

ANTIMICROBIAL ACTION OF PUNICA GRANATUM (POMEGRANATE) AND PASSIFLORA EDULIS (PASSION FRUIT) AGAINST ORGANISMS ISOLATED FROM INTESTINAL INFECTIONS –AN *IN VITRO* STUDY

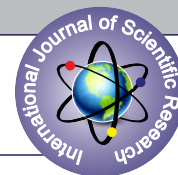
Microbiology

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ABSTRACT

Background and objectives: The overuse and misuse of antibiotics coupled with the intrinsic and acquired resistance of bacteria resulted in the emergence of multi-drug resistant strains. Pomegranate and Passion fruit are two fruits which have been documented to have significant antimicrobial activity against harmful organisms which colonize the gut. The present study evaluates the antimicrobial activity of *Punica granatum* and *Passiflora edulis* against organisms isolated from intestinal infections and compares the antimicrobial efficacy of the ethanolic and aqueous extracts of fruit rind of *Punica granatum* and leaves of *Passiflora edulis*. **Methods:** Fruit rind of *Punica granatum* and the fresh leaves of *Passiflora edulis* plants were dried, ground into powder and made into extracts. A total of 100 gastrointestinal pathogens were isolated and included in the study. The antibacterial efficacy of the aqueous and alcoholic extracts against 100 human faecal isolates was tested by employing Kirby Bauer disc diffusion technique. **Results:** Sensitivity to the ethanolic and aqueous extracts of *Punica granatum* were found to be maximum in *Staphylococcus aureus*. MRSA was sensitive to these extracts. Aqueous extract of *Punica granatum* were effective against a large number of organisms, however, better antimicrobial action was seen with the ethanolic extract of *Punica granatum*. No encouraging results were obtained with the extracts of *Passiflora edulis*. **Interpretation and conclusions:** Our study shows that purified forms of Pomegranate (*Punica granatum*) can be used as an adjunct in the treatment, in conjunction with antibiotics against intestinal pathogens and can open new areas of research.

KEYWORDS

Antibiotic resistance, Antimicrobial efficacy, Passion fruit, Pomegranate

INTRODUCTION

Punica granatum (pomegranate) is a fruit consumed worldwide. Several *in vitro* and *in vivo* studies have proved its antimicrobial, anti-inflammatory and antioxidant effects.^{1,2} Aqueous extracts of pomegranate peel have been shown to have antidiarrheal activity. In spite of all these beneficial effects, very few studies have been conducted on the antimicrobial activity of *P.granatum* peels against organisms isolated from human faecal samples.

Passiflora edulis (passion fruit) is reported to have antimicrobial activity due to 'Passicol', an active compound from the rind of the passion fruit.³ All parts of the *Passiflora* plant are of medicinal value. The leaves are documented to be a rich source of bioactive compounds.

The present study is aimed to investigate the antimicrobial action of the fruit peel of *P.granatum* and leaves of *P.edulis* against intestinal pathogens employing the ethanolic and aqueous extracts of both these plants and comparing their antimicrobial efficacy.

MATERIALS AND METHODS

A total of 100 gastrointestinal pathogens isolated from intestinal infections were included in the study. Samples were processed at the Microbiology department, Father Muller Medical College, Mangalore with a request for routine culture and sensitivity testing.

a) Collection of Plant Materials -

The pomegranate fruit peel was extracted with ethanol and aqueous extract. Fresh leaves of *P.edulis* were collected from the botanical garden of Ayurveda College, Moodabidri. The collected fruit peels and leaves were homogenized to a coarse powder and stored in air-tight bottles at 4°C.

b) Preparation of plant extracts

Fruit rind of *P.granatum* and the fresh leaves of the *P.edulis* plants were dried in the microwave oven in the Pharmacognosy department of Shree Devi College of Pharmacy, Mangalore for a total of ten days and then ground into a powder and stored in sterile glass containers at 4°C.

Ethanolic Extract was prepared by making a powder of the pomegranate peel. It was extracted by ethanol using Soxhlet apparatus⁴. 100gms of the powdered leaves and powdered stem were weighed and extracted with 150 ml ethanol using Soxhlet Extractor. When the solvent was heated up, vapours were liquefied and extract was collected, filter-sterilized and stored at -10°C.

Aqueous extract was prepared by soaking plant material in

chloroform-water mixture in the ratio 1:99 for 5-7 days by Maceration process⁵. The mixture was filtered after shaking and dried at a temperature of 100°C till dark brown in colour.

c) Source of Test Organisms

Bacterial isolates from clinical samples and standard strains were obtained from the Central Microbiology Laboratory of Father Muller Medical College, Mangalore.

ATCC Standard strains that were included in the study were as follows: ATCC *E.coli* – 25922, ATCC *Salmonella Typhi* – 19430, ATCC *Shigella sp.* – 23354, ATCC *Candida albicans* – 90028, ATCC *Klebsiella pneumoniae* – 700603, ATCC *Pseudomonas aeruginosa* – 27853, ATCC *Staphylococcus aureus* - 25923

d) Determination of Antibacterial Activity by Disc Diffusion Method

The antibacterial efficacy of the extracts was tested by Kirby Bauer disc diffusion technique. Bacterial isolates were adjusted to opacity of McFarland 0.5 opacity to obtain a lawn culture in Muller Hinton Agar for bacteria and SDA for *Candida*. Filter paper discs made from Whatman No. 1 filter paper were impregnated with appropriate concentration of herbal extracts were placed on the lawn culture. The zones of inhibition corresponding to the degree of sensitivity of the antimicrobial agent were measured after 24 hours of incubation. The results will be interpreted as follows:

6–9mm	1+ (Minimally activity)
10–13mm	2+ (Partially active)
14–17mm	3+ (Active)
>18mm	4+ (Very active)

The sensitivity of different bacterial strains to aqueous and alcoholic extracts of the peel of *Punica granatum* and leaves of *Passiflora edulis* were calculated by measuring the diameter (in millimeters) of inhibition zone. Bacteria showing a clear zone of inhibition > 6 mm were considered to be sensitive.

Ampicillin, Cefotaxime, Cefoperazone, Ciprofloxacin, Gentamicin and Imipenem were used as positive controls and zones of inhibition were compared with those of the extract.

RESULTS

Table 1 presents the details of the extracts employed in the study.

The sensitivity exhibited by the organisms to the ethanolic and aqueous extracts of leaves of *P.edulis* and fruit rind of *P.granatum* are

shown in Table 2.

It is observed that *Staphylococcus aureus* shows the highest sensitivity (100%) to both extracts of *P.granatum*.

From the results of the study, ethanolic and aqueous extracts of *Punica granatum* are found to have good antimicrobial activity against gastrointestinal pathogens compared to *Passiflora edulis*.

Table 1: Details of herbal products employed in the study
Table – 1

Common name	Scientific name	Part used	Phytochemicals	References
Passion fruit	<i>Passiflora edulis</i>	Leaves	Passicol, alkaloids and passifloricins	Nicholls JM et al ⁶
Pomegranate	<i>Punica granatum</i>	Fruit rind	Phenols, tannins, flavonoids and quionones,	Hasan et al ⁷

Table 2: Percentage of sensitivity elicited by various organisms in the study to the extracts.

Organisms	Total number of isolates	Sensitive to <i>P.edulis</i> ethanolic extract	Sensitive to <i>P.edulis</i> aqueous extract	Sensitive to <i>P.granatum</i> ethanolic extract	Sensitive to <i>P.granatum</i> aqueous extract
<i>E. coli</i>	46	2 (4.3%)	7 (15.2%)	24 (52%)	29 (63%)
<i>Salmonella Typhi</i>	19	-	-	14 (73.6%)	10 (52.6%)
<i>Klebsiella pneumoniae</i>	14	-	1 (7.1%)	4 (28.5%)	8 (57%)
<i>Shigella spp.</i>	5	-	1 (20%)	5 (100%)	4 (80%)
<i>Staphylococcus aureus</i>	5	-	-	5 (100%)	5 (100%)
<i>Pseudomonas aeruginosa</i>	5	-	-	4 (80%)	4 (80%)
<i>Candida albicans</i>	6	-	-	3 (50%)	2 (33.3%)

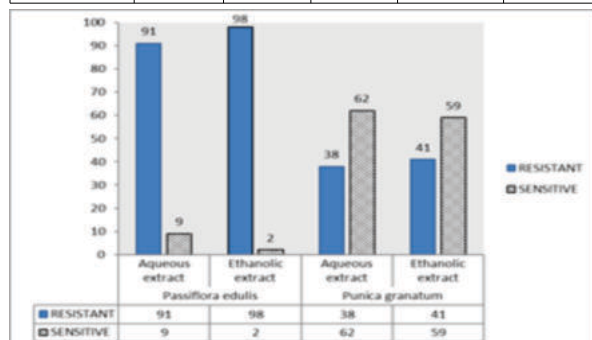


Figure 1: Bar graph of overall antimicrobial activity of the ethanolic and aqueous extracts of *P.edulis* and *P.granatum* against 100 isolates.

DISCUSSION

It is well documented that bacteria are acquiring various resistance mechanisms. The continuous search for newer antimicrobial agents, especially herbal extracts without any side effects, is one of the top areas of priority for researchers. Various investigators demonstrated the antimicrobial efficacy of extracts from different parts of the Pomegranate plant against gastrointestinal pathogens such as *S. aureus* and *E. coli*.^{8,9} Animal experiments and in-vitro studies have shown that the extracts of the Pomegranate plant including peels, flowers and seeds have anti-inflammatory actions on the gastrointestinal tract. Punicalagin isolated from the fruit peel of pomegranate was reported to have antimicrobial activity⁹. Phytochemical compounds such as alkaloids, phenol and flavonoids from this plant were found to have potent antibacterial activities according to certain investigators.¹⁰

Significant activity was seen with both aqueous and ethanolic extracts of *Punica granatum* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It was a remarkable finding that even MRSA was inhibited by the *P.granatum* peel extracts and *Pseudomonas aeruginosa*, which was resistant to some standard antibiotics, was sensitive to the ethanolic extract of *P.granatum*. Aqueous extracts of pomegranate peel

were highly effective against *E. coli* which was resistant to standard antibiotics. *Shigella*, *Salmonella* and *Candida albicans* were all inhibited to a considerable extent by the extracts of *P.granatum*. Analysis of the results of the present study revealed that aqueous and ethanolic extracts of *P.granatum* were sometimes better or equally effective to that of some standard antibiotics used in the experiment, which is in accordance with earlier findings.

However, the ethanolic and aqueous extracts of leaves of *Passiflora edulis* showed either no or minimal activity against all the isolates. These results are similar to those of previous studies.⁶ Hence, extracts from the leaves of *P.edulis* may not be considered to be effective against enteric pathogens. The extracts from other plant parts should be tested to come to a pertinent conclusion regarding the antimicrobial efficacy of this plant which was not available during the study, as it is a seasonal plant.

The results of the present study were encouraging as extracts of Pomegranate peel showed significant antibacterial activity against many enteric pathogens. It also strengthens the claims of the usage of this plant extract as a 'grandmother's remedy' in the past for the treatment of gastrointestinal ailments. This study can be extended by including more bacterial strains and the purified forms of these extracts. To conclude, the results of this study reveals that *P.granatum* extract may be helpful as an adjunct for the treatment of infections caused by enteric pathogens in conjunction with other antibiotics and can open new avenues for research.

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Conflicts of Interest: None.

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