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Phytochemical and Anti-microbial Analysis of *Punica granatum* Peel and Rind extract

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Abstract: ***Punica granatum L. (Pomegranate) is one of the imperative fruit of that has been used since olden days for its medicinal features. It is extensively reported that that plant parts possess antiviral, antioxidant, antiproliferative activities. The aim of the study was to validate biomolecules from Punica granatum peel and rind. Phytochemical analyses of the metabolites were done using standard biochemical procedures. The ethanolic extract showed higher antioxidant and antimicrobial activities. The experimental data obtained show that the Qualitative done, forms a suitable approach to study the potential benefits of Punica granatum peel and rind.***

Keywords: Punica granatum, Flavonoids, Antioxidant Activity.

I. INTRODUCTION

Punica granatum is a small tree predominantly present in Middle East and Mediterranean. It is utilized in traditional medications for the treatment of diarrhea and microbial infections[1]. The extracts of *P. granatum* and its compounds have shown high antioxidant, anti-inflammatory, anticarcinogenic, antimicrobial activity. Entire plant parts have been found to possess innumerable phytochemicals amidst of which peel has surpassing phytochemical compounds [2]

The active ingredient, Punicalagin extracted from Pomegranate has been found to have antimicrobial activity against the *Candida albicans* [3]. Antimicrobial activities against food borne pathogens have also been reported [4].

The contemporary research was done to meticulously report the phytochemical profile and antifungal potential of *Punica granatum* peel extract.

II. MATERIALS AND METHODS

A. Raw Material Preparation

Fresh pomegranate peels and rind were collected from market. Peels were cut into pieces and dried under shade for a period of 10 days at room temperature. Dried peels were pulverized and stored in room temperature [5]. The quantity of dried powder was measured to be 108.50g. Powders were stored in air tight plastic containers and stored at room temperature until used for extraction [6].

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B. Raw Material Processing

About 100 g powders was weighed and extracted by cold maceration method with 100mL of 80% alcohol solution. Powder was homogenized with methanol and ethanol in which filtrate and residue were collected [7]. The extraction was conducted for 3hr in orbital shaker at 140rpm. The extraction obtained by filtration was subjected to liquid-liquid extraction process using chloroform and chloroform- methanol and finally aqueous sample were collected. The extracts were lyophilized prior to phytochemical analysis and anti-microbial assay [8].

III. PHYTOCHEMICALS ANALYSIS

A. Alkaloids:

Two drops of Hager's reagent was added to the 2 ml of the extract along the walls of the test tube. Formation of yellow precipitate confirms the presence of alkaloids [9].

B. Flavanoids:

The extract 25mg was dissolved in lead acetate solution and to this 100ml NaoH solution was added. Existence of flavonoids was indicated by the formation of yellow coloured precipitate[10].

C. Phenolic Compunds & Tannins & Cardinolids:

25mg of the sample was solubilized in 5 ml of sterile double distilled water. Three drops of 0.1% freshly prepared ferric chloride solution was added. Formation of dark blue black color solution indicated the presence of phenolic compound, tannins & cardinolids [10].

D. Test For Terpenoids

To 1ml of extract solution 2ml chloroform and also few drops 1% sulphuric acid freshly prepared was added. It was shaken well and allowed to stand for 10 mins. Reddish brown coloration at interface implies the incidence of terpenoids. [9]

E. Test For Saponins- Froth test

To 1ml of test sample water was added. It is shaken well. The Stable froth which stands atleast for 2mins indicates the presence of saponins[9].

F. Test For Carbhohydrates - Molish Test

Two drops of molish reagent was added to 2 ml of plant extract and shaken well. Concentrated sulphuric acid was added gradually along the sides of test tube till the formation of purple ring [9].

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IV. ANTI-MICROBIAL ASSAY

Based on the results of phytochemical analysis, the antimicrobial assay was done for ethanol, methanol and aqueous fractions [10]. The test sample for the antimicrobial studies was collected from Bhavani river at Sathyamangalam in a sterilized can. The crude extracts were prepared in different concentrations (200, 300, 400, 500, 600 mg/ml) and was added to1 Litre of river water serially diluted. The pH was adjusted to 6.8 in all the test samples.The serially diluted samples were plated onto the nutrient agar and incubated at 37°C. The plates were observed after 24 hours and compared with the control plate [11].

V. STATSITICAL ANALYSIS

The experiments are done in triplicates. Statistical analysis was done using Minitab 17 Statistical software package. Statistical analysis was done by Analysis of Variance. Statistical significance was determined at $p< 0.05$.

VI. RESULTS AND DISCUSSION

The phytochemical analyses of the samples were reported in table 1. The aqueous extract was reported to be the potent source of phytochemicals. The active ingredient of the pomegranate peel, Punicalagin was found to be significantly present in the ethanol fraction. The pomegranate peel and rind from the commercial juice shops and juice manufacturing industries can be reutilized for the extraction of therapeutically important compounds[12].

	Methanol	Ethanol	Chloroform-Methanol	Chloroform	Aqueous
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	-	+
Phenolic compounds	+	+	-	-	+
Tannins	+	+	-	-	+
Terpenoids	+	+	+	-	+
Saponins	-	+	+	+	-
Cardinolids	+	+	-	-	+
Carbohydrates	+	+	+	+	+
Punicalagin	-	+	-	-	-
+ Present - Absent					

Table 1 Results of phytochemical test

The reduction in microbial percentage in the aqueous, methanol and ethanol extracts are graphically represented in figure 1, 2 and 3. Among the three samples, ethanol extract shows potent antimicrobial activity. The potential effect of pomegranate extract against pathogens present in tap water has been reported [13].

The water sample treated with ethanol extract shows significant antimicrobial activity compared with the methanol and aqueous extracts. Water sample treated with 500mg/l concentration gave 45%microbial reduction in10 hours than 400 mg/l dosage. 100% activity of the ethanol extract was observed at 20th hour treatment with 500 mg/l dosage and 400mg/l treated sample shows 95% microbial reduction at the same time. The other concentration and other extracts gave significantly less antimicrobial activity.

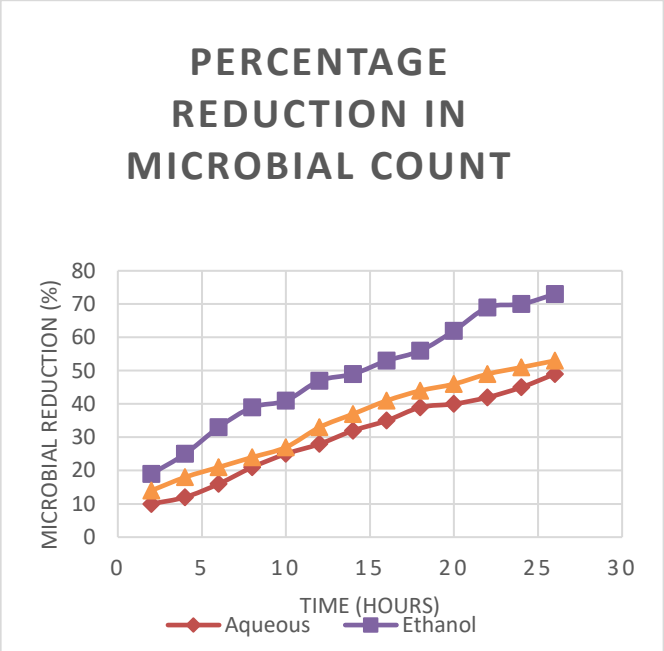

Figure 1: Percentage of microbial reduction in 300mg/l extract

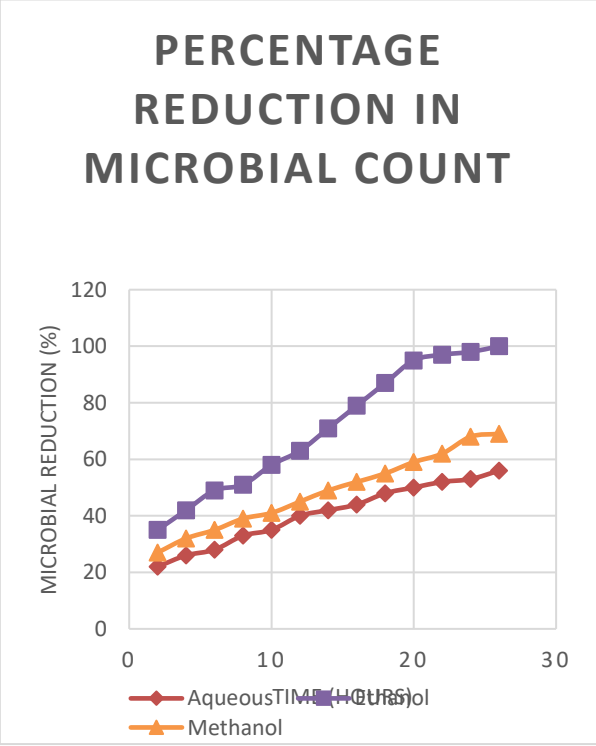

Figure 2: Percentage of microbial reduction in 400 mg/l extract

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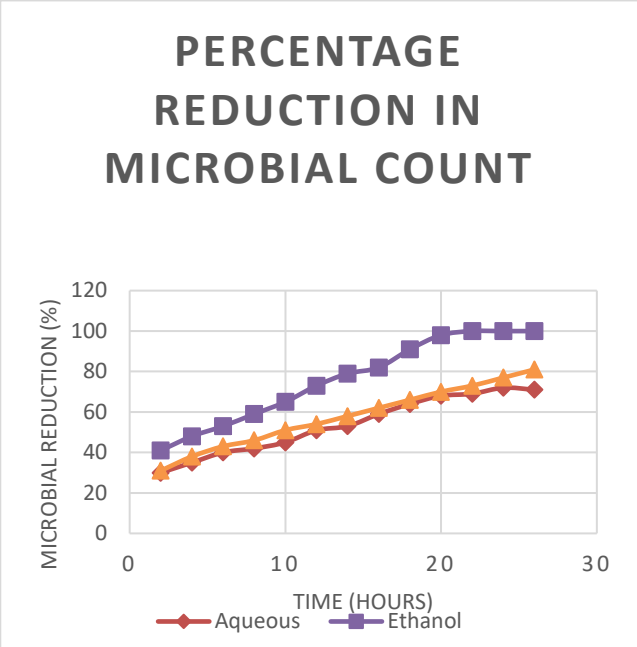

Figure 3: Percentage of microbial reduction in 500 mg/l extract

The concentration 500mg/l showing 100% antibacterial activity in 20 hrs treatment indicates the Minimum bactericidal concentration to be500mg/l.

VII. CONCLUSION

The current findings reveal that peel extract of *Punica granatum* is a potent reservoir of phytochemicals that can be extracted for commercial use. Sensible utilization of these compounds might not only help to overcome the deadly effects of synthetic drugs but also attest to be cost effective strategy in the countries of low economies. The percentage of microbial reduction in the extract treated river water signifies the use of the extract in the treatment of water.

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