Ethno-Pharmacological, Alpha-glucosidase and Biological activities of fruit's peel aqueous extract of *Punica granatum* L.

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Abstract

Since the beginning of human civilization, medicinal plants are utilized for health benefits. However, due to modernization in science and technology, such plants are re-evaluated for their proficiency to treat diseases from different aspects. The dried fruit peel is used as an asthmatic, and cough suppressant and in throat problems by the local community. In the same way, the aqueous peel extract (APE) of Punica granatum L. was used to screen for Alpha-glucosidase, and pharmacological and biological activities, via; in vivo and in vitro approaches. The antipyretic effect of the aqueous peel extract (APE) of Punica granatum was taken under consideration by using albino mice via; in vivo. Similarly, the APE of Punica granatum L. was given orally at concentrations of 50, 100 and 150mg/kg to the mice model to check the fever reduction potential, induced by brewer's yeast, and paracetamol was used as standard. The body temperature of mice was measured rectally using a digital thermometer. All the concentrations were found effective but 150mg/kg was very good with results of 38.8±.15a after 18 hours of fever induce by brewer's yeast. The APE of Punica granatum possesses dose-dependent alpha-glucosidase activity with 500 concentration showed results of 69.1±3.4 with IC50 of 410.37. Additionally, the selected bacterial and fungal strains were positively inhibited by APE with 2mg/mL concentration. It was concluded that the Punica granatum has hidden chemical constituents, which are to be isolated to treat simple to chronic diseases.

Key words: phytochemicals, antipyretic, medicinal plant, pomegranate, pharmacological activities.

Introduction

All organisms respond and perform well, under normal body temperature is normal i.e. 37^{o}C, however, any disruption from normal level disturb the normal physiological and biochemical traits of the body [1, 2]. Among all, pyrexia is the condition in which the temperature is above (above 41^{o}C) normal level, the condition is refer as hyperpyrexia [3]. This increase in body temperature occur due to imbalance heat regulating system of the body. The common and possible reason could be the white blood cells under pressure of toxin directly produce pyrogen in the skin hypothallus interiorly, elevation in body temperature occurs. These results occurred due to some infection in the body [4].

Plants have always been used for medicinal purposes since the prehistoric period [5]. These herbal medicinal plants play a significant role in developing countries for influential therapeutic agents and economy [6]. Similarly, the representation of such rich customs, traditional and herbal biodiversity offers a unique opportunities to the researchers of drug discovery in India, China, Arabic and Pakistan [7]. Among all, Pakistan, is a massive repository to the medicinal plant and medical treatments and about 22,000 medicinal plant species are in record nowadays [8]. Besides other plants *Punica granatum* L. (pomegranate), is of great importance which is a shrub, mostly available in the Mediterranean Sea region. Recently, the fruits of *Punica granatum* have gained the substantial attention of the researchers due to their promising biological activities, like, anti-inflammatory, antibacterial [9], antidiarrheal [10], immune modulatory, antitumor, wound healing and antifungal that have been attributed to various constituents of seeds, bark, juice, pericarp, and leaf of this tree across the globe [11, 12]. Phytochwmical investigation revealed certain phenolic compounds have been documented that possesses a number of prophylactic and therapeutic utilities against various pathological infections as well as non-infectious disorders [13].

Punica granatum L. locally known as Pomegranate or Anar is a member of family Punicaceae, deciduous shrub or small tree, grow up to 1.8-4.6m long. The fruits of pomegranate itself possess various therapeutically relevant constituents and many of these constituents are effective in treatments of various diseases [14]. The seeds are crunchy having acidic pulp enclosed in a membranous skin, said to be cooling and blood producing agents [15]. Recently, *Punica granatum* (Anar) have been studied for various therapeutic and essential uses including,

bacterial infection, anti-arthritic, anti-inflammatory, atherosclerosis or arteriosclerosis, immunomodulation, antioxidants, fungal infection, periodontal disease, parasitic infection, skin disorder, food poisoning and gastro-intestinal infection [16]. There have even been much preliminary toxicity studies and approved data in mice/rats (rodents) to report *Punica granatum* as non-toxic at all concentrations/doses, especially at a high. There are various compounds present in *Punica granatum* fruit, among these compounds, the most therapeutic phytochemicals are polyphenolics, flavonoids, alkaloids, ellagic, as well as gallic acid. The peel contains punicalagin, alkaloids, tannins (approximately 20%), granatins A, granatins B, gallagyldilacton, tellimagrandin I, and corilagin, casuarinin, pedunculagin, having different potential [9]. Compounds like granatins A and B, and punicalagin or punicalin are essential compounds having antimicrobial activities [17]. The potential activities of compounds carried out in a study showed that phenolic compounds relatively at high dose contains the therapeutic potential for antifungal activity [16]. The literature review of *Punica granatum* suggest that all parts of the plant has immense potential to cure a number of diseases. The current study was undertaken to evaluate the antipyretic and alpha glucosidase potentials of the peel extract of the plant.

MATERIALS AND METHODS

Sample collection and processing

Fruits of the *Punica granatum* L. were collected locally and peel was separated and dried in the oven properly. Dried sample was crushed and distal water was added to it and kept in shaking incubator for three days. Filtrate was taken and water was evaporated using water bath. The semi-solid material called crude drug was stored in cool place for further use.

Qualitative Phytochemical analysis

Alkaloids, carbohydrates, flavonoids, phenols, tanins, quinine and saponines were qualitatively analyzed for aqueous extract of *Punica granatum* following standers protocols [18].

Antipyretic activity

Antipyretic activity was carried out using aqueous extract of *Punica granatum* with doses of 50, 100 and 150mg/kg body weight of experimental animal. Albino mice weighing 150-200gms of

both sexes (male and females) were used in experimental assay. During the experiment time animals have free excess to standard food and water.

Brewer's Yeast induced pyrexia

Brewer's yeast induced pyrexia test was taken under consideration for the determination of antipyretic activity [19]. On the same, five different groups were made. Group 1 was injected with saline which served as control group. Brewer's yeast was injected to produce pyrexia in group 2nd 3rd, 4th, and 5th. Similarly, rectal temperature of all mice were taken under consideration before the injection of the yeast. Standard drug Paracetamol was used, percent reduction of the activity was calculated by using formula;

$$\% \text{ reduction} = B - C_n / B - A \times 100$$

Where, B = temperature after pyrexia induction, C_n = temperature after 1, 2, 3, 4 and 5 hours, and A = normal t body temperature.

RESULTS

In the present study, ethno-pharmacological, Alpha-glucosidase and biological activities of peel extract of fruit of *Punica granatum* L. was carried out using aqueous extract (Table 1). The phytochemical results showed that alkaloids, carbohydrates, glycosides, phenols, volatile oils and tannin were found in aqueous extract, while flavonoid and tannin test was found negative.

Table.1 Qualitative Phytochemical analysis of peel of *Punica granatum* L. of aqueous extract.

S.No	Phytochemical test	Presence/absence
1	Alkaloid	+
2	Carbohydrate	+
3	Saponins	+
4	Flavonoid	-
5	Phenol,	+
6	Quinine	+
7	Tannin	-

Note; '+ and -' means presence and absence, respectively.

Anti-pyretic activity of the *Punica granatum* L**.** aqueous extract was carried out by the brewer's yeast induced pyrexia in albino mice (Table 2). Group 1 was injected with saline which served as control group. Brewer's yeast was injected to produce pyrexia in group 2nd 3rd, 4th, and 5th. After 18hrs of the induce pyrexia temperature was checked for all groups and mean was noted which was 39.23±38b, 39.433±1ab, 39.23±14ab and 38.8±15a respectively. Group 2 mice were injected with paracetamol (10mg/kg) as standard and group 3, 4, and 5 were injected with plant extract at a dose of 50, 100 and 150mg/kg, respectively. Then consecutively after 1hr to till 4th hours the data was noted (**Table 2**). The results indicated gradual decrease in body temperature. The reduction in pyrexia was slow with low dose of 50mg/kg (38.20±11a) while it was speedy with high dose of 150mg/kg (37.7±5a). It means the disease is controlled dose dependently.

Table.2 Antipyretic activity aqueous extract of peel of *Punica granatum* L.

Groups	**Dose**	**Normal temp.**	**After 18h**	**After 1h**	**After 2h**	**After 3h**	**After 4 h**
control	…	37.467±0.^{a}	37.86±.08^{b}	37.73±.0^{b}	37.36±0.0^{b}	37.02±.06^{b}	37.76±.12^{b}
standard	10mg/kg	37.5±.15^{a}	39.23±38^{ab}	38.733±.^{a}	38.46±.2a	38.133±.2^{a}	38±.25^{a}
Aqueous	50mg/kg	37.266±.1^{a}	39.433±.1a^{b}	38.96±.14^{a}	38.6±. 15a	38.2±.11^{a}	38.2±.11^{a}
	100mg/kg	37.166±.1^{a}	39.23±.14^{ab}	38.6±.28^{a}	38.06±0.1^{a}	37.83±.29^{a}	37.76±.12 ^{a}
	150mg/kg	37.36±.17^{a}	38.8±.15^{a}	38.46±.1^{a}	38.13±1.0^{a}	38.23±.39^{a}	37.7±.05^{a}

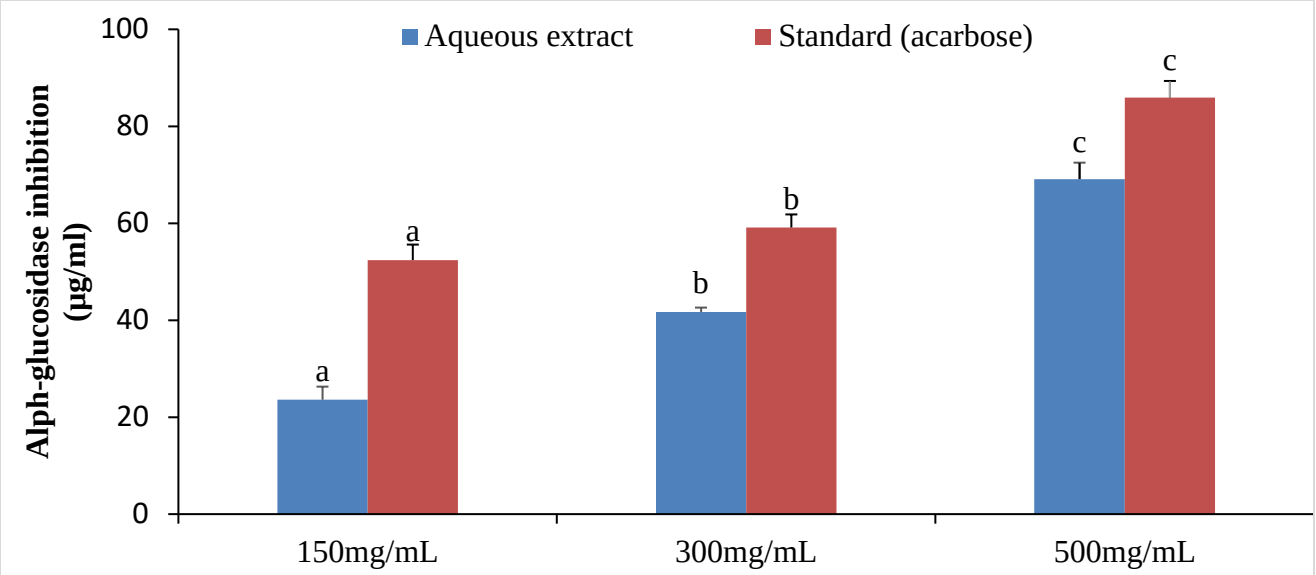

Figure 1 Alpha- glucosidase inhibition activity of aqueous extract of peel of *Punica granatum* L.

The aqueous extract of peel of *Punica granatum* possess dose dependent alpha glucosidase activity. Different concentrations were used to (150, 300 and 500) which gave good results as compared to the standard drug acarbose which shows 85.95±3.01 (Fig. 2)result at 500 concentration with IC^{50} of 155.13(Figure 2). The extract with 500 concentration. showed result of 69.1±3.4 () with IC^{50} of 410.37, indicating that the plant peel has inhibition potential against alpha glucosidase.

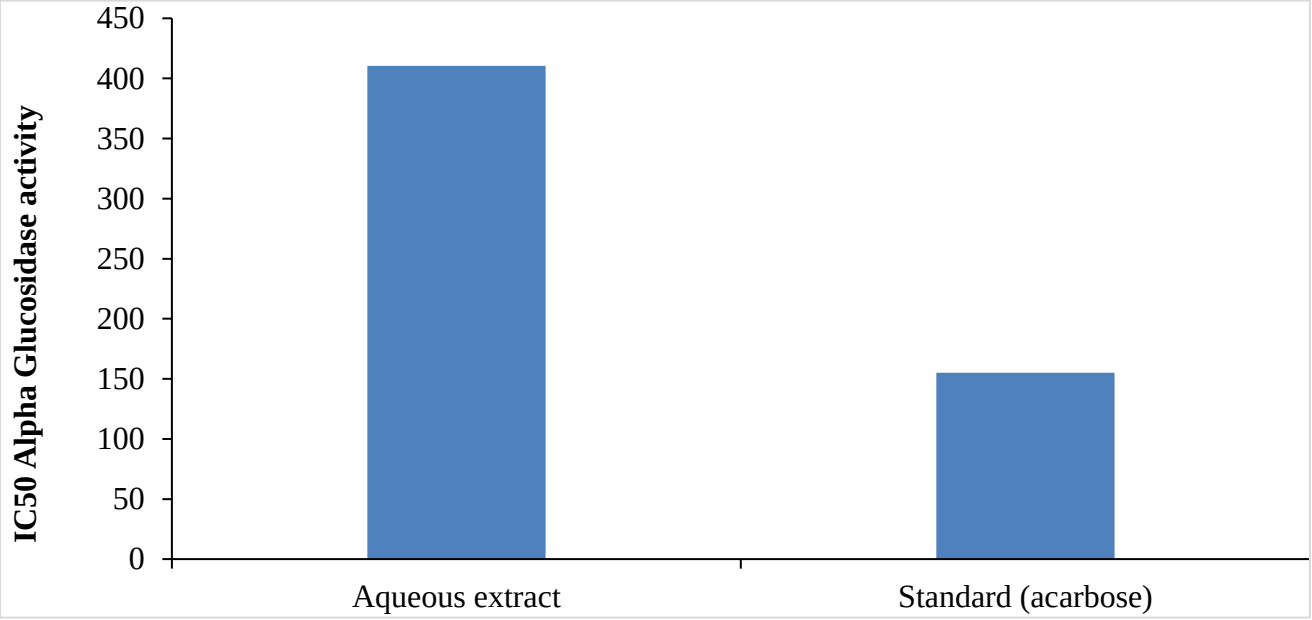

Figure 2. IC^{50} values of Alpha Glucosidase activity of aqueous extract of peel of *Punica granatum*

The antibacterial activity of aqueous extract of dried peel of Punica granatum showed positive inhibition against tested bacterial strains. Two concentrations were used for activity where high concentration (2mg/ml) showed good results as compared to low concentration (1mg/mL). Streptomycin were used as standard (Figure 3).

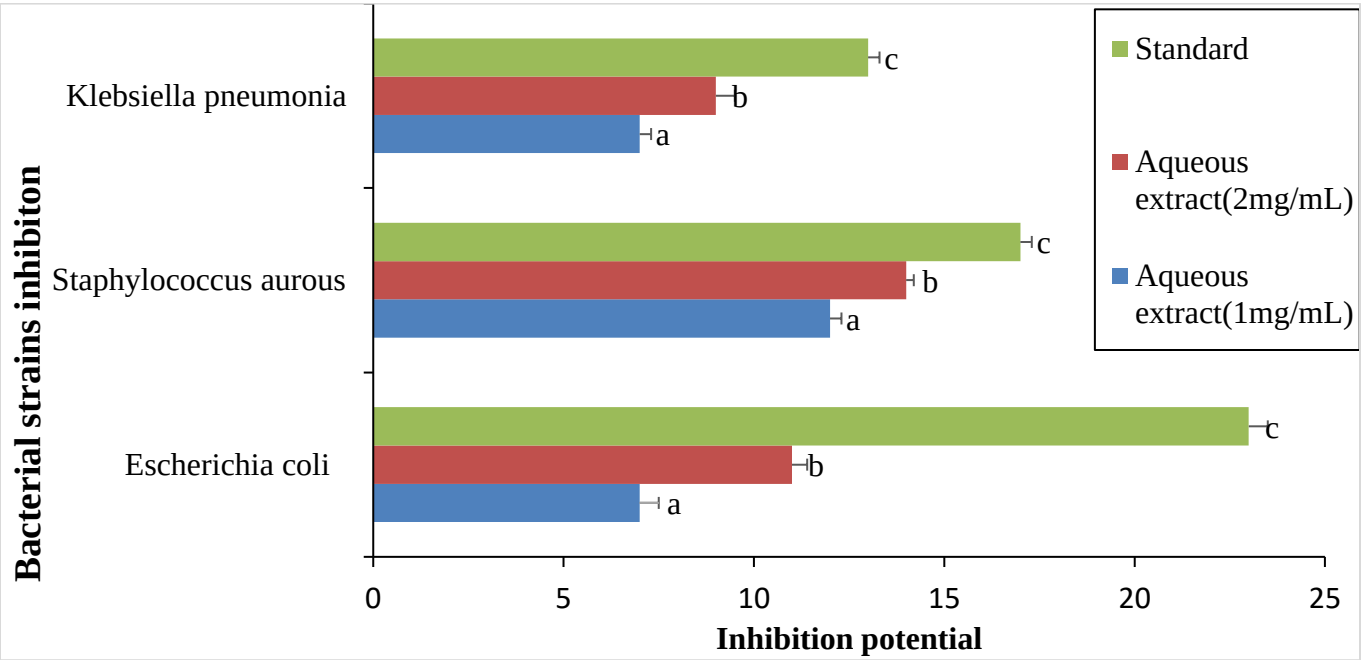

Figure 3 Antibacterial activity of peel extract of *Punica granatum* L.

The antifungal activity of aqueous extract of dried peel of Punica granatum showed mild activity against selected fungal strains as compared to the standard used (Figure 4). The high concentration of extract (2mg/ml) inhibited the fungal organisms *Aspergillus flavus* and *Aspergillus niger* with 18.1±0.9 and 19.1±1.0mm zones respectively.

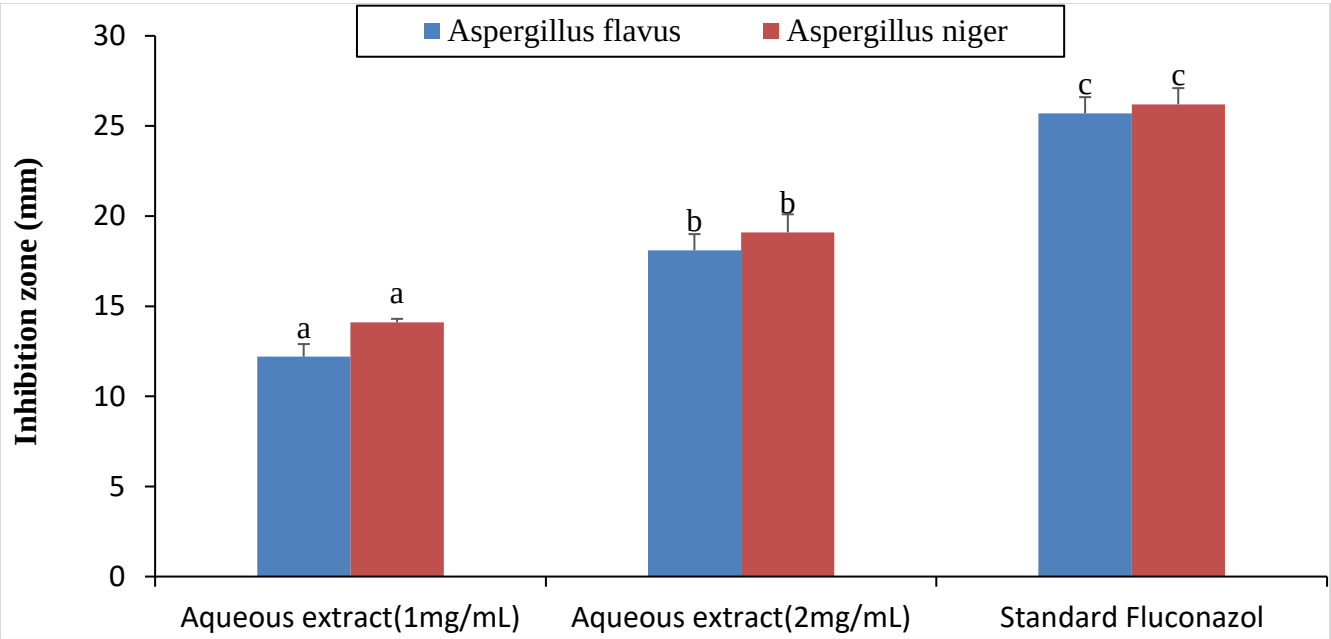

Figure 4 Antifungal activity of peel extract of *Punica granatum* L.

Discussion

In the current study the qualitative phytochemical investigation and pharmacological potential were taken under consideration for aqueous extract of *Punica granatum*. Qualitative phytochemical screening revealed that the aqueous extract of pomegranate peel contained alkaloids, carbohydrates, saponins, flavonoids, phenolic and tannins, while quinine was not detected. Phytochemicals present in plant samples are known to be biologically active compounds that exhibit diverse activities such as antioxidant, antibacterial, anticancer, antifungal, and antidiabetic [20, 21]. Similarly, plant constituents such as alkaloids, flavonoids, phenols, steroids, saponins, tannins and triterpenes vary due to many environmental factors such as climate, altitude and rainfall [22]. Phytochemicals present in plants provide a plethora of medicinal properties [23]. Phenolic compounds found in many plants primarily provide antioxidants, analgesics and antipyretics [24, 25].

The results of this work showed that the plant had a significant antipyretic effect on Saccharomyces cerevisiae-induced fever in mice, and its inhibitory effect was comparable to that of the standard drug paracetamol, with an observed value of 38±.25a after the 4th hour of administration. The highest significant value at 150mg/kg was 37.7±.05a. Saccharomyces cerevisiae releases endogenous substances through cyclooxygenase and prostaglandin

biosynthesis, as well as rectal temperature induction by some other temperature mediators such as arachidonic acid [26]. Flavonoids, terpenoids, and steroids found in plants inhibit the production of prostaglandins, which inhibit rectal temperature induced by Saccharomyces cerevisiae [27].

A fever can be the result of an infection, or it can be one of the screams of tissue damage, inflammation, graft rejection, or another disease state [27]. Antipyretics are drugs that lower an elevated body temperature. Thermoregulation requires a delicate balance between heat production and heat loss, and the hypothalamus regulates the set point for maintaining body temperature. This set point is elevated during fever, whereas medications such as paracetamol do not affect body temperature when elevated due to factors such as exercise or increased ambient temperature [28, 29]. Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermo-regulatory center at a lower temperature [30]. So inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of acetylsalicylic acid (Rawlins et al 2010) (Akio M). Inhibition of any of these mediators may bring about antipyretic [31].

Conclusion

The peel of *Punica granatum* L. is used as a precious medicinal plant since long for its curative purposes. The present study revealed that the plant peel has certain bioactive chemical constituents which have curative properties that are responsible for the curation of the pyrexia in mice. On the same way, for the conformation of some precious pharmacological activities like, antimicrobial (antifungal and antibacterial) have been realized. There are still too many diseases in the lap of nature that remain unexplored and need to be studied. This analysis is, therefore, merely an initiation to include herbal source choices for the treatment of diverse types of diseases.

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Competing interest

Authors declared that don't have any competing interest.

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