Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i3.3491

A COMPARATIVE STUDY ON ANTIMICROBIAL ACTIVITY OF *CITRUS SINENSIS* AND *PUNICA GRANATUM*

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Abstract:
Ayurvedic plants such as *Citrus sinensis* (sweet orange) and *Punica granatum* (pomegranate) have been used for their therapeutic properties in traditional Indian medicine. The study was conducted to asses the antimicrobial activity of chloroform and methanol extracts of *Citrus sinensis* (sweet orange) and *Punica granatum* (pomegranate) peel against various bacterial and fungal strains. The peels were collected and subjected to extraction using organic solvents. The extracts were then analyzed for phytochemical composition using qualitative screening methods. The results revealed the presence of several bioactive compounds such as coumarins, quinones, flavanones, phenols, alkaloids, flavonoids, saponins, steroids, terpenoids, and tannins in both the chloroform and methanol extracts of *Citrus sinensis* peel. *Punica granatum* peel extracts also showed similar phytochemical composition, except for the absence of cardiac glycosides in the methanol extract. The antimicrobial activity of the extracts was evaluated against various bacterial and fungal strains using the agar well diffusion method. The chloroform extract of *Citrus sinensis* peel exhibited significant activity against *S. agalactiae, S. aureus, P. aeruginosa, S. enterica,* and *A. niger*. The methanol extract of *Citrus sinensis* peel showed activity against *E. aerogenes* and *A. chroococcum.* The chloroform extract of *Punica granatum* peel displayed activity against *S. aureus, E. aerogenes, P.aeruginosa,* and *S. agalactiae,* while the methanol extract exhibited activity against *S. agalactiae, S. aureus, P. aeruginosa, A. chroococcum,* and *E. aerogenes.* Overall, both *Citrus sinensis* and *Punica granatum* peel extracts demonstrated antimicrobial activity against the tested strains, highlighting their potential as natural antimicrobial agents.

Keywords: Ayurvedic plants, *Citrus sinensis, Punica granatum,* phytochemical composition, antimicrobial activity, agar well diffusion, natural antimicrobial agents

1. Introduction:

The increasing prevalence of antimicrobial resistance has become a global health concern, necessitating the exploration of alternative sources for antimicrobial agents. Natural products derived from plants have shown promising antimicrobial properties and have been used in traditional medicine for centuries. Ayurvedic plants have been used in traditional Indian medicine

for centuries and are valued for their therapeutic properties. The use of plants in Ayurvedic medicine is based on the principle that each plant has a unique combination of energies and healing properties that can be used to balance the body, mind, and spirit. *Citrus sinensis* and *Punica granatum*are two Ayurvedic plants that have been studied extensively for their health benefits.

Citrus sinensis, commonly known as sweet orange, belongs to Rutaceae family, is a type of citrus fruit that is widely cultivated and consumed around the World. Sweet oranges are used extensively in the food and beverage industry for their flavor, color, nutritional value and also for medicinal purposes due to their high content of bioactive compounds such as flavonoids, carotenoids, and limonoids. Citrus fruits considered a precious resource of soluble andinsoluble fibre with numerous benefits such as removing the toxic effects in the body (RasoolM *et al.,* 2013). The oranges peels are rich in nutrients, which can used as drugs or as food supplements too (Ashok Kumar*etal.,*2011). Citrus fruits are rich in vitamin C, which is an important antioxidant and believed to have anti-inflammatory and antimicrobial effects. (Agrawal M and Vyas N 2016)

Scientific classification:

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsid
Order: Sapindales
Family: Rutaceae
Genus: *Citrus*
Species: *Sinensis*

Image 1.1 *Citrus sinensis*

Punica granatum is popularly known as pomegranate (*Anar*). It is a member of *Lythraceae* family. Different part of pomegranate like bark, leaves, immature fruits, and fruit rind has been used in traditional medicine for thousands of years. The fruit and bark of pomegranate are used against intestinal parasites, dysentery, and diarrhea (Al-SaidFA*etal.,*2009). The juice and seeds are considered a tonic for throat and heart. It is used to stop nose and gum bleeds and treating haemorrhoids (MercolaB2015). It has diverse characteristic of phytochemicals is thought to be responsible for its high antioxidant potential and health benefits (Kahramanoglu*etal.,*2016). Pomegranateis rich in polyphenols and other antioxidants that have been shown to have anti-inflammatory, anticancer, and cardiovascular protective effects. Studies have found that pomegranate may help to reduce inflammation, improve blood pressure, and lower the risk of heart disease. (Jurenka J.S 2009)

Scientific classification:

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsida
Order: Myrtales
Family: Lythraceae
Genus: *Punica*
Species: *granatum*

Image 1.2 *Punica granatum*

While both *Citrus sinensis* and *Punica granatum* have shown promising antimicrobial activity individually, a comparative study evaluating their efficacy against common microbial pathogens is necessary to gain a comprehensive understanding of their potential as antimicrobial agents. This study aims to compare the antimicrobial activity of *Citrus sinensis* and *Punica granatum* extracts against a panel of pathogenic bacteria and fungi commonly associated with human infections. By comparing the efficacy of these two plants, we can identify potential candidates for further development as natural antimicrobial agents.

2.Materials and methods:

2.1. Procurement of plant material:

Citrus sinensis and *Punica granatum* fruits were collected in the month of February 2023 in and around Gajuwaka, Visakhapatnam, Andhra Pradesh, India.

2.2. Extraction:

Citrus sinensis and *Punica granatum* peels was weighed, and grinded. Samples were soaked inorganic solvents (chloroform, methanol) and macerated for 72 hrs. The soaked samples were filtered and solvent was collected. The extract obtained were concentrated using distillation and stored in desiccator till further use.

Image 1.3 *Citrus sinensis, Punica granatum* peel, powder, maceration

2.3.Qualitative phytochemical analysis:

Preliminary analysis were carried out as per standard protocol of Harbone JB 1998

1. Carbohydrate:

To 1mL of sample, few drops of α-naphthol was added and then concentrated H_2SO_4 was added along the sides of test tube. Formation of violet ring indicates the presence of carbohydrates.

2. Alkaloids:

To 1mL of sample treated with dil HCl, Wagner's reagent was added, formation of brown precipitate indicates the present of alkaloids.

3. Cardiac glycosides:

To 1 ml of sample, 2ml of glacial acetic acid and few drops of $FeCl_3$ was added, mixed. and then concentrated H_2SO_4 was added along the sides of test tube. Brown ring at interphase indicates the presence of cardiac glycosides

4. Flavonoids:

To 1 ml of sample, 10%lead acetate was added; formation of yellow precipitate indicates the presence of flavonoids

5. Phenols:

To 1 ml of sample, few drops of 5% $FeCl_3$ was added, appearance of blue- black, blue green colour indicate the presence of phenolics

6.Tannins:

To 1 ml of sample, few drops of 0.1 % $FeCl_3$ was added, appearance of blue- black, blue green colour indicate the presence of tannins.

7. Saponins:

To 1 ml of sample, 5ml of distilled water was added and shaken for 20 min. Appearance of foam indicates the presence of saponins

8. Steroids:

To 1 ml of sample, 10ml of chloroform was added, and then concentrated H_2SO_4 was added along the sides of test tube. Reddish Brown ring at interphase indicates the presence of Steroids

9.Terpenoids:

To 1 ml of sample, 2 ml of chloroform was added, and then concentrated H_2SO_4 was added along the sides of test tube. Reddish Brown ring at interphase indicates the presence of terpenoids

10. Anthocyanin:

To 1 ml of sample, 1 ml of 2N NaOH was added and heated fro for 5 min at 100°C. Formation of bluish green color indicates the presence of anthocyanin.

11. Coumarins:

To 1 ml of sample, 1 ml of 10%NaOH was added. Formation of yellow colour indicates the presence of coumarins.

12. Quinone:

To 1 ml of sample, 1 ml of conc. $H_2SO_4$was added. Formation of red colour indicates the presence of quinones.

13. Flavanones:
To 1 ml of sample, 1 ml of 10%NaOH was added. Color change from yellow to orange indicates the presence of flavanones.

2.4.Microorganisms:
Eight microorganisms were used in this study - Six bacterial strains and two fungal strains. Two were Gram positive bacterium (*Streptococcus agalactiae* and *Staphylococci aureus*) while other four were Gram negative bacteria (*Enterobacter aerogenes, Azotobacter chroococcum, Pseudomonas aeruginosa,* and *Salmonella enterica*) The two fungal strains used are *Aspergillus niger* and *Mucor racemosus.* Microorganism were collected from the department of microbiology in MVR Degree and PG college, Gajuwaka, Visakhapatnam The identities of the test organisms were confirmed to the specie levels using standard biochemical and morphological procedures.

2.4.1.Inoculum preparation:
a. For bacterial strains: The 20 mL of autoclaved nutrient broth was poured conical flask and loopful of the bacterial strain was introduced in it under aseptic conditions. Conical flasks were appropriately labelled and were placed in the incubator at 35 ± 2^0C for about 24 h.
b. For fungal strains: To 20 ml of Sabouraud dextrose agar medium loopful of fungal strain was introduced under aseptic conditions. Conical flasks were appropriately labelled and were placed in the incubator at 25 ± 2^0C for about 48 h.

2.4.2.Inoculation of test plates:
15 ml of sample was added to each petri plate an allowed to solidify. 50 µl of inoculum was transferred onto a sterile swab. In aseptic conditions, the swab was streaked on the whole plate 2-3 times so that inoculum is evenly distributed through the plate.

2.4.3.Screening for antimicrobial activity
Agar well diffusion assay was carried out to determine antibacterial activity of peel extracts. Two Gram positive bacterium *Streptococcus aureus* and *Staphylococci aureus*, four Gram negative bacteria *Enterobacter aerogenes, Azotobacter chroococcum, Pseudomonas aeruginosa, Salmonella enterica,* two fungal strains *Aspergillus niger* and *Mucor racemosus* were screened for their susceptibility to *Citrus sinensis* and *Punica granatum* peel extracts. Wells of 6mm diameter was punched on Inoculated petri plates. Different concentration of extracts and standard were transferred into the wells. The plates were incubated at 37^0C for 24 hours for bacterial and 25^0C for 72 hours for fungal in upright position and the zone of inhibition formed around the wells was measured. All Samples were analysed in triplicates

2.5. Statistical analysis:
Data was analysed in triplicates and give as mean and standard deviation

3.Results and discussion:
The present study evaluated the antimicrobial activity of chloroform and methanol extracts of *Citrus sinensis* and *Punica granatum* peels against various bacterial and fungal strains. The results indicated that the yields of the extracts varied significantly between the two plant species and between the different solvents used for extraction. Citrus sinensis methanol peel extract had the highest yield (24.2%), followed by the chloroform extract (4.93%), while Punica granatum chloroform peel extract had the highest yield (19.3%), followed by the methanol extract (12%).The results were depicted in table 1. The study found the presence of coumarins, quinones, flavanones, phenols, alkaloids, flavonoids, saponins, terpenoids, and tannins in both the chloroform and methanol extracts of *Citrus sinensis* peel. However, anthocyanin and cardiac glycosides were not present in either extract, while steroids were only found in the chloroform extract. In the case of *Punica granatum* peel, both the chloroform and methanol extracts contained coumarins, phenols,

alkaloids, flavonoids, saponins, steroids, terpenoids, and tannins. Additionally, quinones and cardiac glycosides were only present in the chloroform extract. The results were given in table 2.

Table 3. 1: % Yield of chloroform and methanol extracts of *Citrus sinensis* and *Punica granatum* peels

S. No	Plant name	Solvent	Weight of peels	% yield of extract
1.	*Citrus sinensis* peel	Chloroform	100 g	4.93%
		Methanol	100 g	24.2%
2.	*Punica granatum peel*	Chloroform	100 g	19.3%
		Methanol	100 g	12 %

Table3. 2: Phytochemical Screening of chloroform and methanol extracts of *Citrus sinensis and Punica granatum peels*

S.NO	Phytochemicalscreening	*Citrus sinensis* peel		*Punica granatum* peel	
		Chloroform Extract	Methanol Extract	Chloroform Extract	Methanol Extract
1.	Coumarins	+	+	+	+
2.	Quinones	+	+	+	_
3.	Flavanones	+	+	+	_
4.	Anthocyanins	_	_	+	+
5.	Phenol‘s	+	+	+	+
6.	Alkaloids	+	+	+	+
7.	Cardiacglycosides	-	_	+	-
8.	Flavonoids	+	+	+	+
9.	Saponins	+	+	+	+
10.	Steroids	+	_	+	+
11.	Terpenoids	+	+	+	+
12.	Tannins	+	+	+	+
(+)Indicates Presence of phytochemicals, (-) IndicatesAbsence of phytochemicals					

The antimicrobial activity of the extracts was assessed against six bacterial strains and two fungal strains. The chloroform extract of *Citrus sinensis* peel showed the highest activity against *S. agalactiae*(8.02±0.01mm)followed by *P. aeruginosa*(6.04±0.02 mm),*S. enterica* (6 .01±0.02 mm), *A.chroococcum*(4.02±0.02 mm),*S. aureus,E. aerogenes*(3.11±0.02 mm) while in Methanol extract, at 100 µg/ml highest activity was observed in *E. aerogenes*(11.09±0.02 mm),followed by*S. agalactiae*(10.03±0.01mm),*S. aureus*(9.04±0.02mm) , *A.chroococcum*(9 .12±0.01 mm) *P. aeruginosa*(8.01±0.03 mm)*and S. enterica*(8.04±0.04 mm). The chloroform extract of *Punica granatum* peel showed the highest activity against *S. aureus, E.aerogenes* (8.04±0.01mm),followed by *P. aeruginosa* (7.06±0.02 mm), *S. agalactiae* (8.11±0.01 mm), *S. enterica* (5 .02 ±0.02 mm). No zone of inhibition was observed in *A.chroococcum*while in Methanol extract, at 100 µg/ml highest activity was observed in *S. agalactiae*(9.03±0.01mm),followed by *S. aureus, P. aeruginosa, A.chroococcum.*(8.03±0.02 mm), *E.aerogenes* (7.06±0.01 mm) and least in *S. enterica*(4.05±0.02 mm) .The fungal activity of the extracts were also evaluated, and the results showed that chloroform extract of *Citrus sinensis* peel exhibited the highest activity against *A.niger*(8.00 ±0.02 mm) and no zone of inhibition was observed in *M. racemosus*while in Methanol extract, at 100 µg/ml highest activity was observed in*A.niger*(9.06±0.02mm) followed by *M. racemosus*(8.04±0.02 mm). Chloroform extract of *Punica granatum* peel showed the highest fungal activity against *A.niger(*7.03±0.02 mm)followed by *M. racemosus*(5 .11±0.01 mm), while in Methanol extract, at 100 µg/ml highest activity was observed in *M.* The results were displayed in figure

The presence of bioactive compounds such as flavonoids, terpenoids, and phenolic compounds in the extracts might be responsible for thiers antimicrobial acitiviyt. *Citrus sinensis*peel is known to contain flavonoids, terpenoids, which has been associated with its antimicrobial activity (Burt,

2004) The antimicrobial activity of *Punica granatum* has been attributed to the presence of bioactive compounds such as phenolics, anthocyanins (Medjakovic S &Jungbauer A, 2013) Numerous studies have highlighted the inhibitory effects of *Punica granatum* extracts against various pathogenic bacteria and fungi, suggesting its potential as an antimicrobial agent (Nhlanhla Maphetu *et al* ., 2022).

The results obtained from the study indicate that the methanol extracts of pomegranate peels exhibited the highest zone of inhibition compared to the antibiotic penicillin. This suggests that the methanol extract of pomegranate peel may possess potent antimicrobial properties against the tested microorganisms. Furthermore, the results demonstrate that both the chloroform and methanol extracts of *Citrus sinensis* peel and *Punica granatum* peel displayed antimicrobial activity against various bacterial strains. The chloroform extract of *Citrus sinensis* peel showed the highest activity against *S. agalactiae*, while the chloroform extract of *Punica granatum* peel exhibited the highest activity against *S. aureus* and *E. aerogenes*. These findings are consistent with previous studies that have reported the antimicrobial activity of *Citrus sinensis* and *Punica granatum* extracts. The presence of bioactive compounds, such as flavonoids, terpenoids, phenolic compounds, and anthocyanins, in these extracts may contribute to their antimicrobial properties.

4. Conclusion

The study provides important information about the yield, phytochemical composition, and antimicrobial activity of *Citrus sinensis* and *Punica granatum* peel extracts. The results suggest that both plants have potential as sources of antimicrobial agents.The results of this study contribute to the understanding of the therapeutic properties of these traditional medicinal plants and their potential applications in modern medicine.These natural extracts may offer potential solutions to combat bacterial infections, especially considering the increasing concern over antibiotic resistance.However, further research and exploration of these natural extracts may lead to the development of novel antimicrobial agents for therapeutic applications.

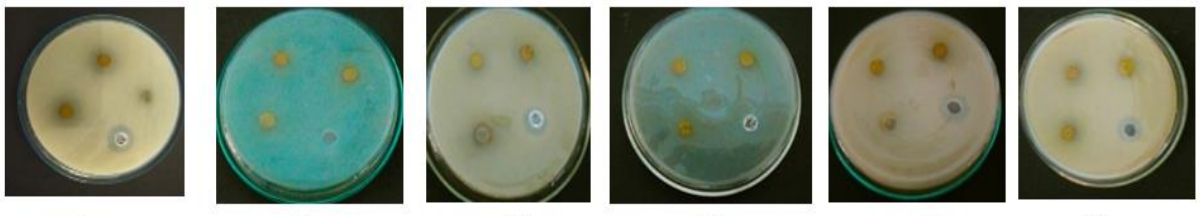


image 4.1 Antibacterial activity of Chloroform extract of *Citrus sinensis* peel

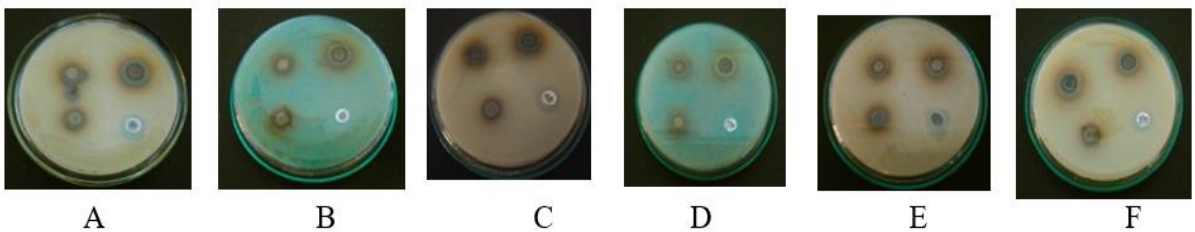
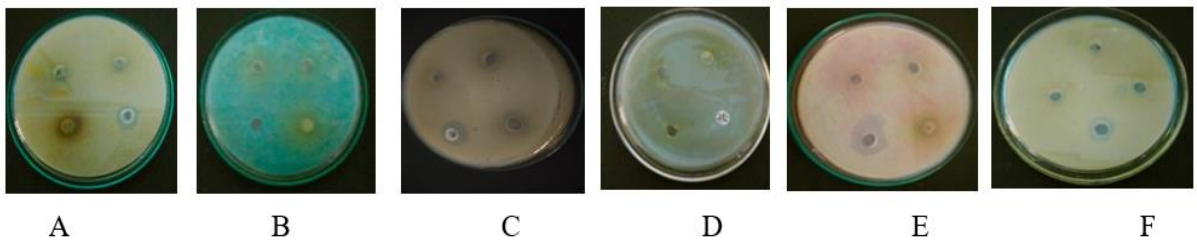


Image 4.2 Antibacterial activity of Methanol extract of *Citrus sinensis* peel



Imagr 4.3 Antibacterial activity of Chloroform extract of *Punica granatum* peel

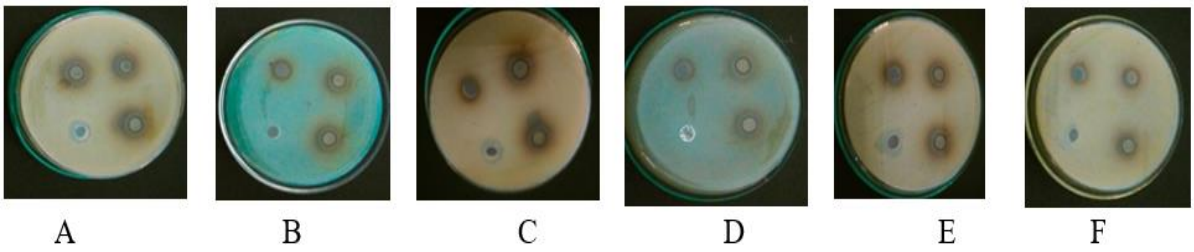


Image 4.4 Antibacterial activity of methanol extract of *Punica granatum* peel

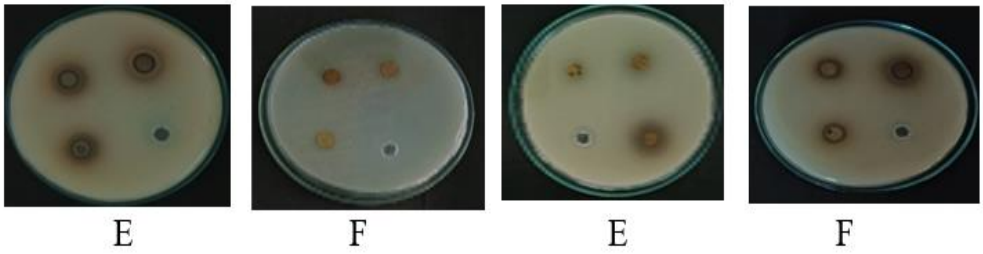


Image 4.5 Antifungal activity of Chloroform extract and methanol extract of *Citrus sinensis* peel

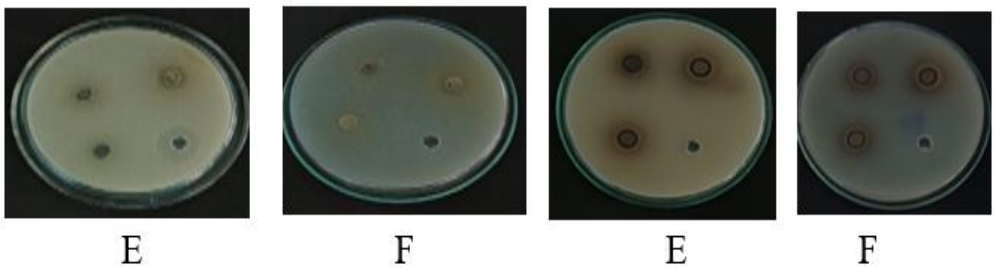


Image 4.6 Antifungal activity of Chloroform extract (A, B) and methanol extract (C, D) of Punica granatum peel

A. *S. agalactiae* B. *S. aureus* C. *E. aerogenes* D. *A. chroococcum*E. *P. aeruginosa*
F. *S. enterica*E.*A.niger*F. *M. Racemosus*

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