



**STUDY OF ANTHELMINTIC AND ANTIMICROBIAL ACTIVITY OF PEEL EXTRACT  
OF PUNICA GRANATUM LINN.**

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**ABSTRACT**

*Punica granatum linn.* (pomegranate) has been used for centuries to confer health benefits in number of diseases. The aim of present study is to identify phytochemical constituents and evaluation of antimicrobial and anthelmintic activities of peel extract of pomegranate. Antimicrobial activity of plant is due to its secondary metabolites. The antimicrobial activity of peel extract in aqueous, ethanol and methanol on *E.coli*, *S.aureus*, and *B.subtilis* was studied at different concentrations (1, 5, 10 mg/ ml). *E. coli* shows significant zone of inhibition in ethanol extract and *S.aureus* in aqueous extract, while *B. subtilis* shows in methanolic extract. The various concentrations (20, 50, 100, and 150 mg/ml) of aqueous, methanol and ethanol extracts were tested *in-vitro* for anthelmintic potency. It was determined by time required for paralysis and time required for death of earth worms. The peel extract shows dose dependant activity. The short time for paralysis and death was observed for methanol extract at concentration 150 mg/ml. From above observations it was concluded that the methanolic extract shows maximum response in dose dependant manner for antimicrobial and anthelmintic activities.

**KEY WORDS:** *Punica granatum linn.*, Pomegranate, Anthelmintic, Antimicrobial.

**INTRODUCTION**

The *Punica granatum linn* (Pomegranate) is an ancient, mystical, unique fruit borne on small, long living tree cultivated throughout the Mediterranean region.<sup>[1]</sup> Fruits are one of the oldest forms of food known to man. There are many references to fruits in ancient literature. Vedas state that fruits from the base of the food of Gods. According to Quran, the fruits like grapes, date, fig, olive and pomegranate are gift and heavenly fruits of God. The people in ancient time regarded fruits to be endowed with magic or devine properties.<sup>[2]</sup> *Punica granatum linn.* is well known by different common name like ayurveda-daadima, dantabijaa, raktakurumaa, unani-anaar, siddha-mathulai, English- pomegranate.<sup>[3]</sup> Modern research suggests that pomegranates might be useful in treating such serious condition such as prostate cancer, skin cancer, osteoarthritis and diabetes. Pomegranate help in preventing heart diseases, heart attack and stroke. This is because pomegranate has a potential to thin the blood, increase blood flow to the heart, reduce blood pressure, reduce plaque in the arteries.<sup>[4]</sup> Various parts of plant like fruit, bark, leaves, peel has wide range of therapeutic properties and can be used in treatment such as antimicrobial activity, analgesic activity, anthelmintic activity, antioxidant activity, antidiarrhoeal activity, anticancer activity, anti-inflammatory activity, antidiabetic activity, cardiovascular activity, dental activity, antiatherosclerotic effect, erectile dysfunction, male infertility, infectious disease estrogen like-activity,

Alzheimer's disease, dermal wounds, hepatoprotective, antibacterial, neuroprotective activity, antiproliferation, dysentery, antispermatogenic activity. The popularity of using plant derived medicines is increasing from last century due to having lower side effects, low cost and availability of the product. The current research focus on study of anthelmintic and antimicrobial activity of pomegranate.

**MATERIALS AND METHODS**

**Collection of plant material:** Fruits of *Punica granatum Linn.* (Pomegranate) family punicaceae were collected from local farm of village Dhavalivihir, Malegaon, Maharashtra, India. Fresh peels of fruits were separated from fruit and collected. Collected material was washed in running tap water and then rinsed with distilled water. Peels were subjected to drying at room temperature for a week in open air. The dried peels were powdered using mixer grinder and passed through sieve no 85. Powder then stored in refrigerator until further use.

**METHODS**

**Preparation of peel extract**

**A) Organic Solvent Extracts:** The dried peels were subjected to soxhlet apparatus extraction using 70 gm powder in 400 ml of 99% ethanol and methanol for 24 hrs. The mixtures were concentrated in a rotary flash evaporators and stored in refrigerator.

**B) Aqueous Extracts:** Peel powder was extracted by maceration for 72 hrs using 100 gm powder in 350 ml of distilled water and filtered through Whatman filter paper no.2. Filtrate was evaporated to dryness in hot air oven and stored in refrigerator.

#### Phytochemical analysis of peel<sup>[1, 5-7]</sup>

Peel extracts are subjected to various phytochemical analysis to find the chemical constituent of peels. Then, antimicrobial, anthelmintic activity of peel extract of *Punica granatum linn* was done by following methods

#### A. Antimicrobial test<sup>[8-12]</sup>

The agar well diffusion method was used to study the effect on growth of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* by measuring the diameter of zone of inhibition. Pure tested bacterium was first subculture in nutrient broth at 37°C for 24 hrs. 10 ml of sterile assay agar was added to each petridish with slow shaking. One hundred microliters (100µl) of standardized inoculum of each bacterium added in warm agar and allow to harden in refrigerator at 4°C for 1hr.

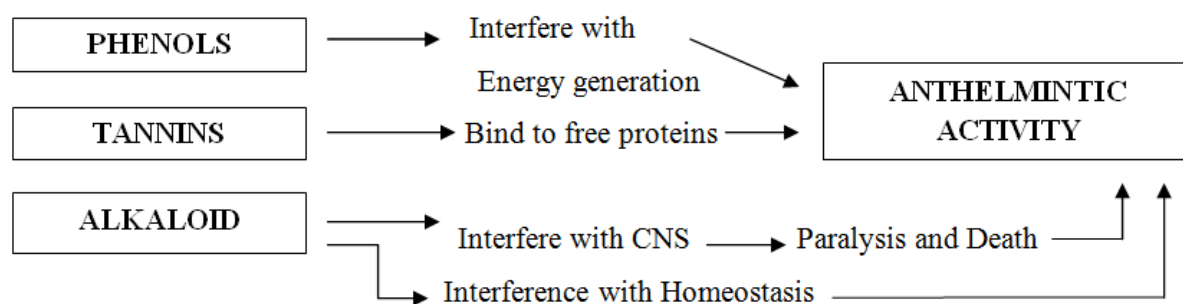
Afterwards three equidistance holes were made in the agar using sterile cork borers and 1, 5, 10 mg/ml was added on the top of inoculated agar layer then dried at 25°C for 30 min. Plates were kept at 4°C and then inoculated at 37°C for 24 hrs. At the end of this period, inhibition zones formed on medium accurately measured in millimeter.

#### B. Anthelmintic Test<sup>[13-15]</sup>

Helminthiasis is a macro parasitic disease observed in humans and animals. Helminth infections are among the commonest infections in the man affecting a large proportion of the world. In this infection body part is get infested with parasitic worm such as nematode (round worm) and flate worms (flukes and tape worm). These worms reside in the GI tract.

#### Screening of anthelmintic activity<sup>[15]</sup>

The worms used in the *in-vitro* assay are *Pheretima posthuma*, *Ascaridia galli*, *Ascaris lumbricoids*, *Raillietina spiralis*.



**Figure 1: Mechanism of Phytoconstituents of Pomegranate (Anthelmintic activity).<sup>[14]</sup>**

**Worm collection:** Anthelmintic activity performed on adult Indian earth worm *pheretima posthuma* as it has anatomical and physiological resemblance with intestinal round worm.

#### Procedure<sup>[13-14,16]</sup>

Indian earth worm collected from moist soil and washed with normal saline. The earth worm of 6-8 cm was used for experimental protocol. The worms were divided into 5 groups containing six worms in each group. Six equal size worms were released in each 50 ml formulation containing different concentrations of aqueous and organic solvent extract such as 25, 50, 100,150 mg/ml respectively in distilled water. First group was treated as control. All the test suspension was prepared freshly before starting the experiment. 2% Gum acacia was used as a suspending agent in all formulation. Observations

were made for the paralysis time (PT) and subsequently for death time (DT). Time for paralysis was noted when no movement of any sort could be observed except when worms were shaken vigorously. Death time was noted when worms lost their motility followed by fading away of their body color.

## RESULT AND DISCUSSION

**Phytochemical analysis of peel extract:** Phytochemical analysis of the aqueous extract of peels shows the presence of steroids, terpenoids, cardiac glycoside, flavonoids, carbohydrate and vitamin C etc. The metabolic extract of peels exhibit positive results for steroids, cardiac glycoside, flavonoids, tannins and carbohydrates. Ethanolic extract shows the presence of flavonoid, tannins and carbohydrates (table 1).

**Table 1: Phytochemical analysis of peel extract**

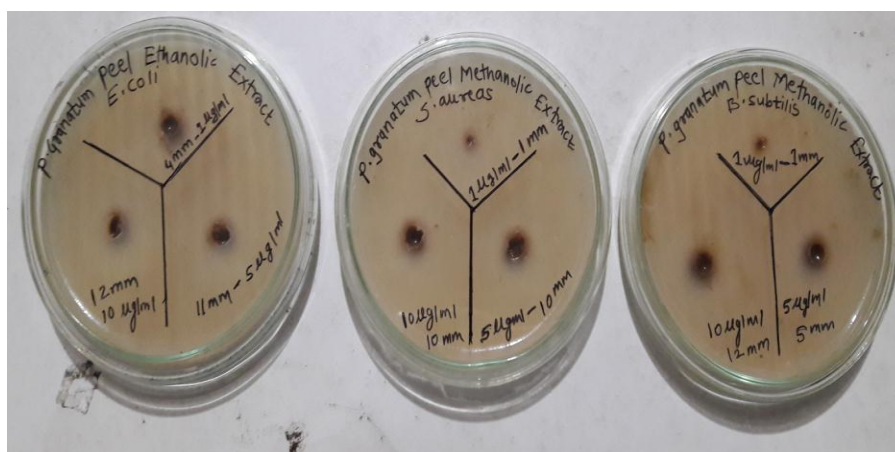
Sr.no.	Test	Aqueous extract	Methanol extract	Ethanol extract
1	Steroids	+ve	+ ve	- ve
2	Terpenoids	+ ve	- ve	- ve
3	Cardiac Glycoside	+ ve	+ ve	- ve
4	Saponins	- ve	- ve	-ve

5	Alkaloids	- ve	- ve	- ve
6	Flavonoids	+ ve	+ ve	+ ve
7	Amino acids	-ve	- ve	- ve
8	Tannins	-ve	+ ve	+ ve
9	Proteins	- ve	- ve	- ve
10	Carbohydrates	+ ve	+ ve	+ve
11	Vitamin C	+ ve	- ve	- ve

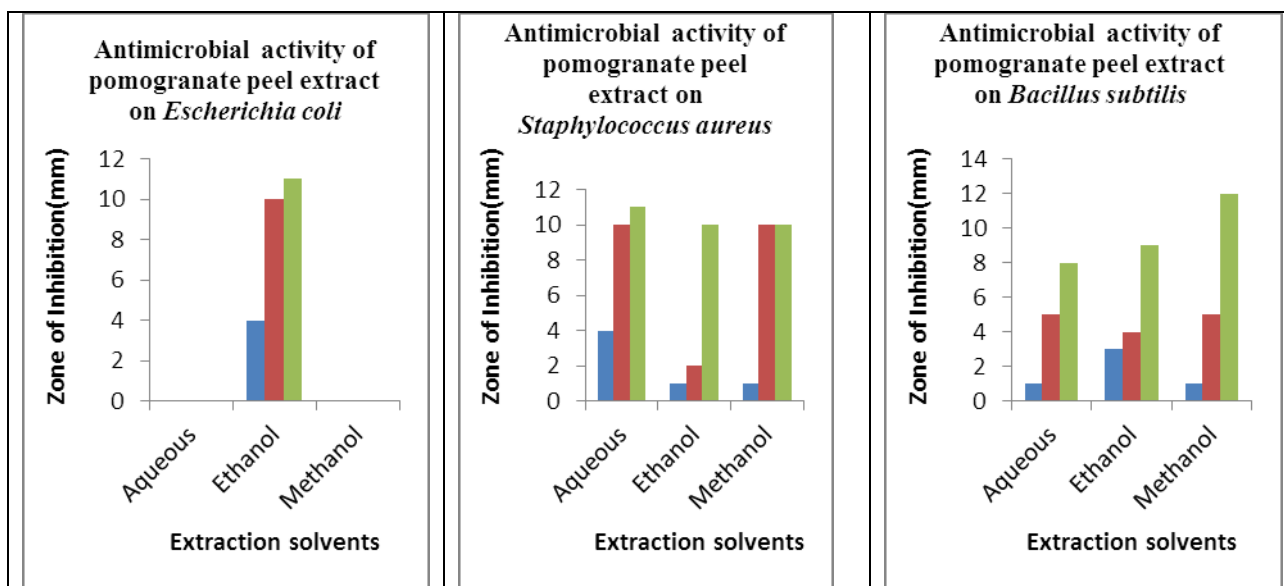
**A. Antimicrobial test:** The antimicrobial activity of pomegranate peel extract on *E. coli*, *S. aureus* and *B. subtilis* was studied at different concentration as shown in table 2 and figure 2 - 3.

**Table 2 : Antimicrobial activity of pomegranate peel extract.**

Sr.No.	Organism	Aqueous extract (mg/ml)			Ethanol extract (mg/ml)			Methanol extract (mg/ml)		
		1	5	10	1	5	10	1	5	10
1	<i>Escherichia coli</i>	-	-	-	4mm	11mm	12mm	-	-	-
2	<i>Staphylococcus aureus</i>	4mm	10mm	11mm	1mm	2mm	10mm	1mm	10mm	10mm
3	<i>Bacillus subtilis</i>	1mm	5mm	8mm	3mm	4mm	9mm	1mm	5mm	12mm



**Figure 2: Antimicrobial activity of pomegranate peel extract**



**Fig. 3: Antimicrobial activity of pomegranate peel extracts on *Escherichia coli*, *Staphylococcus*, *Bacillus subtilis*.**

Antimicrobial activity of plant extract may be due to presence of plant secondary metabolite such as flavonoids. Peel extract of pomegranate shows presence of glycoside, tannins and flavonoid. For *E. coli* zone of

inhibition for aqueous and methanol extract was not found while ethanol extract shows inhibition zone range from 4 mm to 12 mm for 1mg/ml and 10mg/ml respectively as shown in figure 2 - 3. *Staphylococcus*

*aureus* and *Bacillus subtilis* shows inhibition zone in all three extract. For *Staphylococcus aureus* maximum zone of inhibition obtained in aqueous extract > methanol extract > Ethanol extract as shown in fig. 2 - 3. For *Bacillus subtilis* zone of inhibition in Methanol extract > Ethanol extract > aqueous extract as shown in figure 2 - 3.

From the above study it is concluded that pomegranate peel extract shows antimicrobial activity for *E. coli*, *Staphylococcus aureus* and *Bacillus subtilis*. As the concentration of drug increases zone of inhibition increases. *E.coli* shows zone of inhibition in ethanol extract, *S. aureus* in aqueous extract while *B. subtilis* in methanol extract.

**B. Anthelmintic Test:** The experimental data it was found that aqueous, ethanol and methanol extract from peel of *punica granatum Linn.* shows anthelmintic activity against earth worm. Dose dependant activity was observed in all three extracts as shown in table 3.

The time of paralysis and death was noted against various concentrations of extracts. The time for paralysis of earth worm for aqueous, ethanol and methanol at low concentration (25 mg/ml) was 66, 50 and 34 minutes respectively and at high concentration (150 mg/ml) was 14, 12 and 09 minutes respectively. Death time for aqueous, ethanol and methanol at low concentration (25 mg/ml) was 86, 62, 25 minutes and at high concentration (150 mg/ml) 37, 23 and 12 minutes respectively.

**Table 3: Anthelmintic activity of peel extracts of *Punica granatum Linn.***

Sr. no.	Test Extract of <i>Punica granatum Linn.</i>	Concentration (mg/ml)	Time of paralysis in min. (Mean )	Time of Death in min. (Mean )
1	Aqueous extract of fruit peel	25(mg/ml)	66	86
		50(mg/ml)	44	69
		100(mg/ml)	24	65
		150(mg/ml)	14	37
2	Ethanol extract of fruit peel	25(mg/ml)	50	62
		50(mg/ml)	37	48
		100(mg/ml)	24	39
		150(mg/ml)	12	23
3	Methanolic extract of fruit peel	25(mg/ml)	34	25
		50(mg/ml)	26	18
		100(mg/ml)	18	15
		150(mg/ml)	9	12



**Figure 4 :**It shows death of worms after addition of methanolic extract of *punica granatum linn peel* (150mg/ml)



**Figure 5 :**It shows death of worms after addition of ethanolic extract of *punica granatum linn peel* (150mg/ml)



**Figure 6 :**It shows death of worms after addition of aqueous extract of *punica granatum linn peel* (150 mg/ml )

The study of Anthelmintic activity of pomegranate peel extracts shows activity in dose dependant manner. The shortest time of paralysis and death was observed for all extracts at 150 mg/ml. Methanolic extract shows shortest time for paralysis (9 min) and death (12 min) as

compared to aqueous and ethanolic extract. The present study indicates that aqueous, ethanol and methanol extracts have been confirmed to display anthelmintic activity. Methanolic extract shows activity in shortest time

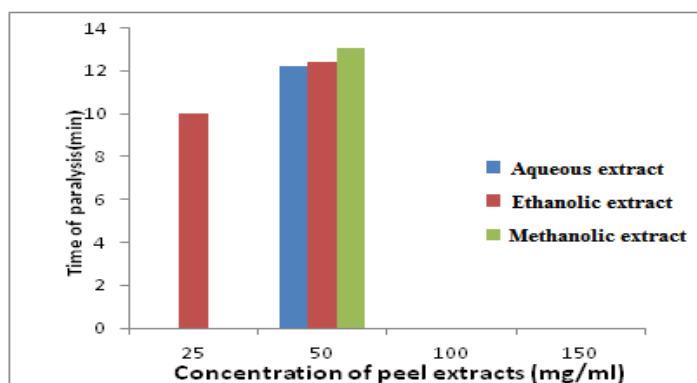


Figure 7: Indicate paralysis time(min) for earth worm at various concentration for aqueous, ethanolic and methanolic peel extract of *punica granatum linn.*

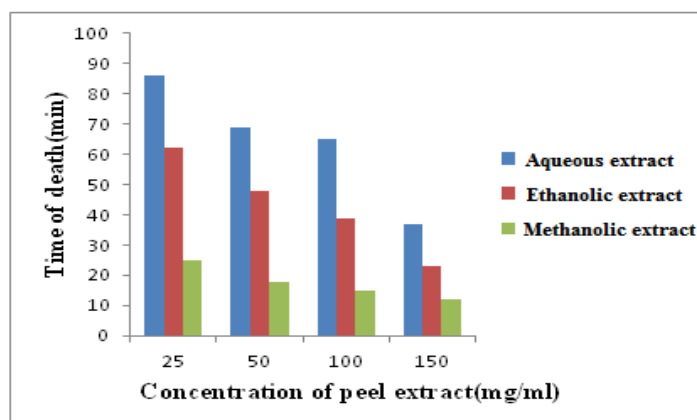


Figure 8: Indicate death time (min) for earth worm at various concentrations for aqueous, ethanolic and methanolic peel extract of *punica granatum linn.*

## CONCLUSION

From phytochemical analysis of various extracts of pomegranate peels, the secondary metabolites found are steroids, terpenoids, glycosides, flavonoids, carbohydrates and vitamins. The extracts (i.e. aqueous, methanolic, ethanolic) showed significant antimicrobial activity specially against *E.coli*, *staphylococcus aureus* and *bacillus subtilus*. Also the above extract proved having anthelmintic activity against earthworms by their paralysis and death. It was very clear that above mentioned activities were dose dependant and showing enhanced activities when dose is increased and vice-versa.

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