



## ORGANOLEPTIC AND WORMICIDAL EVALUATION OF VARIOUS CRUDE EXTRACTS OF POMEGRANATE (*PUNICA GRANATUM*) PEEL

Sikandar Khan Sherwani<sup>1\*</sup>, Tasveer Zahra Bokhari<sup>2</sup>, Yasmeen Bibi<sup>3</sup>, Syed Aneel Gilani<sup>4</sup>, Shahzad Munir<sup>5</sup>, Muhammad Ajmal Shah<sup>6</sup>, Ikramullah<sup>7</sup>

<sup>1</sup>Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan

<sup>2</sup>Institute of Pure and Applied Biology, BZU-Multan, Pakistan

<sup>3</sup>Department of Chemistry, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan

<sup>4</sup>Pakistan Museum of Natural History, Islamabad-Pakistan

<sup>5</sup>Department of Microbiology, Kohat University of Science and Technology, KPK, Pakistan

<sup>6</sup>Department of Pharmacognosy, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan

<sup>7</sup>Department of Microbiology, Hazara University, Mansehra, KPK, Pakistan

\*Corresponding Author Email: sikander\_biology@hotmail.com

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### ABSTRACT

In the present study, organoleptic evaluation of Pomegranate (*Punica granatum* L) peel powder was done and found pink in color having no smell and slightly bitter taste. Moreover, aqueous and methanolic extracts of Pomegranate (*Punica granatum* L) peel were also prepared and investigated for their anthelmintic activity using a model worm i.e. *Pheretima posthuma*. Three concentrations (25, 50 and 100 mg / ml) of each extracts were studied in activity, which involved the determination of time of paralysis and time of death of the worm. Both nature of extracts exhibited anthelmintic activity at highest concentration of 100 mg / ml; however, the response of methanolic extract was more profound. Piperazine citrate in same concentration as that of extract was included as standard reference and distilled water as control.

**Keywords:** Anthelmintic activity, wormicidal, *Pheretima posthuma*, *Punica granatum* L.

### INTRODUCTION

Anthelmintic drugs or wormicidal agents are those that expel parasitic worms from the body, either by stunning or direct killing<sup>1</sup>. In spite of the fact that a number of wormicidal agents are available and of high cost, yet their efficacy is always a big concern<sup>2</sup>. Moreover, in the same connection, the emerging developments of resistance to a number of commercially available anthelmintic drugs have now become a growing concern worldwide in terms of management of infections clinically<sup>3</sup>. Pomegranate is the oldest known fruit in human history<sup>4</sup>. The pomegranate is native from the Himalayas in northern India to Iran. It is also found in India, Southeast Asia, the East Indies and tropical Africa<sup>5</sup>. Moreover, similarly the tree is also planted for its fruit in the drier regions of California and Arizona<sup>6</sup>. Pomegranate has been used in various regions as a folk medicine as well as food supplement because of its rich compound diversity along with lots of activity and without toxicity<sup>7</sup>. Almost all parts of Pomegranate (*Punica granatum* L) are used in traditional medicine for the treatment of various diseases. In particular, bark and rind of the fruit are used in dysentery, diarrhea piles, bronchitis, to reduce the risk of cardiovascular disease and as an anthelmintic<sup>8-9</sup>. Furthermore, the decoction has been used as mouth wash, enema and for stomatitis<sup>10-11</sup>. Antibacterial and antifungal activity has also been reported against a number of pathogenic microorganisms<sup>12-13</sup>. The most widely use of Pomegranate (*Punica granatum* L) globally has been as a vermifugal agent<sup>14</sup>. It selectively kills and expels out intestinal worms<sup>15</sup>. The current study was carried out to evaluate organoleptic property as well as for endorsing ethno botanically the wormicidal potential of Pomegranate (*Punica granatum* L) using earthworm as a model worm.

### MATERIALS AND METHODS

#### Plant Materials

Pomegranate was purchased from the local market in Karachi, Pakistan. The fruits were brought in the Research laboratory of Federal Urdu University of Arts, Science and Technology-Karachi, Pakistan; where they were thoroughly washed and then peeled off nicely by the help of sharp knife. The peels were later dried under direct sunlight for 15 days and crushed them first into small pieces and later finely ground by high speed kitchen electrical grinder for making its aqueous and methanolic extract.

#### Extract Preparation

The extract was prepared in 5 % concentration (5 g of weighted Pomegranate peel powder added in 100 ml of distilled water)<sup>16</sup>. The aqueous extract was prepared by boiling method of tea in water bath by constant agitation of for 15 minutes<sup>17-18</sup>. After extract preparation, coarse suspended particles of tea were removed by passing through strainer and later by passing via 0.22 µm filter<sup>19</sup>. The extract was stored in refrigerator in small vials as aliquots for further use.

#### Methanol Extraction

The methanol extractions of the active ingredient of the leaves were carried out using Harbone method<sup>20</sup>. 25 g of the grinded leaves were soxhelt extracted using 250 ml of 95 % methanol. The extraction lasted for six hours. The volatile oil obtained was concentrated by evaporation using water bath at 100°C<sup>17-18</sup>.

**Table 1: Organoleptic Evaluations of Pomegranate (*Punica granatum* L) Peel Powder**

Name of Plant	Part of the Plant	Colour	Odour	Taste
<i>Punica granatum</i> L	Peel	Pink	No smell	Slightly bitter

**Table 2: Anthelmintic Activity of Control and Standard**

Concentration mg / ml	Control		Standard	
	Paralysis time (minutes)	Death time (minutes)	Paralysis time (minutes)	Death time (minutes)
25 mg / ml	96.46 ± 0.46	160.06 ± 0.11	18.36 ± 0.15	54.36 ± 0.40
50 mg / ml	78.23 ± 0.25	142.2 ± 0.34	12 ± 0.00	46.46 ± 0.40
100 mg / ml	62.43 ± 0.11	111.7 ± 0.26	08.84 ± 0.03	13.6 ± 0.17

Values are the mean ± S.E.M. of control and standard drug on three earthworms. Control is normal saline while standard drug is Piperazine citrate.

**Table 3: Anthelmintic Activity of Aqueous Crude Extract of Pomegranate Peel**

Concentration mg / ml	Crude Extract of Pomegranate ( <i>Punica granatum</i> L) Peel	
	Paralysis time (minutes)	Death time (minutes)
25 mg / ml	39.20 ± 0.26	132.73 ± 0.30
50 mg / ml	19.33 ± 0.45	100.1 ± 0.17
100 mg / ml	7.5 ± 0.4	92.4 ± 0.36

Values are the mean ± S.E.M. of aqueous extract of on three earthworms Pomegranate peel.

**Table 4: Anthelmintic Activity of Methanolic Crude Extract of Pomegranate Peel**

Concentration mg / ml	Methanolic Crude Extract of Pomegranate ( <i>Punica granatum</i> L) Peel	
	Paralysis time (minutes)	Death time (minutes)
25 mg / ml	20.1 ± 0.47	67.6 ± 0.05
50 mg / ml	13.6 ± 0.30	48.7 ± 0.20
100 mg / ml	10.7 ± 0.32	15.9 ± 0.11

## RESULTS AND DISCUSSION

Helminthes are infact considered as the most common infections in man, affecting seriously a large percentage of people globally and also highly responsible of acute as well as chronic illness among human beings as well as in cattles and other livestock<sup>21-22</sup>. Certain problems have been evolved by the wide spread use of chemotherapeutic control practices as parasites are acquiring resistance to chemical anthelmintics<sup>23</sup>. In a study, it has been explored lately that anthelmintic substances having significant toxicity to human beings and also are present in foods derived from livestock, thus a serious threat to human health<sup>24</sup>. Medicinal plants have always been a rich and attractive source to search for new remedies for human health issues and ailments<sup>25</sup>. The plants are well known to provide a rich source of botanical anthelmintics<sup>26</sup>. Today plants now consider as a source of many potent and powerful drugs<sup>27</sup>. In the current study, organoleptic evaluation of Pomegranate (*Punica granatum* L) peel powder was done and found be pink color having no smell and slightly bitter in taste as mentioned in Table 1. In this study, in earthworms, both aqueous and methanolic extracts of Pomegranate (*Punica granatum* L) peel showed anthelmintic activity and the activity was recorded in dose dependent way in all groups including control, standard drug and both nature of extracts. Earthworms belonging to control group showed paralysis time at the concentration of 25 mg / ml, 50 mg / ml and 100 mg / ml i.e. 96.46 ± 0.46 minutes, 78.23 ± 0.25 minutes and 62.43 ± 0.11 minutes while; death time 160.06 ± 0.11 minutes, 142.2 ± 0.34 minutes and 111.7 ± 0.26 minutes respectively. On the other hand, the standard reference compound Piperazine citrate showed the time of paralysis time at the concentration of 25 mg / ml, 50 mg / ml and 100 mg / ml i.e. 18.36 ± 0.15 minutes, 12 ± 0.00 minutes and 08.84 ± 0.03 minutes respectively while; death time at 54.36 ± 0.40 minutes, 46.46 ± 0.40 minutes, 13.6 ± 0.17 minutes respectively as indicated in Table 2. In case of aqueous extract of Pomegranate (*Punica granatum* L) peel group, its paralysis time was noted at the concentration of 25 mg / ml, 50 mg / ml and 100 mg / ml i.e. 39.20 ± 0.26 minutes, 19.33 ± 0.45 minutes, 7.5 ± 0.4 minutes respectively and death time 132.73 ± 0.30 minutes, 100.1 ± 0.17 minutes and 92.4 ± 0.36 minutes respectively as indicated in Table 3. Similarly, on the other hand, in case of methanolic extract, its paralysis time was noted at the concentration of 25 mg / ml, 50 mg / ml and 100 mg / ml i.e. 20.1 ± 0.47 minutes, 13.6 ± 0.30 minutes, 10.7 ± 0.32 minutes respectively and the death time recorded as 67.6 ± 0.05 minutes, 48.7 ± 0.20 minutes and 15.9 ± 0.11 minutes respectively as mentioned in Table 4. The results of the methanolic extracts were more promising as compared to aqueous extract, most probably due to its treatment with methanol, an organic solvent. It is believed that various treatments for extract preparation release different nature and classes of chemical bioactive substances. Moreover, it can be observed from results that higher concentration of crude extract of pomegranate showed paralytic effect much earlier and the time to death was shorter for almost all the worms. Piperazine citrate infact causes flaccid paralysis and expels out the worm by means of peristalsis<sup>19</sup>. It actually generates paralysis by increasing the chloride ion conductance of worm muscle membrane resulting in the hyper polarization and reduced excitability, resulting to muscle relaxation thus a worm expels out by the action of peristalsis<sup>28</sup>. The medicinal importance of pomegranate is due to the presence of some special substances like alkaloids, glycosides, resins, volatile oils, gums and tannins, etc<sup>29</sup>. It has been observed that screening of medicinal plants for their anthelmintic activity remains of great scientific significance despite extensive use of synthetic chemicals in modern clinical practices all over the world<sup>30</sup>.

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## CONCLUSION

Conclusively, in view of results achieved, both crude aqueous as well as methanolic extract of Pomegranate (*Punica granatum* L) peel extracts showed anthelmintic activity that endorsed categorically the ethno botanical application for the treatment of helminthic infections. However, it is the preliminary screening that paved the way and a source of

encouragement to plan detailed experiments to find out the mode of action of pomegranate extract.

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