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Anthelmintic activity of essential oil of *Pimenta dioica* (Linn.) Merill, Family: Myrtaceae, collected in Summer from South Canara, India.

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ABSTRACT

Helminthic infections are among the most common infections affecting a large sector of the world's population. Various concentrations of the essential oil of leaves of Pimenta dioica (Linn.) Merill, Family: Myrtaceae, collected in summer during months of January to May (AOS1 - 5) from South Canara district, Karnataka, India were studied for their in vitro anthelmintic potential against Pheritima posthuma (Earth worm). The helminthes were found to be more susceptible to the oil sample AOS4 when compared to Albendazole standard. Variation in the activity was noted based upon the month of collection which may be due to the changes in the chemical constituents. The present investigation throws light on the versatile use of allspice leaf essential oil against helminthes, which indicates that the leaf can be used in culinary practices providing additional health benefits.

Keywords: *Pheritima posthuma*, Allspice leaf oil, Myrtaceae, Anthelmintic activity.

INTRODUCTION

Infections due to helminthes are a common malady affecting the human population. Mortality rate due to parasitic infections is increasing coupled with poor management practices [1, 2]. As there is increased development of resistance of helminthes to currently available anthelmintic drugs, treatment of helminthic infections becomes a major concern today [3, 4].

Extensive work has been carried out on *in vitro* anthelmintic activity of essential oils and extracts of various plants against *Pheritima posthuma* (Earth worm) according to available literature [5-20].

Allspice was used by the early Central American civilizations as a flavouring for chocolate. Amongst almost all of the aromatic spices, Allspice has a worldwide reputation and was first brought to Europe by Christopher Columbus in the 15th century, who found it in Jamaica, its true country of origin. It was first known as 'Piment', probably from the word "Pimento", which was used in the Middle ages for every spice [21]. The name "Pimienta" was coined by the Spanish explorers of the 17th as the berries have peppery flavour [22]. As a medicine, Allspice leaves have much

the same use as Cloves and their oils are similar. It works well as a digestive and has an antiseptic and slightly anaesthetic action [22].

Allspice berry oil is reported to show good nematicidal activity against *Burasa-phelenchus xylophilus* [23]. Allspice leaf oil from Jamaica was reported to contain eugenol, methyl eugenol, β-caryophyllene and Myrcene as the major constituents and is also reported to have good antioxidant activity [24].

The present work reports the *in vitro* anthelmintic activity of essential oil of Pimento leaf collected from South Canara district of Karnataka, India and compares the variation in the activity based upon the month of collection.

MATERIALS AND METHODS

Plant material:

Procurement of leaves

The leaves used for the investigation were collected from a private garden at a place called Puttur in Dakshina Kannada district, Karnataka in the months of Jan-May. The leaves were shade-dried and powdered and stored in air-tight containers to prevent the loss of volatile oil till further use. A voucher specimen PD08-01 is stored with us.

Identification

The plant under investigation was identified with the help of flora and also with the help of two local taxonomists, viz., Prof. Rajagopal, from Mahatma Gandhi Memorial College, Udupi and Prof. Gopalakrishna Bhat, from Poorna Prajna College, Udupi, Karnataka.

Extraction of the oil

The leaf powder was hydrodistilled in a Clevenger's apparatus to yield yellowish brown oil. The oils collected during months of January, February, March, April and May will be known henceforth as AOS1, AOS2, AOS3, AOS4 and AOS5 respectively.

Evaluation of anthelmintic activity

Evaluation of anthelmintic activity was carried out according to the standard method [25]. Indian adult earth worms (*Pheretima posthuma*) of 4-6 cm length and 0.1-0.2 cm width were used for the study due to their anatomical and physiological resemblance with the intestinal parasites of human beings [26, 27].

The earthworms were divided into six groups containing six worms in each group. The essential oil was dissolved in water using Tween 80, to give 0.0125, 0.025, 0.050, 0.10 and 0.15 ml/ml respectively. Albendazole was used as the standard at 10mg/ml.

10 ml of freshly prepared oil and standard solution were poured into petridishes. The worms were washed and released into the petridishes and the time taken for the worms to get paralyzed and killed was noted.

Statistical Analysis

The statistical analyses were performed using GraphPad Prism 2.01 statistical software (GraphPad Software, San Diego, CA, USA). The statistical significance was determined by multiple regression analysis. A p value of <0.05 was considered statistically significant. All the data are expressed as mean ± SEM (standard error of the mean).

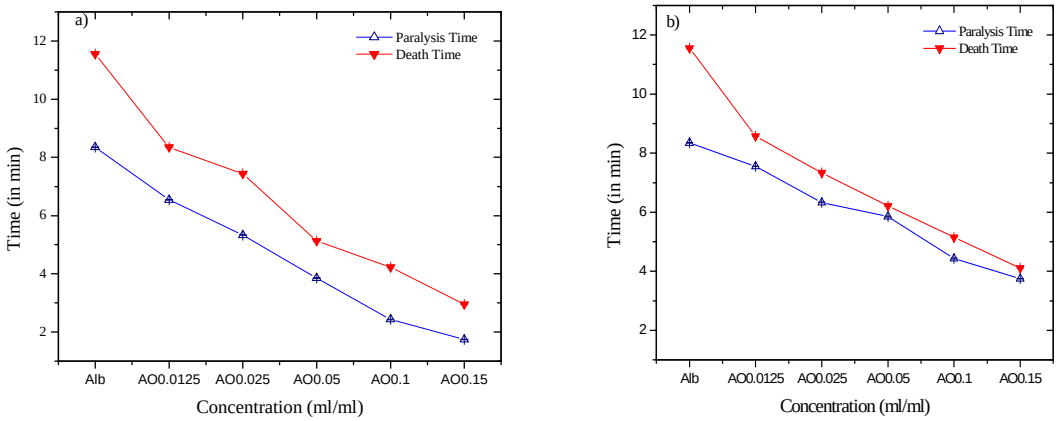
RESULTS AND DISCUSSION

The results of anthelmintic activity are shown in Table 1 and Figure 1. All oil samples showed significant anthelmintic activity in a concentration dependant manner. AOS4 collected in the month of April, showed significant paralysis as well as death time at all concentrations. Among all the concentrations evaluated for the oils, AOS1, AOS2, AOS3, AOS4 or AOS5, 0.15ml/ml solution exhibited the most potent anthelmintic activity (Table 1, Fig 1) when compared with the positive control albendazole (Alb). Though eugenol is considered as the main constituent, other constituents may have contributed to the anthelmintic activity.

Table 1: ANTHELMINTIC ACITIVITY OF ALLSPICE OIL (SUMMER SAMPLE)

S.No	Sample	Concentration	Time (min, Mean±SEM)	
			Paralysis	Death
1	**Negative Control**	-	-	-
2	**AOS1**	0. 0125 ml/ml	6.55±0.0077	8.35±0.011
		0.025 ml/ml	5.33±0.0103	7.44±0.009
		0.05 ml/ml	3.85±0.0184	5.14±0.010
		0.10 ml/ml	2.43±0.0112	4.23±0.008
		0.15 ml/ml	1.75±0.0071	2.96±0.019
3	**AOS2**	0. 0125 ml/ml	7.55±0.0077	8.57±0.008
		0.025 ml/ml	6.33±0.0103	7.33±0.009
		0.05 ml/ml	5.85±0.0184	6.21±0.011
		0.10 ml/ml	4.43±0.0112	5.15±0.009
		0.15 ml/ml	3.75±0.0071	4.11±0.011
4	**AOS3**	0. 0125 ml/ml	8.13±0.006	9.41±0.008
		0.025 ml/ml	7.23±0.012	7.41±0.009
		0.05 ml/ml	6.45±0.011	6.44±0.011
		0.10 ml/ml	4.61±0.011	5.23±0.009
		0.15 ml/ml	3.15±0.008	4.54±0.011
5	**AOS4**	0. 0125 ml/ml	5.56±0.006	6.51±0.010
		0.025 ml/ml	4.33±0.010	5.33±0.008
		0.05 ml/ml	3.15±0.014	4.23±0.012
		0.10 ml/ml	2.03±0.011	3.16±0.006
		0.15 ml/ml	1.05±0.009	2.89±0.011
6	**AOS5**	0. 0125 ml/ml	7.91±0.0081	9.41±0.008
		0.025 ml/ml	6.16±0.0103	7.41±0.009
		0.05 ml/ml	5.33±0.018	6.44±0.011
		0.10 ml/ml	4.61±0.0112	5.23±0.009
		0.15 ml/ml	3.15±0.0071	4.54±0.011
7	**Albendazole (Positive Control)**	10 mg/ml	9.35±0.00792	11.55±0.011

**Results are expressed as mean ±S.E.M. (n=6) for each group; significance at p< 0.05, as compared to Standard.*



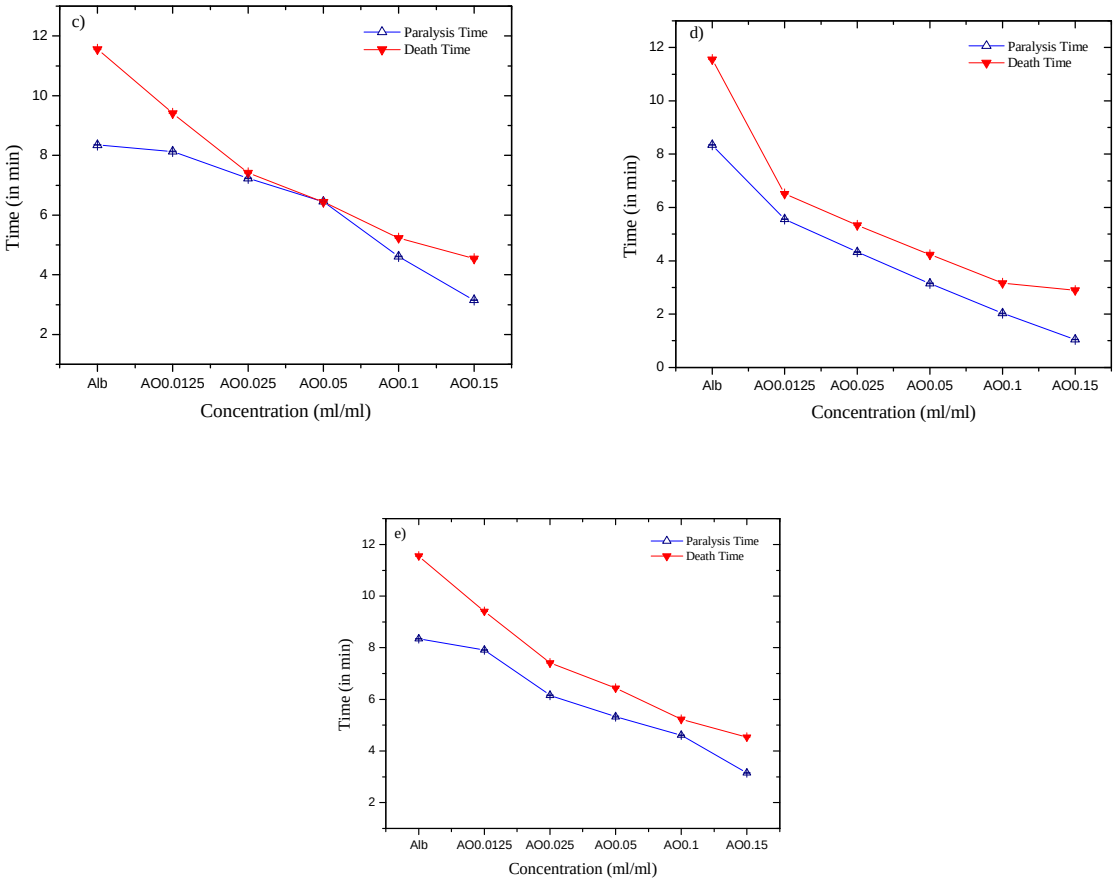


Figure 1: Anthelmintic activity of Essential oil of Pimenta dioica (Allspice) Leaves collected in Summer (AOS) a) AOS1; b) AOS2; c) AOS3; d) AOS4 and e) AOS5

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