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PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITY OF INDIVIDUAL AND COMBINED SEED EXTRACTS (*VITIS VINNIFERA* AND *PUNICA GRANATUM*) AGAINST INDIAN EARTHWORMS

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ABSTRACT

The present study aimed at the *in-vitro* comparative study of anthelmintic activity of aqueous extracts of seeds of *Vitis vinnifera*, *Punica granatum* and mixed. The seeds were extracted separately with distilled water by maceration method. The various concentrations of the extract (50, 100, 200, 300, 400mg/ml) respectively were screened for their anthelmintic activity using *Pheretima posthuma*. The activity was similar to that of the common medication albendazole. An observable rise in anthelmintic activity occurs with increasing extract concentrations. The worms' times of paralysis (P) and death (D) were measured as part of the investigation. Aqueous extract of *Vitis vinnifera*, *Punica granatum* and mixed showed anthelmintic activity but mixed extract was more efficient anthelmintic activity against Indian earthworms. Using a one-way ANOVA, the findings were determined to be statistically significant ($P < 0.0001$).

KEYWORDS

Vitis vinnifera, *Punica granatum*, *Pheretima posthuma*, Anthelmintic activity and Albendazole.

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INTRODUCTON

Helminthiasis is a worm infestation of humans and other animals even life stock and crops affecting health and food production respectively and have impact on global economic factor. The term "helminths" refers to the worms that cause helminthiasis, while "anthelmintics" are the medications used to treat the condition. There are various types of worms such as hook worms, fluke worms, round worms, tape worms which causes helminthiasis. The names are assigned in accordance with 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 hookworm helminthiasis causes anemia and intestinal hemorrhage. *Pheretima* is a genus of earthworms. *Pheretima posthuma* are long, cylindrical worms that measure between 15 and 30 centimeters in length. They are primarily found in wet soil and are the source of humus and vegetables. Their life span is 3 to 10 years¹.

Punica granatum is a deciduous shrub that bears fruit and is a member of the Lythraceae family. 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 wounds².

Vitis vinnifera L. commonly known as grape belongs to the family *Vitaceae*. Grape is one of the largest commodities in agriculture. The grape farming is called as viticulture. Around 10,000 varieties of grape are there in this world. The varieties include seedless, no seedless and also come in white, red, green colors. *Vitis vinnifera* species dominate the other species of grape by 90%. Grapes have been used for thousand years because of their nutritional and medicinal benefits. These are rich in sugars, flavonoids, anthocyanin sand proanthocyanins, organic acids, tannin, mineral salts and vitamins. Grapes have been used traditionally in Pakistan, Italy and Turkey as laxatives, carminatives and as drug therapy for cold, flu, anaemia, wound care, allergies and bronchitis. Researches has proven that the bioactive compounds present in grapes has led to the

pharmacological activities such as antioxidant, antidiabetic, anticancer, anti-inflammatory, anti-acne, anti-aging, antiplatelet, antiasthma, anti-obesity and anti-sunburn and wound healing properties³.

MATERIAL AND METHODS

Collection

The seeds of *Vitis vinnifera* and *Punica granatum* was identified and purchased from local market of Nuzvid.

Preparation of Extract⁴

The dried seeds of *Vitis vinnifera* and *Punica granatum* was collected and then dried seeds were powdered to get a coarse powder. The dried powder seeds (250gm) were taken in beaker and add 1000 ml of distilled water. Then it was kept for maceration for 7 days. The extract was concentrated by evaporating it over a water bath after being doubly filtered through muslin cloth and Whatman No.1 filter paper. The extract was dried and used.

Preliminary Phytochemical Screening⁵

The preliminary phytochemical investigation was carried out with aqueous extracts of *Vitis vinnifera*, *Punica granatum* and mixed seeds for identification of phytochemical constituents. Standard procedures were used to conduct phytochemical testing.

Test Organism⁶

The experiment was conducted using adult Indian earthworms, or *Pheretima posthuma*. 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 -0.5cm width were used for all the experiment protocol. Due to its easy accessibility and anatomical and physiological similarities to *Pheretima posthuma*, it was first employed for the in-vitro assessment of its anthelmintic activity. Time of paralysis was observed either when any movement could not be observed except when the worms where 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 – Albendazole⁷ -50, 100, 200, 300, 400mg/ml

Group III

Test-I -*Vitis vinnifera* aqueous extract [VVAE] - 50, 100, 200, 300, 400mg/ml]

Group IV

Test -II - *Punica granatum* aqueous extract [PGAE] - 50, 100, 200, 300, 400mg/ml]

Group V

Test- III- Mixed seed aqueous extract of *Vitis vinnifera* and *Punica granatum* [MSAE] 50, 100, 200, 300,400mg/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 haloperidol 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: 7.0.

RESULTS AND DISCUSSION

In this study, we found that aqueous seed extract of *Vitis vinnifera*, *Punica granatum* and mixed possess the following chemical constituents (Table No.1).

Anthelmintic activity

The Aqueous extract of *Vitis vinnifera*, *Punica granatum* and mixed produced a significant anthelmintic activity in dose dependent manner as shown in below Table No.2.

Table No.1: Phytochemical screening of VVAE, PGAE and MSAE

S.No	Phytochemical constituents	Aqueous seed extract of <i>Vitis vinnifera</i>	Aqueous seed extract of <i>Punica granatum</i>	Aqueous mixed seed extract
1	Alkaloids	+	+	+
2	Carbohydrates	+	+	+
3	Flavonoids	+	+	+
4	Phenols	-	-	-
5	Saponins	+	+	+
6	Terpenoids	-	-	-
7	Sterols	-	-	-
8	Tannins	+	+	+
9	Proteins	-	-	-
10	Amino acids	-	-	-
11	Glycosides	+	+	+
12	Fixed oils and fatty acids	+	+	+

+ indicate the compulsory present and – indicate the absent.

Table No.2: Anthelmintic activity of *Vitis vinnifera*, *Punica granatum* and mixed extracts and Standard drug on earthworm

S.No	Groups	Concentration	Time Taken in minutes	
			Paralysis (P)	Death (D)
	Control		-	-
1	Standard (Albendazole)	50mg/ml	219.2 ± 0.58	231.4 ± 0.51
		100mg/ml	94.4 ± 1.12	98.2 ± 0.37
		200mg/ml	31.6 ± 0.92	54.8 ± 0.37
		300mg/ml	28.2 ± 0.37	49.4 ± 0.51
		400mg/ml	17.0 ± 0.31	30.2 ± 0.37
2	Test-I [VVAE]	50mg/ml	234.4 ± 0.40	242.2 ± 0.97
		100mg/ml	83.6 ± 0.74	89.0 ± 0.44
		200mg/ml	27.4 ± 0.40	55.2 ± 0.37
		300mg/ml	23.4 ± 0.51	41.2 ± 0.37
		400mg/ml	20.2 ± 0.37	30.4 ± 0.51
3	Test-II [PGAE]	50mg/ml	239.2 ± 0.37	245.8 ± 0.86
		100mg/ml	82.4 ± 1.03	88.4 ± 0.51
		200mg/ml	25.0 ± 0.70	50.0 ± 0.31
		300mg/ml	19.8 ± 0.37	44.4 ± 0.51
		400mg/ml	15.0 ± 0.44	34.4 ± 0.40
4	Test-III [MSAE]	50mg/ml	210.4 ± 0.51	218.8 ± 0.58
		100mg/ml	75.0 ± 0.31	80.6 ± 0.40
		200mg/ml	19.2 ± 0.58	35.8 ± 0.37
		300mg/ml	14.4 ± 0.51	30.8 ± 0.37
		400mg/ml	11.4 ± 0.51	25.6 ± 0.51



Figure No.1: Test-I (VVAE) - 50, 100, 200, 300 and 400mg/ml – Paralysis



Figure No.2: Test-I (VVAE) - 50, 100, 200, 300 and 400mg/ml – Death

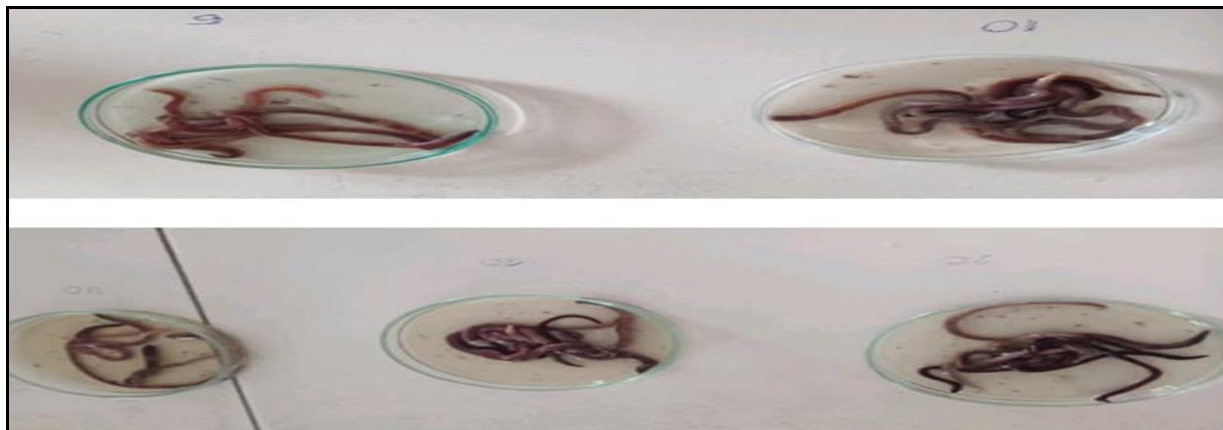


Figure No.3: Test-II (PGAE) - 50, 100, 200, 300 and 400mg/ml – Paralysis



Figure No.4: Test-II (PGAE) - 50, 100, 200, 300 and 400mg/ml- Death



Figure No.5: Test-III (MSAE)-50, 100, 200, 300 and 400mg/ml- Paralysis



Figure No.6: Test-III (MSAE) -50, 100, 200, 300 and 400mg/ml

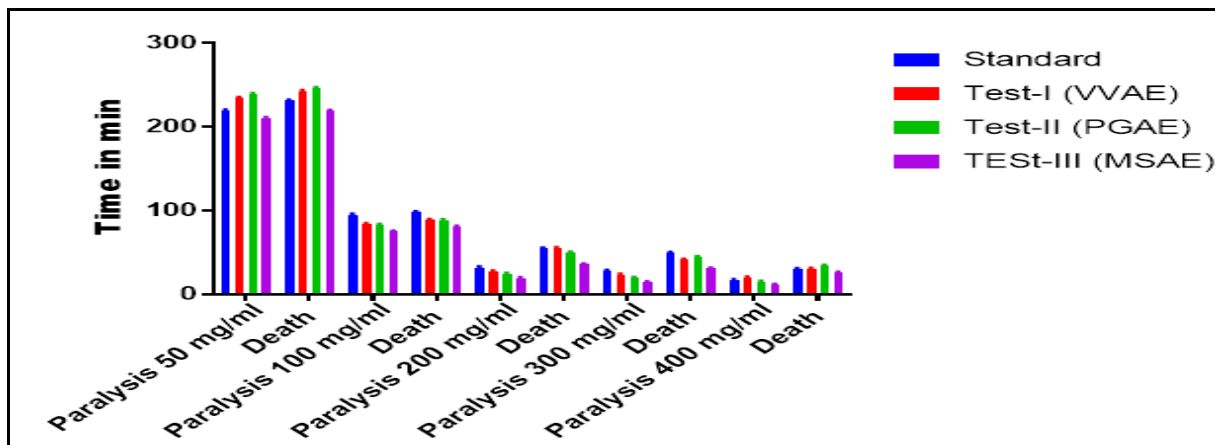


Figure No.7: Anthelmintic activity of standard, VVAE, PGAE and MSAE. Values are expressed as Mean \pm SEM, P < 0.0001

CONCLUSION

In the present investigation, *Vitis vinnifera*, *Punica granatum* and mixed seed possess the presence of alkaloids, carbohydrates, saponins, tannins, Flavonoids and glycosides.

Tannins are a chemically polyphenolic molecule that has been demonstrated to have anthelmintic effects. It has also been reported that tannins can attach to free proteins in the animal's gastrointestinal system or to glycoproteins on the parasite's cuticle, which can result in the death of the parasite.

These facts suggest that tannins present in the aqueous fruit extract of *Vitis vinnifera*, *Punica granatum*, and mixed showed the anthelmintic effect by above mentioned mechanisms.

From the result shown in Table No.2 aqueous seed extract of *Vitis vinnifera*, *Punica granatum* and mixed showed anthelmintic activity in dose dependent manner giving shortest time of paralysis and death.

The aqueous seed extract of *Vitis vinnifera*, *Punica granatum* and mixed at normal concentration i.e. 50 mg/ml to higher concentration i.e. 400 mg/ml, and this was compared to the effects of a standard reference medication albendazole.

The study finally concluded aqueous mixed fruit extract i.e. combination of *Vitis vinnifera*, *Punica granatum* showed marked and potent anthelmintic activity than the aqueous extract and standard drug albendazole.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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