

## Original Research Article

## In Vitro Antihelmintic Activity of Individual and Combined Leaf Extracts (*Punica granatum* and *Psidium guajava*) Against *Pheretima Posthuma*

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**Abstract:** *Punica granatum* and *Psidium guajava* leaves were easily available and commonly used in our day-to-day life. The present study aimed at the in-vitro comparative study of anthelmintic activity of aqueous extracts of leaves of *Punica granatum*, *Psidium guajava* and mixed. The fruits were extracted separately with distilled water by maceration method. The various concentrations of the extract (50,100, 200mg/ml) respectively were screened for their anthelmintic activity using *Pheretima posthuma*. The activity was comparable with the standard drug albendazole. When the concentrations of the extract are increased, a gradual increase in anthelmintic activity is observed. The study involved the determination of time of paralysis (P) and time of death (D) of the worms. Aqueous extract of *Punica granatum*, *Psidium guajava* and mixed showed anthelmintic activity but mixed extract was more efficient anthelmintic activity against Indian earthworms. The data were found statistically significant by using one way ANOVA ( $P < 0.001$ ).

**Keywords:** *Punica granatum*, *Psidium guajava*, *Pheretima posthuma*, Anthelmintic activity, Albendazole.

## INTRODUCTION

Helminthiasis is a worm infestation of humans and other animals even life stock and crops affecting health and food production respectively and has impact on global economic factor (Kumar, 2014). The worms which causes helminthiasis are called as helminths and the drugs which are used for treating helminthiasis are nothing but anthelmintics (Abongwa, 2017). There are various types of worms such as hook worms, fluke worms, round worms, tape worms which causes helminthiasis. The names are given according to their shapes. The major organs which get affected in helminthiasis are stomach and intestine and major symptoms of severe helminthiasis include diarrhea, abdominal pain, general malaise and impaired cognitive development. Chronic helminthiasis by hook worm lead to intestinal bleeding and anemia (Hedley, 2015). *Pheretima* is a genus of earthworms. *Pheretima posthuma* are long cylindrical shaped worms having length of 15-30cm. they are mostly found in moist soil and responsible for vegetables and humus. Their life span is 3 to 10 years (Kutschera, 2010) [1].

*Punica granatum* belonging to family Lythraceae, is a fruit bearing deciduous shrub. Fruits are consumed fresh or used for the preparation of fresh juice, jelly and jam, and beverage products. In several systems of medicine *Punica granatum* fruit is used for variety of ailments. Its fruit juice have various phytoconstituents whose functional and medicinal effects such as hepatoprotective, antibacterial, antioxidant, anticancer, antidiabetic, anti-atherosclerotic effects, estrogen-like activity had been confirmed. In Ayurveda, the peels of the fruit are used for stomach ailments including diarrhoea and dysentery. The peels has wide range of therapeutic properties and can be used in treatment of diabetes, cancer, cardiovascular disease, dental conditions, erectile dysfunction and male infertility, infectious diseases, Alzheimer's disease and dermal wounds [2].

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*Psidium guajava* (L.) (Myrtaceae family) presents high nutritional value [3]. *Psidium guajava* is well known tropic tree which is abundantly grown for fruit (Dakappa *et al.*, 2013). All parts of *Psidium guajava* have an old history of medicinal value (Nwinyi *et al.*, 2008). Traditionally, preparations of the leaves have been used in folk medicine in several countries, mainly as anti-diarrheal remedy and as anti-bactericidal in Nigeria (Sanda *et al.*, 2011). *Psidium guajava* L. is consumed not only as food but also as folk medicine in subtropical areas all over the world due to its pharmacologic activities (Miyazaki *et al.*, 2010). The ability of guava leaf extract on the treatment of various diseases has been proven scientifically, but the mechanism hasn't been fully explained (Denny *et al.*, 2013) [4].

## MATERIALS AND METHODS

### Collection of plant material

The fruits of *Punica granatum* and *Psidium guajava* was identified and purchased from local market of Nuzvid.

### Preparation of aqueous extract (Maceration method) [5]

The 500gm of dried leaves of *Punica granatum* and *Psidium guajava* was collected and powdered to get a coarse powder and was kept for maceration with 1000 ml of distilled water for 7 days. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used.

### Preliminary phytochemical screening [6-8]

The preliminary phytochemical investigation was carried out with aqueous extracts of *Punica granatum* and *Psidium guajava* and mixed fruits for identification of phytochemical constituents. Phytochemical tests were carried out by standard methods.

### Test organism [9]

Indian adult earthworms (*Pheretima posthuma*) were used during the experiment. The earthworms were collected from a local supplier. Worms were washed with normal saline to remove all fecal matter. The earthworms of 8-10 centimeter (cm) in length and 0.2 -0.5 cm width were used for all the experiment protocol. Ready availability, anatomical and physiological resemblance of (*Pheretima posthuma*) made it to be used initially for *in-vitro* evaluation of anthelmintic activity. Time for paralysis was noted either when any movement could not be observed except when the worms were shaken vigorously. Death was included when the worms lost their motility followed by white secretions and fading away of their body colour.

### Evaluation of Antihelmintic activity

The antihelmintic activity was evaluated on adult Indian earthworm. The earthworms were randomly chosen and divided into five groups having five earthworms in each as follows:

**Group I:** Control Group

**Group II:** Standard Group – Albendazole [10] -50,100, 200 mg/ml

**Group III:** Test-I -Aqueous leaves extract of *Punica granatum* and [PGAE- 50,100, 200 mg/ml]

**Group IV:** Test -II - Aqueous leaves extract of *Psidium guajava* [PGAE- 50,100, 200 mg/ml]

**Group V:** Test -III- Mixed leaves aqueous extract of *Punica granatum*, *Psidium guajava* [MLAE- 50,100, 200 mg/ml]

Observations were made for the time taken by worms to paralyze and death was observed. Time for paralysis was noted when no movement could be observed with a slight pin prick method. Death was ascertained by applying external stimuli which stimulate and induce movements in worms as well as fade of the body color was noted.

### Statistical Analysis

The values are expressed as mean± SEM. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Comparisons were made between control group and test/standard groups. P-values <0.05 was considered statistically significant. The statistical analysis was done by using Graph pad prism version no: 6.0.

## RESULTS AND DISCUSSION

In this study, we found that aqueous fruit extract of *Punica granatum*, *Psidium guajava* and mixed possess the following chemical constituents (Table 1).

**Table 1: Phytochemical screening of PGAE, PGAE & MLAE**

Phytochemical constituents	Aqueous leaves extract of <i>Punica granatum</i>	Aqueous leaves extract of <i>Psidium guajava</i>	Aqueous mixed leaves extract
Alkaloids	+	+	+
Carbohydrates	+	+	+
Flavonoids	+	+	+
Phenols	=	=	=
Saponins	+	+	+
Terpenoids	=	=	=
Sterols	=	=	=
Tannins	+	+	+
Proteins	=	=	=
Amino acids	=	=	=
Glycosides	+	+	+
Fixed oils and fatty acids	+	+	+

+ indicate the compulsory present and – indicate the absent.

PGAE- *Punica granatum* aqueous leaves extract.

PGAE - *Psidium guajava* aqueous leaves extract.

MLAE- Mixed leaves aqueous extract.

#### Anthelmintic activity:

The Aqueous extract of *Punica granatum*, *Psidium guajava* and mixed produced a significant anthelmintic activity in dose dependent manner as shown in below table.

**Table 2: Anthelmintic activity of *Punica granatum*, *Psidium guajava* and mixed extracts & Standard drug on earth worm**

Groups	Concentration	Time Taken in minutes	
		Paralysis (P)	Death (D)
Control		-	-
Standard	50mg/ml	31.6±0.510	55.4±0.509
	100mg/ml	27.8±0.383	49.0±0.707
	200mg/ml	18.2±0.374	28.8±0.383
Test-I [PGAE]	50mg/ml	28.4±0.510	56.2±0.374
	100mg/ml	24.6±0.400	43.0±0.548
	200mg/ml	21.4±0.509	31.4±0.510
Test-II[PGAE]	50mg/ml	25.2±0.374	51.4±0.509
	100mg/ml	20.8±0.383	44.2±0.860
	200mg/ml	13.6±0.510	35.4±0.509
Test-III [MLAE]	50mg/ml	20.8±0.374	36.20±0.383
	100mg/ml	15.6±0.400	31.0±0.316
	200mg/ml	13.2±0.707	25.6±0.509

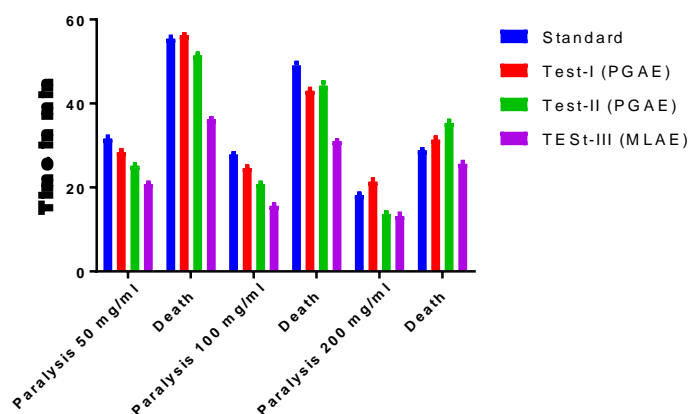


Figure 1: Anthelmintic activity of standard, PGAE, PGAE & MLAE. Values are expressed as Mean ± SEM, P < 0.001.

## CONCLUSION

In the present investigation, *Punica granatum*, *Psidium guajava* and mixed leaves possess the presence of alkaloids, carbohydrates, saponins, tannins, Flavonoids and glycosides. Tannins are chemically polyphenolic compound and were shown to produce anthelmintic activities and reported the effect of tannin can bind to free proteins in gastro intestinal tract of host animal or glycoproteins on the cuticle of parasite and may cause death. These facts suggest that tannins present in the aqueous leaves extract of *Punica granatum* and *Psidium guajava* and mixed showed the anthelmintic effect by above mentioned mechanisms. From the result shown in table-2 aqueous leaves extract of *Punica granatum*, *Psidium guajava* and mixed showed anthelmintic activity in dose dependent manner giving shortest time of paralysis and death. The aqueous fruit extract of *Punica granatum*, *Psidium guajava* and mixed at normal concentration i.e. 50 mg/ml to higher concentration i.e. 200mg/ml showed good anthelmintic activity and this is compared with effect produced by reference standard drug albendazole. The study finally concluded aqueous mixed leaves extract i.e. combination of *Punica granatum* and *Psidium guajava* showed marked and potent anthelmintic activity than the aqueous extract and standard drug albendazole.

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