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ASSESSMENT OF THE ANTIPYRETIC POTENTIAL OF *PUNICA GRANATUM* LEAVES IN INDUCED PYREXIA MODELS

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ABSTRACT

Background: *Punica granatum* (pomegranate) is widely used in traditional medicine for its therapeutic properties, including antipyretic activity. The present study aimed to scientifically evaluate the antipyretic potential of *Punica granatum* leaves along with their phytochemical and antioxidant properties. **Materials and Methods:** The leaves were extracted using Soxhlet extraction with ethanol and petroleum ether. Phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), and DPPH antioxidant assay were performed. Acute oral toxicity was assessed as per OECD 423 guidelines. Antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in Wistar rats, with paracetamol (150 mg/kg) as the standard drug. **Results:** The ethanolic extract showed higher yield (6.71%) compared to petroleum ether (1.84%) and contained major phytoconstituents such as alkaloids, flavonoids, and glycosides. TPC and TFC were found to be 29.5 mg/g and 27.6 mg/g, respectively. The extract exhibited notable antioxidant activity (IC_{50} = 48.73 µg/ml). In vivo studies demonstrated a significant, dose-dependent reduction in rectal temperature, with the 300 mg/kg dose showing marked antipyretic activity, though less effective than paracetamol. **Conclusion:** The study confirms that the ethanolic extract of *Punica granatum* leaves possesses significant antipyretic and antioxidant activities, supporting its traditional use in fever management. The observed effects may be attributed to its rich phenolic and flavonoid content, warranting further pharmacological investigation.

KEYWORDS: *Punica granatum*, antipyretic activity, Brewer's yeast, phytochemical analysis, antioxidant activity, flavonoids.

1. INTRODUCTION

In addition to helping to preserve cultural customs and biodiversity, traditional knowledge about medicinal plants and how indigenous societies use them is beneficial for community healthcare and future medication development. Synthetic tropical therapies have several adverse effects and are too expensive for most people to afford. Without scientific proof, plants that grow nearby are used to solve this **issue (Gurib-Fakim, 2006).** *Punica granatum* (pomegranate) have been revered in traditional medicine for centuries, not only as a nutritional fruit but also as a versatile therapeutic plant **(Scalici et al., 2023).** It possesses a remarkable range of pharmacological activities, including anti-inflammatory, antimicrobial, antioxidant, and antipyretic effects, which have been documented across various cultures and traditional systems. The therapeutic potential of pomegranate is attributed to its rich phytochemical profile, which varies across plant parts—leaves, fruits, seeds, and bark—containing phenolic acids, flavonoids, alkaloids, tannins, and glycosides **(Gómez et al., 2013).** Pyrexia or fever is usually caused as a secondary impact of infection, tissue damage, inflammation, graft rejection and malignancy or other diseased states. The body by its natural defense mechanism creates an environment where infectious agent or damaged tissue cannot survive. Pyrexia or fever is usually caused as a secondary impact of infection, tissue damage, inflammation, graft rejection and malignancy or other diseased states **(Jan & Khan.**

2016). The body by its natural defense mechanism creates an environment where infectious agent or damaged tissue cannot survive **(Saper and Breder, 1994**). Therefore this study validates the traditional use of *Punica granatum* leaves for fever management and highlights their potential as a natural antipyretic, supporting further phytochemical and pharmacological research.

2. MATERIALS AND METHODS

2.1 Plant collection

The botanical identity and authenticity of the selected traditional medicinal plant, Punica granatum were confirmed by a qualified plant taxonomist. Authentication ensured the purity and correct identification of the plant material prior to its use in experimental or therapeutic applications **(López et al., 2020)**

2.2 Extraction process

The extraction of the plant material in this study was performed using the Soxhlet apparatus, employing the continuous hot percolation method to ensure efficient recovery of phytochemicals. Initially, the Soxhlet thimble was loaded with powdered *Punica granatum* leaves **(Joshi et al., 2013).**

2.3 Phytochemical investigation

Using a thorough qualitative phytochemical analysis, the experiment was conducted to determine if certain phytoconstituents were present or absent. Medical reactions to tests were measured by the precipitate formation or the color intensity. Standard operating procedures were applied **(Goyal et al., 2012).**

2.4 Determination of Total Phenolic Content

The total phenolic content of *Punica granatum* extracts was quantified using the Folin–Ciocalteu colorimetric assay. The total phenolic content in the plant extract was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract **(Babbar et al., 2011).**

2.5 Total Flavonoid Content

The total flavonoid content of Punica granatum extract was determined using the aluminum chloride colorimetric method. The total flavonoid content of the extract was then calculated from this standard curve and expressed as milligrams of Rutin equivalent per gram of dry extract (mg RE/g) **(Ghafar et al., 2017).**

2.5 DPPH Radical Scavenging Assay

The antioxidant potential of Punica granatum extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. The decrease in absorbance of DPPH in the presence of the extract, compared to the control, was used to calculate the free radical scavenging activity. This method allowed for quantitative assessment of the extract's antioxidant capacity **(Xie & Schaich, 2014).**

2.6 Acute oral toxicity

The acute oral toxicity of the ethanolic extract of *Punica granatum* was evaluated according to OECD Guideline 423 using a stepwise procedure. Groups of three animals of the same sex received oral doses of 5, 50, 300, and 2000 mg/kg body weight, with 50 mg/kg as the starting dose. Animals were observed for signs of toxicity and mortality immediately after dosing, during the first 24 hours, and daily for 14 days. Parameters assessed included behavioral changes, physical appearance, and neurological symptoms **(Bhandary et al., 2013).**

2.7 Experimental Work

All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC). Wistar rats of either sex (200 ± 50 g) were used and housed in groups of six under standard laboratory conditions (22 ± 2 °C, normal light–dark cycle) after acclimatization. Animals were fed a standard pellet diet (Golden Feed, New Delhi) and had free access to water ad libitum. Proper hygiene and regular cage cleaning were maintained to minimize stress and ensure reliable results.

6.8 *In-vivo* Antipyretic Activity

Brewer's Yeast Induced Pyrexia in Rats

The antipyretic activity of *Punica granatum* extract was evaluated in male Wistar rats divided into five groups (n = 6). Pyrexia was induced by subcutaneous injection of Brewer's yeast (1 ml/kg), except in the normal control group. Rectal temperature was recorded before yeast administration and after 18 hours to confirm fever induction. Following treatment, temperatures were measured at 1, 2, 3, and 4 hours. The antipyretic effect of the extract was assessed by comparing temperature changes with the standard drug, paracetamol Following this.

Group I (Normal Control): Received vehicle (1% v/v Tween 80 in distilled water).
Group II (Yeast Control): Received Brewer's yeast (1 ml/kg.) only, without treatment.
Group III (Standard Drug): Received paracetamol at a dose of 150 mg/kg orally.
Group IV (Test Group I): Received *Punica granatum* extract at 200 mg/kg orally.
Group V (Test Group II): Received *Punica granatum* extract at 300 mg/kg orally.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield

Table 1: Percentage yield.

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1.	*Punica granatum*	Ethanol	365	24. 5	6.71 %
2.		Pet ether	250	4.60	1.84 %

3.2 Preliminary Phytochemical study

Table 2: Phytochemical testing of the extract of methanol.

Sr. no.	Experiment	Presence or absence of phytochemical test	
		Ethanolic Extract	Pet. Ether extract
1.	Alkaloids	Present	Absent
2.	Glycoside	Present	Absent
3.	Carbohydrates	Present	Absent
4.	Flavonoids	Present	Present
5.	Tannin and Phenolic Compounds	Absent	Absent
6.	Saponin	Absent	Absent
7.	Test for Triterpenoids and Steroids	Absent	Absent

3.3 Total Phenolic content (TPC) estimation

Table 3: Standard table for Gallic acid.

S. No.	Concentration (µg/ml)	Absorbance
1.	15	0.156
2.	25	0.187
3.	35	0.218
4.	45	0.246
5.	55	0.283

Table 4: Total Phenolic Content in extract of *Punica Granatum*.

S. No.	Concentration (µg/ml)	Absorbance
1.	0.168	29.5 mg/gm
2.	0.194	
3.	0.229	

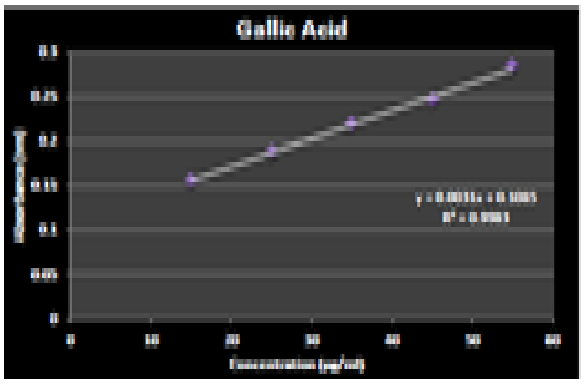


Figure 1: Represent standard curve of Gallic acid.

3.4 Total Flavonoids content (TFC) estimation

Table 5: Standard table for Rutin of *Punica granatum*.

S. No.	Concentration (µg/ml)	Absorbance
1.	15	0.119
2.	25	0.148
3.	35	0.176
4.	45	0.212
5.	55	0.243

Table 6: Total Flavonoid Content in extract.

S. No.	Absorbance(nm)	TFC in mg/gm equivalent of Rutin
1.	0.114	27.6 mg/gm
2.	0.154	
3.	0.191	

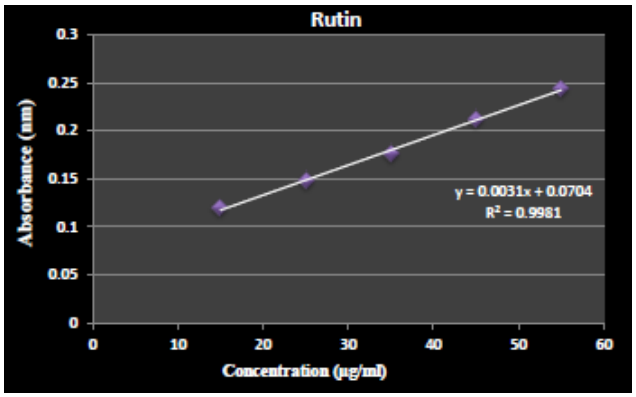


Figure 2: Represent standard curve of Rutin.

3.5: *In vitro* Antioxidant Assays.

Table 7: DPPH radical scavenging activity of Std. Ascorbic acid.

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.491	50.702
40	0.439	55.923
60	0.380	61.847
80	0.269	72.991
100	0.187	81.224
Control 0.996	**IC50 22.81**	

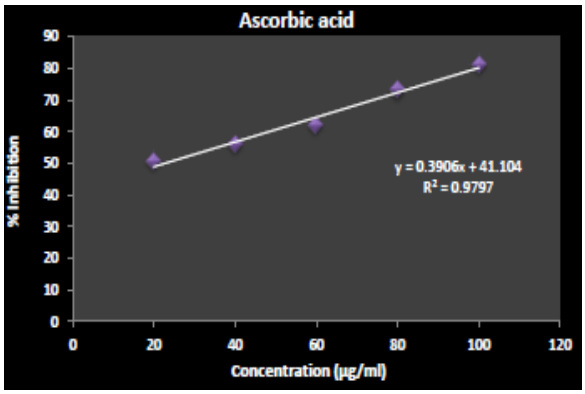
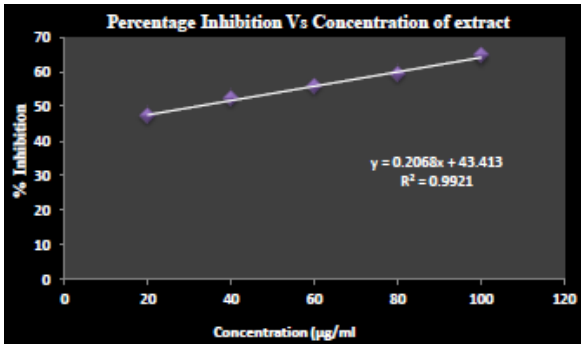


Table 8: DPPH radical scavenging activity of methanol extract of *Punica granatum*.

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.525	47.289
40	0.474	52.409
60	0.442	55.622
80	0.406	59.236
100	0.353	64.558
Control 0.996		**IC50 48.73**



3.6 *In vivo* acute oral toxicity (OECD 423)

Table 9: Parameter of acute oral toxicity, extract dose 300mg/kg per body weight.

Extract Dose 50 mg/kg							
Tc. Parameter	**1 Day**	**3 Day**	**5 Day**	**7 Day**	**9 Day**	**11 Day**	**14 Day**
Body weight	165 gm	170 gm	174 gm	176 gm	180 gm	182gm	185 gm
Skin & fur	Normal	Red color	Red color	Acute redness	Acute redness	Acute redness	Normal
Eye	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Stool	Normal	Hard	Hard	Hard	Hard	Hard	Hard
Urination	Normal	Normal	yellowish	Colour change	yellowish	Normal	Normal
Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Behaviour	Normal	Normal	aggressive	aggressive	aggressive	Normal	Normal

3.7 Brewer's Yeast Induced Pyrexia in Rats

Table 10: **Brewer's Yeast Induced Pyrexia in Rats.**

Rectal temperature (°C)					
Groups	**18 h after Yeast administration**		**Temperature after treatment**		
	0 h	**1 h**	**2h**	**3h**	4 h
Normal control	37.32±0.1	37.59±0.2	37.76±0.1	37.62±0.2	37.55±0.1
Yeast induced pyrexia group	42.78±0.1	42.09±0.3	42.05±0.2	42.94±0.1	42.81±0.3
Paracetamol (150mg/kg bw)	39.78±0.2	39.49±0.2	37.66±0.2	36.55±0.1	36.12±0.2
Punica granatum extract (200mg/kg)	42.29±0.1	41.41±0.3	41.28±0.1	41.40±0.2	41.04±0.3
Punica granatum extract(300mg/kg)	40.86±0.1	40.06±0.1	38.63±0.2	38.99±0.1	37.18±0.2

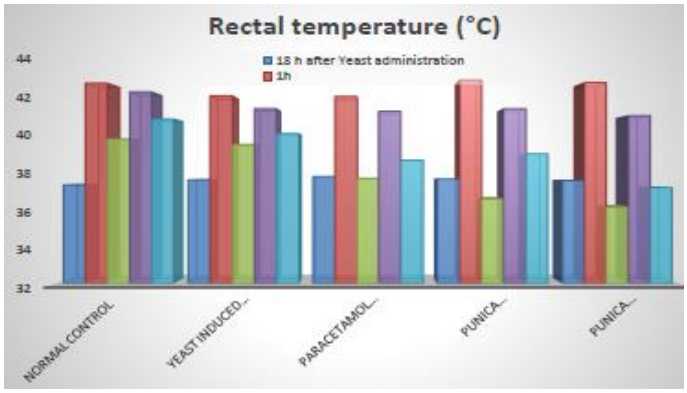


Figure 2: **Brewer's Yeast Induced Pyrexia in Rats.**

DISCUSSION

The study evaluates the antipyretic efficacy of *Punica granatum* leaves using induced pyrexia models in rats, alongside their phytochemical composition and antioxidant activity. Ethanol proved to be a more effective solvent than petroleum ether for extracting bioactive compounds, yielding 6.71% compared to 1.84%. The ethanolic extract contained significant amounts of carbohydrates, alkaloids, tannins, phenolics, flavonoids, and glycosides, but lacked triterpenoids, steroids, saponins, and cardiac glycosides **(Guyton et al., 1967).** Notably, the extract displayed high levels of total phenolics (29.5 mg/g) and total flavonoids (27.6 mg/g), contributing to its pharmacological effects. *In vitro* antioxidant activity demonstrated significant free-radical scavenging with an IC_{50} of 48.73 µg/ml **(Mishra et al., 2012).** *In vivo* tests confirmed that the extract effectively reduced febrile temperatures in a dosage-dependent manner, especially at 300 mg/kg, affirming its traditional use in fever management, though it was less potent than paracetamol **(Jethani et al., 2011).**

1. CONCLUSION

The findings demonstrate that the ethanolic extract of *Punica granatum* leaves possesses significant antipyretic activity, supported by its rich phytochemical profile and strong antioxidant potential. The extract effectively reduced fever in a dose-dependent manner, particularly at 300 mg/kg, although its efficacy was lower than that of paracetamol. These results validate its traditional use in fever management and suggest that the antipyretic effect may be attributed to the presence of phenolics and flavonoids with antioxidant properties.

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