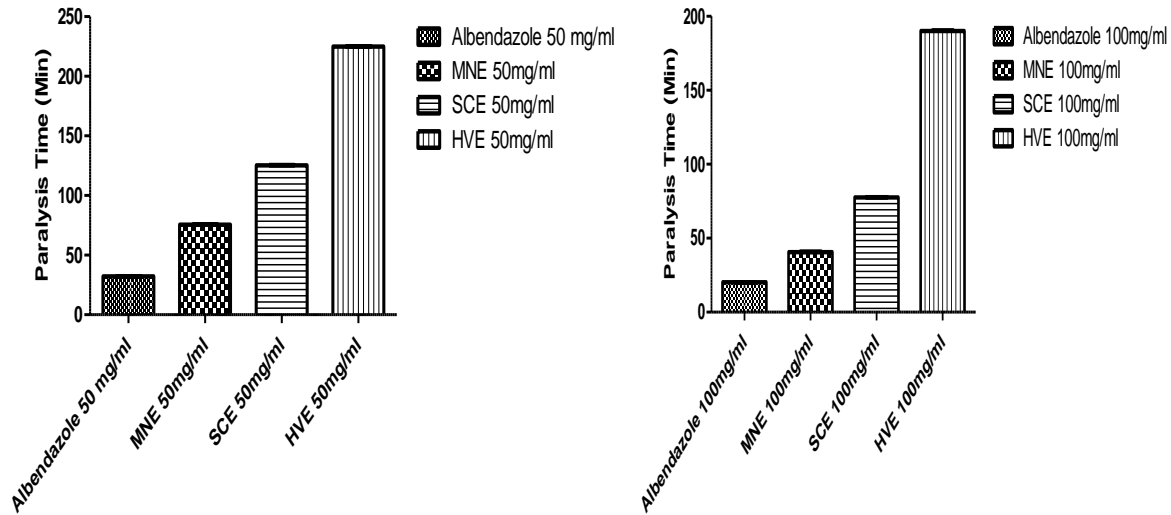
+ = Present, - = Absent

**Table 2: Anthelmintic activity of MNE, SCE and HVE.**

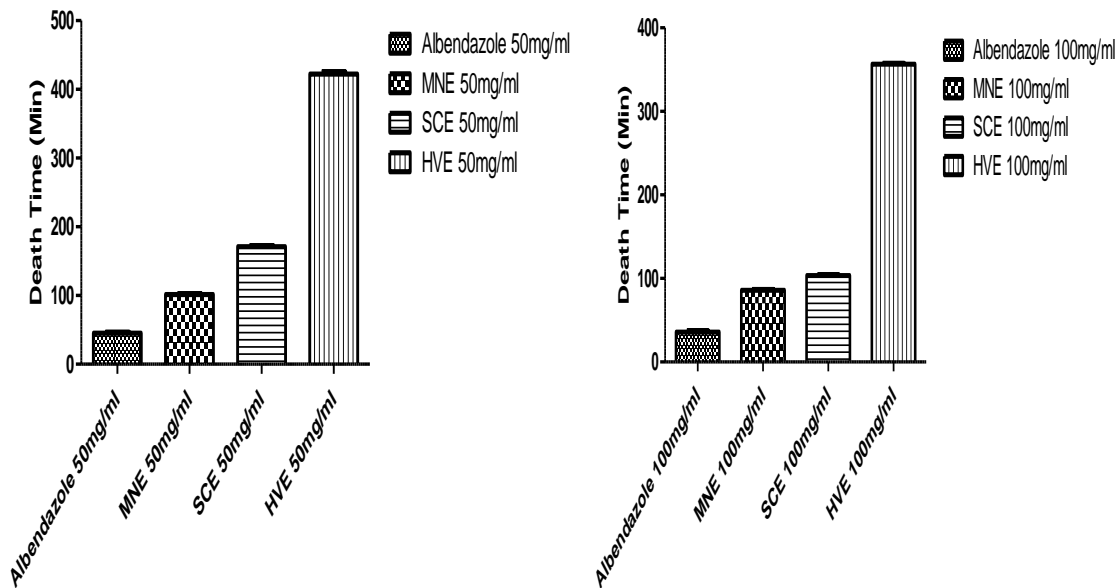
Groups/treatment	Concentration (mg/ml)	Paralysis time (Min)	Death time (Min)
Control(1% w/v CMC)in distilled water	-	-	-
Albendazole(standard)	50 mg/ml	32.11±0.13*	45.25±1.11*

MNE	100mg/ml	20.23±0.10*	35.85±0.9*
	50mg/ml	75.46±0.15*	101.40±1.05*
SCE	100mg/ml	40.59±0.12*	85.83±1.12*
	50mg/ml	125.22±0.22*	171.22±1.24*
HVE	100mg/ml	77.55±0.15*	103.44±0.71*
	50mg/ml	224.96± 0.15*	422.16±3.18*
	100mg /ml	190.15±0.17*	356.43±1.14*

\* Values are expressed as mean ± SEM ; n=6



Graph 1: Paralysis Time for Standard (Albendazole) and MNE, SCE, HVE



Graph 2: Death Time for Standard (Albendazole) and MNE, SCE, HVE





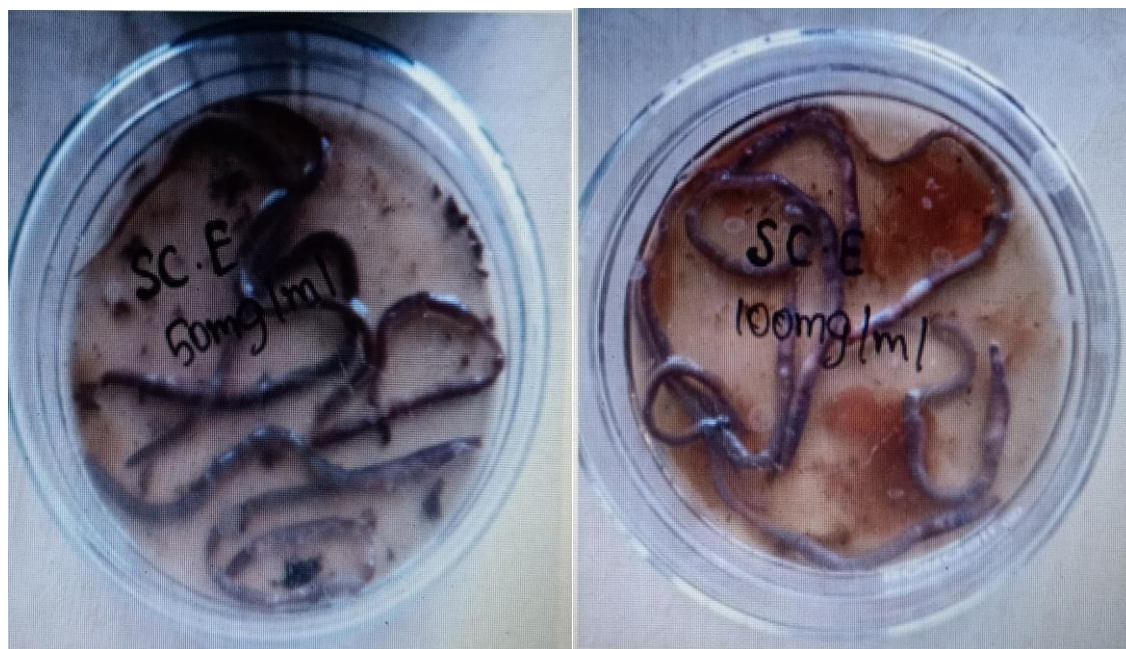
**Figure 1: Control**



**Figure 2: Anthelmintic activity of Standard (Albendazole) at 50 mg/ml and 100 mg/ml**



**Figure 3: Anthelmintic activity of MNE at 50 mg/ml and 100 mg/ml**



**Figure 4: Anthelmintic activity of SCE at 50 mg/ml and 100 mg/ml**





**Figure 5: Anthelmintic activity of HVE at 50 mg/ml and 100 mg/ml**

## CONCLUSION

The whole plant extract of *Mollugo nudicaulis* Lam has showed potent anthelmintic activity than the seeds of *Syzygium cumini* Linn at the concentration of (100mg/ml), when compare to standard Albendazole. The whole plant extracts of *Hibiscus vitifolius* Linn has showed a least anthelmintic activity. The highest activity exhibited that herb act as vermifuge and vermucidal which assures the traditional use of this plant as anthelmintic. The plant may be further explored for isolation of active constituents to bring a new active lead for suitable anthelmintic activity.

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