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AERVA LANATA (LINN.) JUSS.: AN ANTHELMINTIC HERB

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***ABSTRACTS*:** There is an increasing demand of herbal medicines globally, majority of herbs were used against different diseases. WHO estimated that two million of people harbour parasitic worm infections. Helminthes infections are one of the common health problems in India. These infections affect most population with major economic and social consequences. In view of this attempts have been made to study anthelmintic activity of *A. lanata*. The aerial parts of *Aerva lanata* is widely used in the treatment of helminthiasis in folklore medicine. In this present study we investigated the folklore claim methodically and scientifically. The efficacy of plant extracts was proved by determining the time period for paralysis and death of earthworm and roundworm against different extracts (Acetone, chloroform, petroleum ether and aqueous) with different doses, i.e., 5, 15, 25, 50 and 75 mg/ml. Result revealed that Chloroform extract of *A. lanata* showed significant anthelmintic activity against *Pheretima posthuma* and Acetone extract for *Haemonchus contortus* than the standard drug. However, the efficacy of extracts was found to be considerably high at higher concentrations. For earthworm, standard drug at 75mg /ml showed paralysis at 3.75 ± 0.5 min and death time was 4.8 ± 0.8 min and for *Haemonchus contortus*, standard drug at 75mg /ml showed paralysis at 3 ± 0.8min and death time was 4.6 ± 1.6 min respectively. Among the various concentrations tested, Chloroform extract at 75mg/ml showed efficient paralytic effect at (4.6 ± 1.7 min) and death effect at (7.2 ± 2 min)for *Pheretima posthuma* while Acetone extract at the same concentration found efficient for *Haemonchus contortus* paralytic effect at (1.8 ± 0.9 min) and death effect at (2.8 ± 0.8 min). Therefore, it may conclude from experimental evidences observed in this study *A. lanata* possesses significant anthelmintic properties and supports their use against intestinal helminthes infections in traditional medicine.

Keywords: (*Aerva lanata*, Anthelmintic, medicinal plants, earthworms and roundworms)

1 INTRODUCTION

Helminthes infections are most prevalent diseases affected large population in the world. In developing countries, they pose a major health problem. Heavier infections of helminthes can cause abdominal pain and diarrhea, anemia, eosinophilia, pneumonia, weakness undernourishment and malnutrition (Bundy, 1994). Parasite load also affects other health outcomes i.e., allergy and autoimmunity (Tara *et al*., 2014). If helminthes infections left untreated, it results into chronic inflammatory disorders (Budke *et al*., 2005; Benthony *et al*., 2006; King, 2007).

An anthelmintic (also known as de-wormer) is a medicine intended to paralyses or kill (vermicide) the parasitic worms or expel (vermifuge) infesting helminthes in their mammalian host (Mohamed *et al*., 1999; Satyanarayan *et al*., 2013). But most of the existing anthelmintics produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache, diarrhea, gastrointestinal disturbances, drowsiness, irreversible liver failure, neurotoxicity, allergic reactions, general malaise and weakness (Goodman and Gilman, 2001; Tripathi, 2008). Ancient classical literature has described the use of plants in traditional system of medicines for the treatment of helminthic infections. Many reports revealed that some of the plant not only affects the nutrition of host but also have antiparasitic effects (Waghorn and Mcnabb, 2003). Herbal anthelmintics are expelling or killing worms from body. Thus, there is an urgent need for new, safe and inexpensive drugs that are able to act for longer periods.

For our studies, we used *Aerva lanata* that belongs to the Amaranthaceae family and are traditionally used as vermifuge drugs (Kumar *et al*., 2010). *A. lanata* is commonly known as “Kapuri madhuri” in Marathi. *A lanata* is a prostrate to decumbent, sometimes erect herb of 0.8 to 1.2 m height, woolly, tomentose throughout and is found as weed abundantly in fields and west lands. It is being used in the indigenous systems of medicine to treat many diseases such as Kidney stone, diarrhea, cholera

snakebite and helminthes infections etc. (Ranjan and Deokule, 2013). Variety of pharmacological activities of this ethno medicinally important plant has been reported as for various pharmacological activities. The plant is recorded for its antimicrobial property (Soundararajan, 2006; Rajesh *et al*., 2010; Vijaylakshmi and Ravindhran, 2013) and anthelmintic (Singh *et al*., 2011).

In continuation with our interest in anthelmintics and herbal medicinal plants were an attempt to investigate anthelmintic activity of acetone, chloroform, petroleum ether and aqueous extracts of *A. lanata* was carried out different five concentrations (5, 15, 25, 50, 75 mg/ml) against Indian earthworm and roundworm using piperazine citrate as standard and normal saline as a control.

2 MATERIALS AND METHODS

2.1 Collection and identification plant material

Fresh leaves of *Aerva lanata* were collected from G.V.I.S.H. campus, Amravati (M.S.) in the month of Dec 2015. The plant was identified and authenticated by Dr. Milind Sirdesai**,** and a voucher specimen was deposited in the herbarium of Department of Botany, Dr. Babasaheb Aambedkar, Marathwada University, Aurangabad.

2.2 Extraction of plant material

The extracts were prepared as per the method described by Thimmaiah (1999). The collected plant material was washed, shade dried and crushed to produce coarse powder. The crude powdered was subjected to extract exhaustive extraction with different solvents like acetone, chloroform, petroleum ether, and water by using Soxhlet apparatus. These extracts were concentrated under reduced pressure in oven to yield semisolid mass. Stored for further analysis in cool and dry place. The different concentrations of extracts (5, 15, 25, 50, 75 mg/ml) were prepared for study. The extracts were used for anthelmintic activity against *Pheretima posthuma* and *Haemonchus contortus.*

2.3 Preliminary photochemical screening

The leaf powder was subjected to preliminary Phyto-chemical tests to detect the presence of various Phyto-constituents. A phytochemical screening was carried out as per the standard methods (Kokate *et al.*, 1998; Kulkarni and Apte, 2000; Sadasivam and Manickam, 2005; Thimmaiah, 1999). Responses to various tests were denoted by +, ++ and +++; indicating weak, moderate and strong reactions respectively.

2.4 Organisms

Pheretima posthuma and Haemonchus contortus.

2.5 Drugs and chemicals

Piperazine citrate, acetone, chloroform, petroleum ether.

3 EVALUATIONS OF ANTHELMINTIC ACTIVITY

The anthelmintic assay was carried as per the method of Deore et al with minor modifications (Deore *et al*., 2009). The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (Dash *et al*., 2002; Szewezuk, 2003). Earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro because of its easy availability (Sollmann, 1918).

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and roundworm (*Haemonchus contortus*) collected from Mominpura abattoir of Amravati. *H.contortus* is commonly used as animal model to evaluate the anthelmintic activity of medicinal plants (Ciulei, 1982; Jabbar *et al*., 2006). The worms washed with normal saline to remove all faecal matter and were used for the anthelmintic activity. The worms were divided into six groups containing four worms of both types. The experiment was performed at the dose of various concentrations i.e., 5, 15, 25, 50, 75 mg/ml of each extract in distilled water. Saline water as a control group I and standard reference drug piperazine citrate treated as group II. Determination of time of paralysis and time of death of the worms was observed. Time for paralysis was recorded when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death was concluded when the worms lost their motility completely while dipped in warm water (50 ^{0}c) followed with fading away of their body colors.

Table 1: Anthelmintic activity *of Aerva lanata* against earthworms and roundworms.

Groups	Treatments	Conc. (mg/ml)	*Pheretima posthuma*		*Haemonchus contortus*	
			(Earthworm)		**(Roundworm)**	
			P (min)	**D (min)**	**P (min)**	**D (min)**
I	**Normal saline / Control**	_	_	_ _	_	_ _
II	**Standard (Piperazine citrate)**	5	13.55±2	17.5±3.8	9.6±1.14	14.3±2.4
		15	12.57±2.7	15.6±2.3	7.5±2	11.5±1.2
		25	10.5±2.3	13.75±2.8	6±0.9	7.1±1.70
		50	6.7±1.70	9±1.8	3.9±0.8	5.5±1.2
		75	**3.25±0.5**	**4.8±0.8**	**3±0.8**	**4.6±1.6**
III	**Acetone (ALAC)**	5	11.1±1.5	17.6±3.17	4.2±1.7	11.4±2.1
		15	10.5±1.29	15±1.8	3.4±1.6	8.8±1.3
		25	9.1±3.14	12.67±2.2	3±0.8	6.7±0.9
		50	7.5±2.3	10.67±3.2	2.6±0.4	4.9±0.8
		75	**6.8±2.9**	**9.8±3.7**	**1.8±0.9**	**2.8±0.8**
IV	**Chloroform (ALCL)**	5	7.06±0.9	16.12±4.13	16.1±4.1	21.9±6.4
		15	5.8±2.01	13.2±2.5	13.2±2.5	18±2.6
		25	6±1.8	12.5±5	12.25±5	16.2±5.7
		50	5.7±1.7	7.5±2.3	4.1±0.6	8.5±1.3
		75	**4.6±1.7**	**7.2±2**	**3.1±1.6**	**6.4±2.2**
V	**Petroleum ether (ALPE)**	5	10±2.1	13.7±1.2	5.5±2	27.2±6.5
		15	8.8±2.01	12.17±3.9	3.8±0.9	25.3±3.3
		25	7.6±2.8	9.2±2.2	3±0.8	23.8±3.5
		50	6.5±1.9	11.9±3.7	2.6±0.6	21.6±7.5
		75	**6±1.4**	**7.8±1.6**	**2.4±0.5**	**18.8±2.3**
VI	**Aqueous (ALAQ)**	5	25.5±5.5	30.2±7.04	6.1±2	27±8.9
		15	24±6.4	26.2±6.5	4.6±2.1	17.5±2.6
		25	22.3±8.6	23.6±9.01	3.6±0.7	11.8±2.3
		50	17.6±2.8	19.3±2.8	3.5±1.2	7.5±1.9
		75	**10.3±2.2**	**15.5±4.8**	**3.3±0.4**	**5.3±1.1**

Values are expressed in mean ± SD (n=4); _No paralysis, _ _ No death

Table 2: Phytochemical screening of *Aerva lanata.*

S.N.	Test	Response	Intensity	Inference
1	**Alkaloids**			
	a) Mayer's Reagent	White ppt	++	Present
	b) Dragendorff's Reagent	White ppt	+++	Present
	c) Wagner's Reagent	White ppt	+++	Present
2	**Anthraquinones**			
	Test – a	Red colour	+	Present
	Test – b	Red colour	+	Present
	Test – c	Pink colour	++	Present
3	**Simple Phenolics**			
	Test a) With NaoH	Red	++	Catechol present
4	**Flavonoids**			
	a) Shinoda Test	Pink	+	Present
	b) Flavononol Test	Magenta colour	++	Present
	c) Flavanol Test	Yellowish Green	++	Present
	d) Flavone, Flavonol	Orange	++	Present
	Flavonone Test			
	e) Flavanone (Rao & Sheshadri	Blue green	++	Present
5	**Tannin** Test a- Test b-	 White ppt No ppt	 +++ –	 Present Absent
6	**Saponins**	Froth Formation	++	Present
7	**Steroid/ Triterpenoids Test** Test a Test b Test c	 Blue Brown No change	 + ++ –	 Present Present Absent

(Test was noted as +++ Strong, ++ moderate, + weak, – negative).

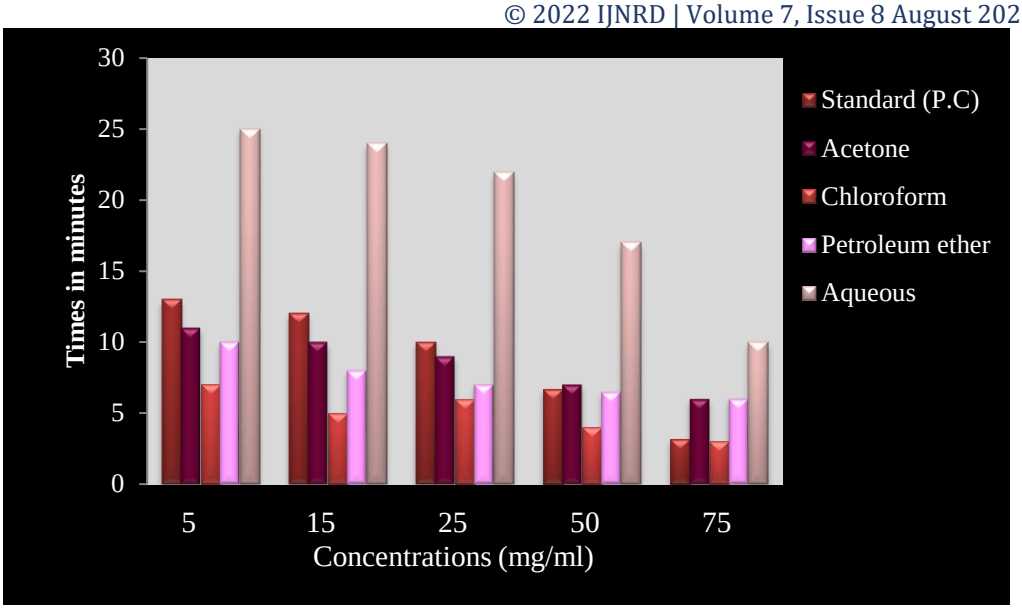

Fig 1. Comparative *in vitro* anthelmintic effect of different concentrations of *A. lanata* against illustrating paralysis time of *P. posthuma*.

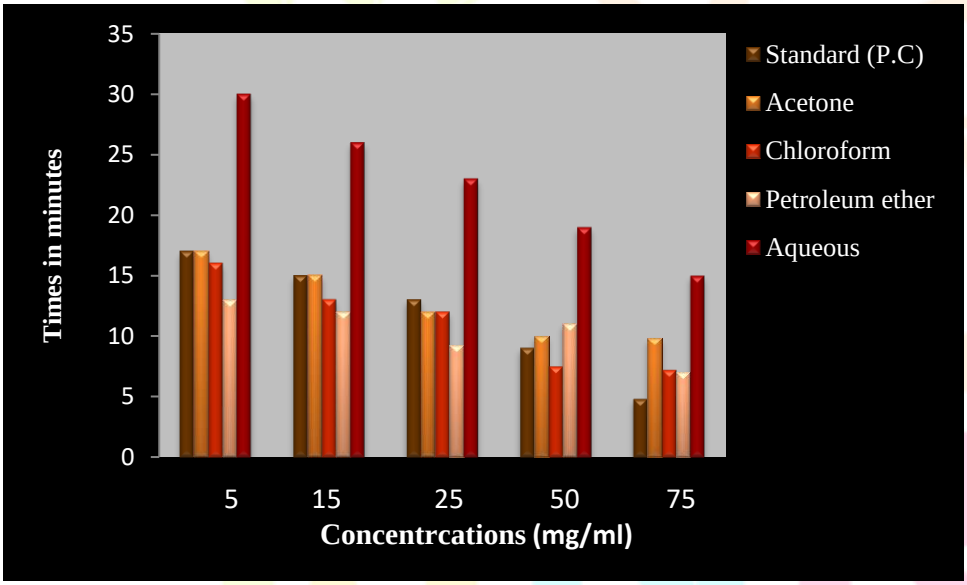

Fig 2. Comparative *in vitro* anthelmintic effect of different concentrations of *A. lanata* against illustrating death time of *P. posthuma*.

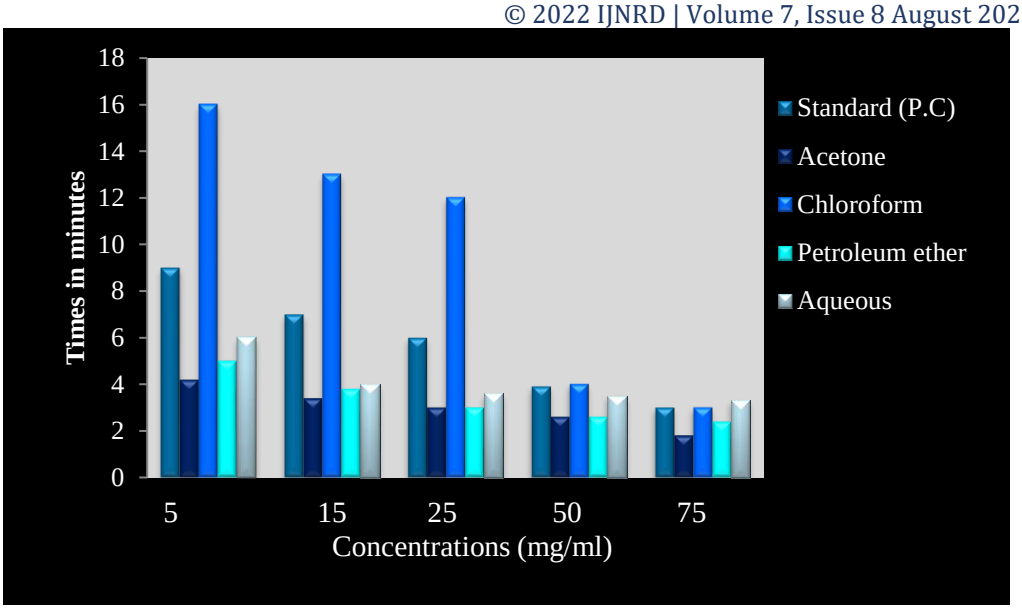

Fig 3. Comparative *in vitro* anthelmintic effect of different concentrations of *A. lanata* against illustrating paralysis time of *H. contortus*.

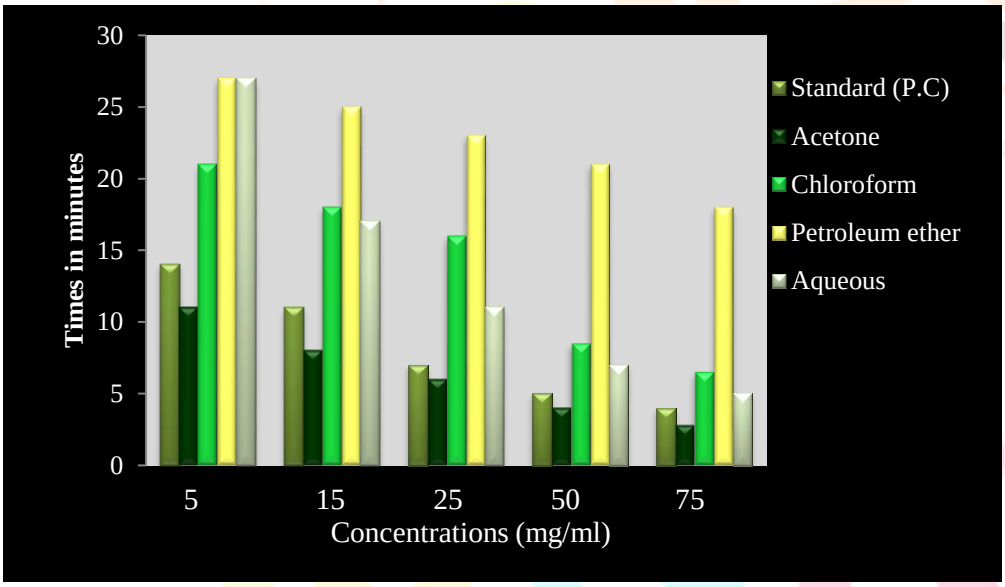

Fig 4. Comparative *in vitro* anthelmintic effect of different concentrations of *A. lanata* against illustrating death time of *H. contortus*.

4 RESULTS

Aerva lanata were screened for potential anthelmintic activity. The results of *in vitro* trials proved its potential at higher dose. Chloroform and Acetone extract of *A. lanata* exhibited time and dose-dependent *in vitro* anthelmintic activity (**Table 1**).

In aqueous extract at concentration of 75 mg/ml, paralysis and death time of *Pheretima posthuma* was found to be 10.3 ± 2.2 min and 15.5 ± 4.8 min respectively. In the same extract at the concentrations of 50, 25, 15, 5 mg/ml, time to paralysis was reported at 17.6 ± 2.8 min, 22.3 ± 8.6 min, 24 ± 6.4 min, 25.5± 5.5 min and death time at 19.3 ± 2.8 min, 23.6 ± 9.01 min, 26.2 ± 6.5 min, 30.2 ± 7.4 min respectively (**Fig 1 and 2**). In aqueous extract at concentration of 75 mg/ml, paralysis and death time of *Haemonchus contortus* was found to be 3.3 ± 0.4 min and 5.3 ± 1.1 min respectively. In the same extract at the concentrations of

50, 25, 15, 5 mg/ml, time to paralysis was reported at 3.5 ± 1.2 min, 3.6 ± 0.7 min, 4.6 ± 2.1 min, 6.1 ± 2 min and death time at 7.5 ± 1.9 min, 11.8 ±2.3 min, 17.5 ± 2.6 min, 27 ± 8.9 min respectively (**Fig 3 and 4**).

In Acetone extract at concentration of 75 mg/ml, paralysis and death time of *Pheretima posthuma* was found to be 6.8 ± 2.9 min and 9.8 ± 3.7 min respectively. In the same extract at the concentrations of 50, 25, 15, 5 mg/ml, time to paralysis was reported at 7.5 ± 2.3 min, 9.1 ± 3.14 min, 10.5 ± 1.2 min, 11.1 ± 1.5 min and death time at 10.67 ± 3.2 min, 12.67 ± 2.2 min,15 ± 1.8 min, 17.6 ± 3.1 min respectively (**Fig 1 and 2**). In Acetone extract at concentration of 75 mg/ml, paralysis and death time of *Haemonchus contortus* was found to be 1.8 ± 0.9 min and 2.8 ± 0.8 min respectively. In the same extract at the concentrations of 50, 25, 15, 5 mg/ml, time to paralysis was reported at 2.6 ± 0.4 min, 3 ± 0.8 min, 3.4 ± 1.6 min, 4.2 ± 1.7 min and death time at 4.9 ± 0.8 min, 6.7 ± 0.9 min, 8.8 ± 1.3 min, 11.4 ± 2.1 min respectively (**Fig 3 and 4**).

In Chloroform extract at concentration of 75 mg/ml, paralysis and death time of *Pheretima posthuma* was found to be 4.6 ± 1.7 min and 7.2 ±2 min respectively. In the same extract at the concentrations of 50, 25, 15, 5 mg/ml, time to paralysis was reported at 5.75 ± 1.7 min, 6 ± 1.8 min, 5.8 ± 2.01 min, 7.6 ± 0.9min and death time at 7.5 ± 2.3 min, 12.5 ± 5 min, 13.2 ± 2.5 min, 16.1 ± 4.1 min respectively (**Fig 1 and 2**). In Chloroform extract at concentration of 75 mg/ml, paralysis and death time of *Haemonchus contortus* was found to be 3.1 ± 1.6 min and 6.4 ± 2.2 min respectively. In the same extract at the concentrations of 50, 25, 15, 5 mg/ml, time to paralysis was reported at 4.1 ± 0.6 min, 12.5 ± 5.06 min, 13.25 ± 2.5 min, 16.1 ± 4.1 min and death time at 8.5 ± 1.3 min, 16.2 ± 5.7 min, 18 ± 2.6 min, 21.9 ± 6.4 min respectively (**Fig 3 and 4**).

In Petroleum ether extract at concentration of 75 mg/ml, paralysis and death time of *Pheretima posthuma* was found to be 6 ±1.4 min and death in 7.8 ± 1.6 min. respectively. In the same extract at the concentrations of 50, 25, 15, 5 mg/ml, time to paralysis was reported at 6.5 ± 1.9 min, 7.6 ± 2.8 min, 8.8 ± 2 min, 10 ± 2.1 min and death time at11.9 ± 3.7 min, 9.2 ± 2.2 min, 12.17 ± 3.9 min, 13.7 ± 1.2 min respectively (**Fig 1 and 2**). In Petroleum ether extract at concentration of 75 mg/ml, paralysis and death time of *Haemonchus contortus* was found to be 2.4 ± 0.5 min and death in 18.8 ± 2.3 min respectively. In the same extract at the concentrations of 50, 25, 15, 5 mg/ml, time to paralysis was reported at 2.6 ± 0.6 min, 3 ± 0.8 min, 3.8 ± 0.9 min, 5.5 ± 2 min and death time at 21.6 ± 7.5 min, 23.8 ± 3.5 min, 25.3 ± 3.3 min, 27.2 ± 6.5 min respectively (**Fig 3 and 4**).

Among the various concentrations tested, Chloroform extract at 75mg/ml showed efficient paralytic effect at (4.6 ± 1.7 min) and death effect at (7.2 ± 2 min) for *Pheretima posthuma* while Acetone extract at the same concentration found efficient for *Haemonchus contortus* paralytic effect at (1.8 ± 0.9 min) and death effect at (2.8 ± 0.8 min). This investigation revealed that Chloroform extract of *A. lanata* showed significant anthelmintic activity against *Pheretima posthuma* and Acetone extract for *Haemonchus contortus* than the standard drug.

For *Pheretima posthuma*, standard drug at 75mg /ml showed paralysis at 3.75 ± 0.5 min and death time was 4.8 ± 0.8 min and for *Haemonchus contortus*, standard drug at 75mg /ml showed paralysis at 3 ± 0.8 min and death time was 4.6 ± 1.6 min respectively.

PHYTOCHEMICAL SCREENING

Present study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as Flavonoids, Tannins, Saponins, Alkaloids, Triterpenoids and Phenols (Catechol) were present in the samples. The result of the phytochemical analysis shows that plants are rich in Alkaloids and Tannins followed by Flavonoids and Saponins (**Table 2**).The previous phytochemical analysis and present study showed nearly the similar results. Ranjan and Deokule (2013) have been reported for phytochemicals like Saponins, Tannins, Sugars and Alkaloids etc. Major Phytochemicals in *A. lanata* are Tannin, Cyanogenic glycosides reported by Gupta *et al*. (2005). Amino acids and Phenolics, Flavonoids by Gujjeti and Mamidala (2013) which may responsible for possible anthelmintic activity of *A. lanata*. Its Tannin content which has been proven for anthelmintic properties (Anantha *et al*., 2010). The present study confirms the traditional use of *A. lanata* as an anthelmintic.

CONCLUSION

The traditional used of *A. lanata* have been confirmed as its anthelmintic activity in present study. The current study evidenced that the Chloroform and Acetone extracts of aerial parts of *A. lanata* have a promising *in vitro* anthelmintic activity against earthworm and roundworms. However, the acetone extracts of *A. lanata* was superior to Chloroform, Petroleum and Aqueous extracts. In this present study we investigated the folklore claim methodically and scientifically. Plant species could be a potential source of new lead anthelmintic agents. Studies on such a medicinal plant used for animal health could contribute to increase livestock production also.

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