DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION AND ANTIMICROBIAL ACTIVITY OF SOME WILD CITRUS PLANT SPECIES OF UTTARAKHAND

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Abstract; Citrus plant is the source of vitamine C, high nutrient value and enormous antioxidant activity. Citrus plant such as *Punica granatum Linn, Punica granatum, Citrus pseudolimon*, and *Citrus sinensis* were used for present study. Ethanol and hexane solvant system were used to prepare peel extract. Antimicrobial activity of peel extract and MICs of juice were performed against three pathogenic strains of *C. ablicans, S. aureus* and *E. coli*. Ethanolic peel extract show highest antimicrobial activity (zone of inhibition) compare to hexane peel extract. Among the plant species *Punica granatum Linn* express highest zone of inhibition against the microorganism with 9 mm of inhibition zone. 75% concentration of *Citrus sinensis* juices showed minimum inhibitory concentration (MIC) against *S. aureus* compare to *E coli*. All the citrus plants exhibited positive control against microbes so the peel extract and fruit juice utilized as potential microbial resistant resource.

***Keywords*:** *Punica granatum Linn, Citrus pseudolimon, Citrus sinensis*, antimicrobial activity.

I. INTRODUCTION

In developing countries, 80% of total population exploited plant based traditional medicine system. Present day Herbal drug is key health care because they have not any side effects **(Chaudhari et al, 2012)**. In US pharmaceutical industries, produced various type of new drug from plant souces **(Pandey et al, 2011)**. Various type of fungal and microbial disorder was cure through medicinal plant product because of their diversified antimicrobial profile. Different phytochemical isolate from the plants such as terpenoids, alkaloids, phenols and flavonoids present in essential oil also shows bacterial confrontation **(Hwang et al, 2001)**.

Rutaceae is important family having medicinal properties and economical value. This family has 140 type of genera and nearly 1,300 citrus species **(Afroja et al, 2017)**. Many tropical and subtropical counties like India, Mexico, Brazil, Pakistan and China wildly cultivate citrus plant for their valuable products **(Mahmood 2005; Khan 2005)**. Medicine, food and cosmetic industries produces product from citrus plant **(Silalahi 2002)**. Flavonoids are glycosylated derivative, isolated directly from citrus fruits act as free radical scavenger and antioxidants along with enzymatic and cell inhibitory profile **(Duthie and Crozier 2000; Sohan et al, 2004)**. Fibres of citrus plant possess identical bioactive polyphenol (vitamin C) compound, use to cure scurvy type disease **(Aronson, 2001)**.

The present study was to estimate the potential of different citrus peel extracts on microbial strains by using common antibacterial assay methods.

II. MATERIAL AND METHODOLOGY

The study was conducted at the Department of Biotechnology, Uttaranchal University Dehradun, in the month of November 2018 to January 2019.

Collection of fruit Material

The fruit peel of *Punica granatum*, *Citrus sinensis*, *Punica granatum* L and *Citrus Pseudolimon* were collected from Uttarakhand regions. The peels were shade dry for 25 days.

Extraction

The dried peel material was pulverized into fine powder using a grinder (mixer). About 15 g powder was used for extraction in soxhlet extraction apparatus with 250 ml of each of the solvents; hexane and ethanol. The extracts obtained with each solvent were filtered through whatman filter paper and the respected solvents were evaporated at 40ºC with moisture heating on water bath. The sticky brownish and yellowish substances were obtained and stored in refrigerator for further use.

Preparation of Microorganism

All organisms were obtained from the Department of Microbiology of Uttaranchal College of Applied and Life Science and include:

Gram positive – *S.aureus*

Gram negative-*Escherichia coli*

Fungal strain – *Candida ablicans*

The strain were revived in 20 ml Luria Bertani Broth (HiMedia) and incubated at 37ºC

Dilutions and Inoculums preparations

The dried peel extracts powder of selected plants (*Punica granatum*, *Citrus sinensis*, *Punica granatum* Linn. and *Citrus Pseudolimon*) were dissolved in their respective solvents (Ethanol and Hexane) and make dilution different concentration (2mg/ml,4mg/ml,6mg/ml) and control The inoculums of *S. aureus*, *E .coli*, ,*C. ablicans* were stored at 2-8°C for further analysis.

Disc Diffusion Method

Petri plates sterilized at 121ºC for 15 minutes. Pore 20ml of media into each sterile petri plates and allow them to solidify. After the solidifying of the media, 10µl inoculum was spreaded uniformly over the agar medium and distributes the microbes with the help of a sterile glass rod. The readily prepared sterile discs were loaded with concentrations of about 2mg/ml, 4mg/ml,6mg/ml and control of peel extract of into each .The paper diffuse discs were loaded on to the medium with their respective concentrations and the plates were incubated at 37º C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc **(Bayer *et al*, 1966)**.

Minimum inhibitory Concentration

Minimum inhibitory concentration (MIC) is the final concentration of an antimicrobial that inhibits the detectable growth of microorganism. MIC is generally measured as the most elementary laboratory measurement of the action of an antimicrobial agent against the microorganism. Four different concentrations of fruit juices (*Punica granatum,Punica granatum Linn, Citrus sinensis and Citrus pseudolimon*) were prepared 100%,75%,50% and 25%. Nutrient broth was ready for each test tube which labelled as 100%, 75%, 50% and 25%, 9 ml of broth was aseptically transferred into each test tube. Test tubes containing 9 ml nutrient, 1ml of each fruit concentration was added into the respective test tubes and 0.1ml of the test pathogen suspension was inoculated into the respective labelled test tubes. After the inoculation, the test tubes were kept at the shaker incubator for overnight at 37ºc and take OD at 600 nm in spectrophotometer.

III. RESULT AND DISCUSSION

Two type of citrus fruit peel extracts (ethanol and hexane) were used for antimicrobial activities against selected strains. The zone of inhibition is measured in mm (millimeter) after the of 24 hour incubation period at 37ºC. *Punica granatum Linn* (F_4, wild species) ethanolic peel extract shows maximum antimicrobial activity against all microbial strains *S. aureus* (12mm), *E. coli* (8mm) and *C. ablicans* (13mm), While ethanolic peel extract of *Citrus pseudolimon* (F_3) show minimum zone of inhibition (3 mm) with 6 mg/ml concentration against *S. aureus*, 6 mm with 6 mg/ml against *E.coli* and *C. ablicans* **(Table 1, Figure 1 and 2)** . Whereas *Punica granatum Linn* (F_4, wild species) hexane peel extract show highest antimicrobial activity (zone of

inhibition) again *S. aureus* (8mm), *E.coli* (7mm) and *C. albicans* (7mm), while *Citrus sinensis* (F1) hexane peel extract show lowest antimicrobial activity against *S. aureus* (5 mm), *E.coli* (6 mm) and *C. albicans* (6 mm) **(Table 2, Figure 1and 3)**.0

This study supports previous findings that the antimicrobial activities have a direct relation to increasing the extracts concentration **(Bhalodia and Shukla, 2011).** It has been also reported previously that the extracts from several plants such as oregano, cumin, cinnamon, sage, and other spices possessed significant antibacterial and antifungal activities against Gram-positive and Gram-negative bacteria , as well as yeast **(Nassan et al, 2015; Liu et al, 2017)**. The antibacterial activities of ethanol extract from some plant against bacteria were similar to our results **(Liu et al., 2017)**. The microorganism *E.coli*, having the property against multi resistant drug, was also resistant to the plant extracts tested. Similar result was noted with extract of dry C. limon, S. agalactiae and C. albicans to the juice of C. limon **(Nada et al, 2013)**.

Some important phytochemical components are in large quantities available in citrus peel, including ascorbic acid, phenolic acids, polyphenols, and dietary fiber **(Gorinstein et al, 2001)**. **Matasyoh et al (2007)** and **Mahmud et al (2009)** reported antioxidant, antiviral, antibacterial, antifungal, and anticancer activities have also been found in citrus. Similar study found that have described the inhibitory activities of citrus plants against fungi, yeasts and food pathogens **(Nannapaneni et al., 2008; Lee and Najiah, 2009)**.

Table 1: Antimicrobial activities of different ethanolic peel extract against selected strain

	Zone of inhibition in mm of ethanol fruit extracts															
	F1			**F2**			**F3**			**F4**			**Control**			
Conc. in mg/ml	**2**	**4**	**6**	**2**	**4**	**6**	**2**	**4**	**6**	**2**	**4**	**6**	**F1**	**F2**	**F3**	**F4**
S. aureus	5	7	8	4	3	3	5	7	9	10	10	12	2	2	1	1
E. coli	3	4	6	5	4	6	7	8	9	5	6	8	2	2	1	1
C. ablicans	3	3	4	6	5	6	5	7	8	8	13	13	1	1	1	1

F1-*Citrus sinensis*, F2- *Citrus pseudolimon*, F3- *Punica granatum* and F4- *Punica granatum* L

Table 2: Antimicrobial activities of different hexane peel extract against selected strain.

	Zone of inhibition in mm of hexane fruit extracts															
	F1			**F2**			**F3**			**F4**			**Control**			
Concentration in mg/ml	**2**	**4**	**6**	**2**	**4**	**6**	**2**	**4**	**6**	**2**	**4**	**6**	**F1**	**F2**	**F3**	**F4**

S. aureus	2	4	5	2	4	6	2	4	6	6	6	8	1	1	2	1
E. coli	3	6	6	5	4	7	3	5	7	5	6	7	1	1	1	1
C. ablicans	3	3	6	3	5	6	2	4	6	2	5	7	1	2	1	2

F1--*Citrus sinensis*, F2- *Citrus pseudolimon*, F3- *Punica granatum* and F4- *Punica granatum* L.

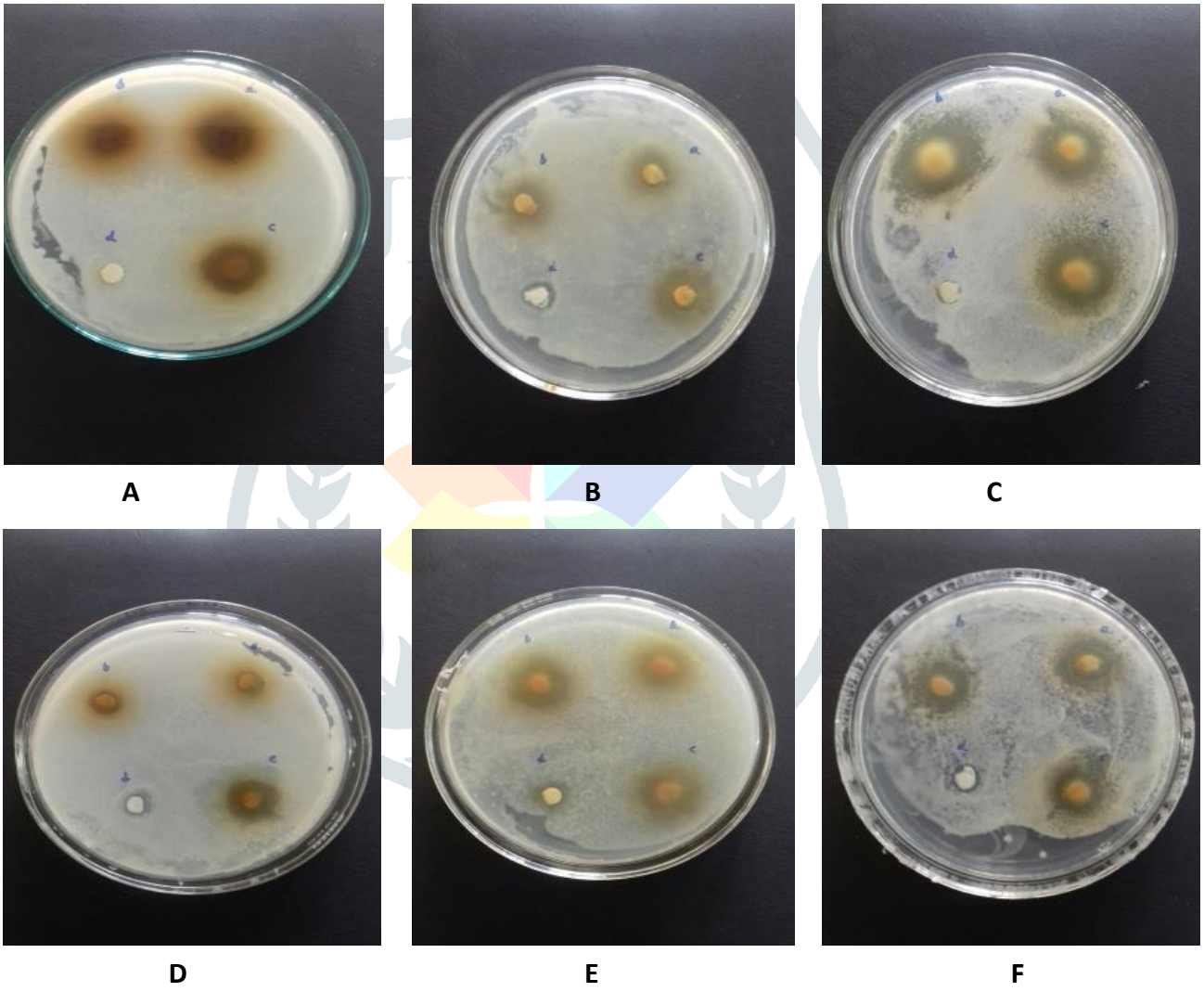

Figure 1: A-Antimicrobial effect of *Punica granatum Linn* ethanolic peel extract against *S. aureus,* B- Antimicrobial effect of *Punica granatum* ethanolic peel extract against *E. coli,* C-Antimicrobial effect of *Punica granatum Linn* ethanolic peel extract against *C. albicans,* D-Antimicrobial effect of *Punica granatum Linn* hexane peel extract against *S. aureus,* E-Antimicrobial effect of *Punica granatum* hexane peel extract against *E. coli,* F-Antimicrobial effect of *Citrus pseudolimon* hexane peel extract against *C. albicans.*

Hayes and Markovic (2002) investigated the antimicrobial properties of lemon and found that lemon possesses significant antimicrobial activity against *S. aureus*, *Escherichia coli*, and C. albicans. These results showed resistance to most of these extracts except that of S. aureus and Klebsiella. Moreover, **Al-Ani et al (2009)** showed excellent bacterial inhibition by C. limon against *S. aureus*.

Quantitative evaluation of the MICs, in mg/ml, of the four citrus fruits against *S. aureus* and *E.coli* were presented in **Table 3, Figure 4 and 5**. It has been stated that gram negative bacteria are more resistant to various antimicrobials than gram-positive microorganisms due to their exterior lipopolysaccharide (LPS) membranes **(Ahmad and Beg, 2001; Negi and Jayaprakasha, 2003)**.In current study it has been estimated that the MIC values are seen higher in gram positive bacteria, such as *S. aureus* 0.03± 0.011 then gram native bacteria *E. coli* 0.016±0.003 in 100% concentration of citrus fruit juice.

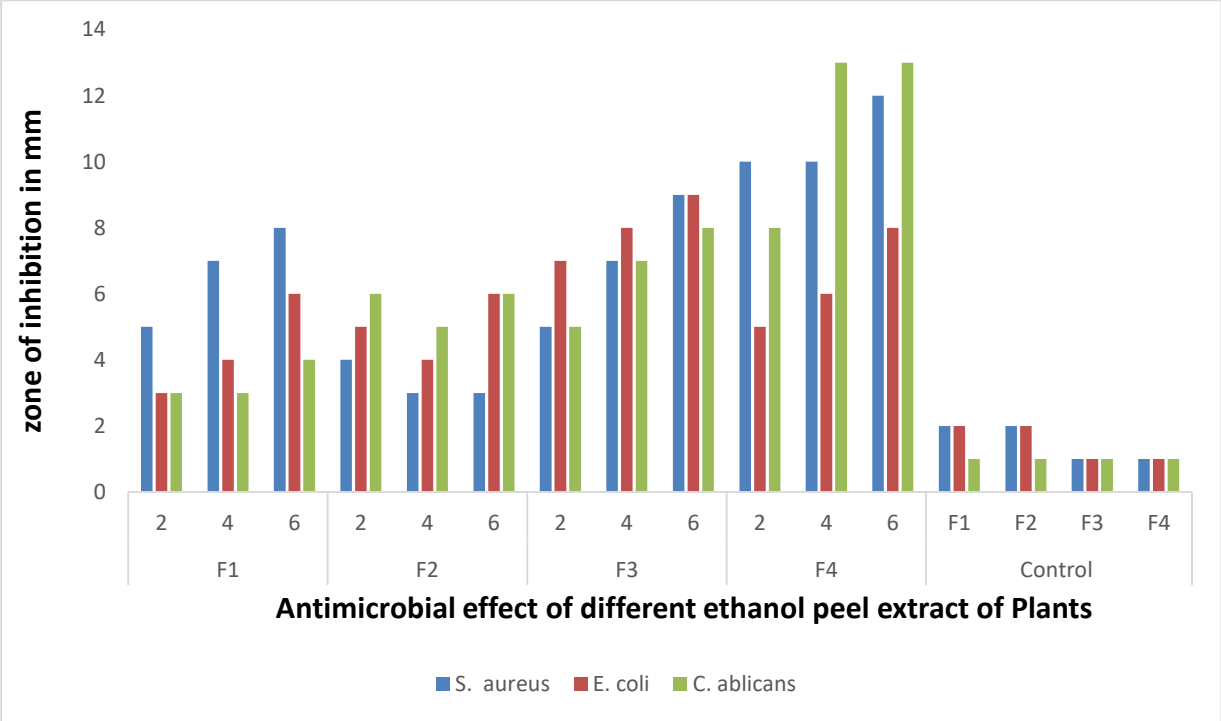

Figure 2: Comparative antimicrobial analysis of different plant peel ethanolic extract

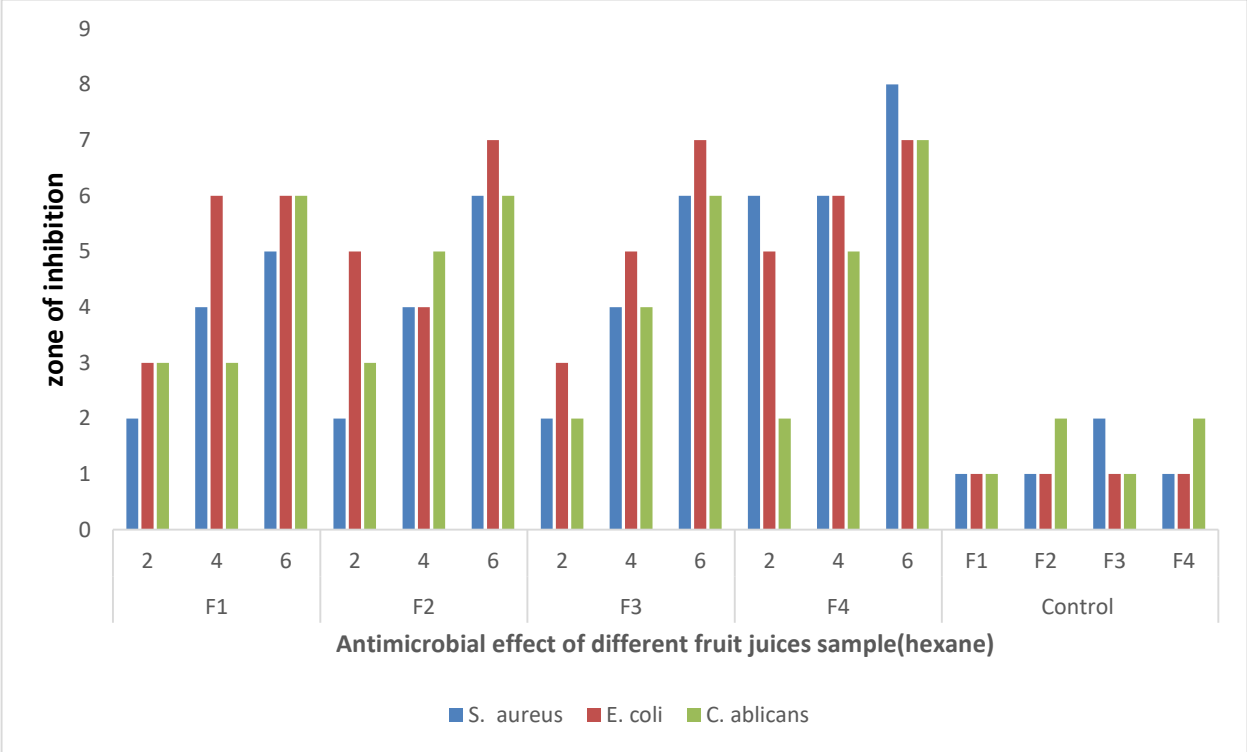

Figure 3: Comparative antimicrobial analysis of different plant hexane peel extract

Table 3: Minimum Inhibitory Concentration (µg/ml) of different selected plant juice sample against bacterial and fungal strain.

Conc.	*Citrus sinensis* (F1)		*Citrus pseudolimon* (F2)		*Punica granatum* (F3)		*Punica granatum* L. (F4)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
100%	0.118±0.001	0.536±0.003	0.016±0.003	0.03±0.011	0.11±0.01	0.653±0.035	0.243±0.024	0.073±0.006
75%	0.118±0.004	0.643±0.012	0.103±0.054	0.13±0.005	0.253±0.029	0.726±0.024	0.276±0.003	0.236±0.008
50%	0.224±0.007	0.906±0.008	0.146±0.017	0.174±0.004	0.260±0.025	0.796±0.035	0.33±0.01	0.486±0.03
25%	0.223±0.008	1.126±0.012	0.206±0.014	0.22±0.011	0.316±0.012	0.816±0.031	0.356±0.012	0.49±0.01

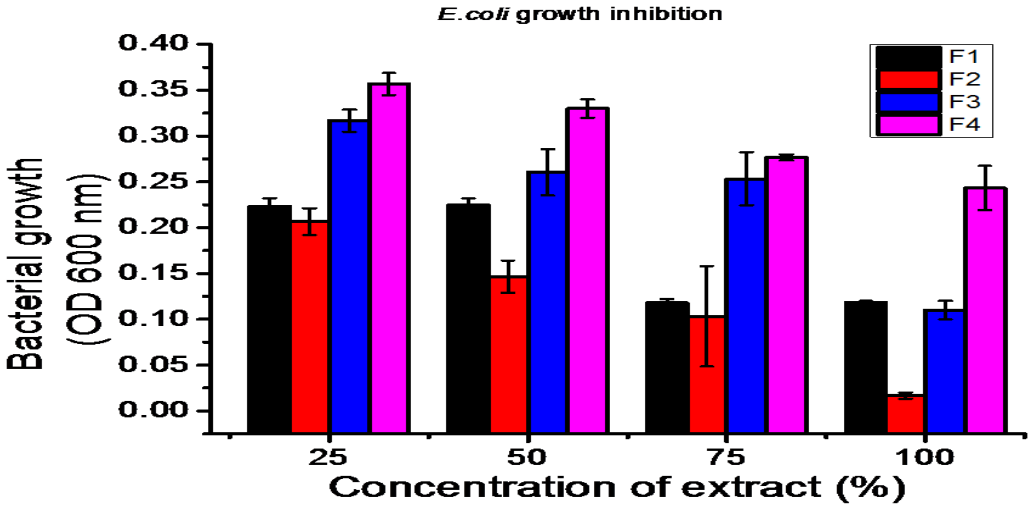

Figure 4: Minimum inhibitory concentration of different juice sample against *E.coli*.

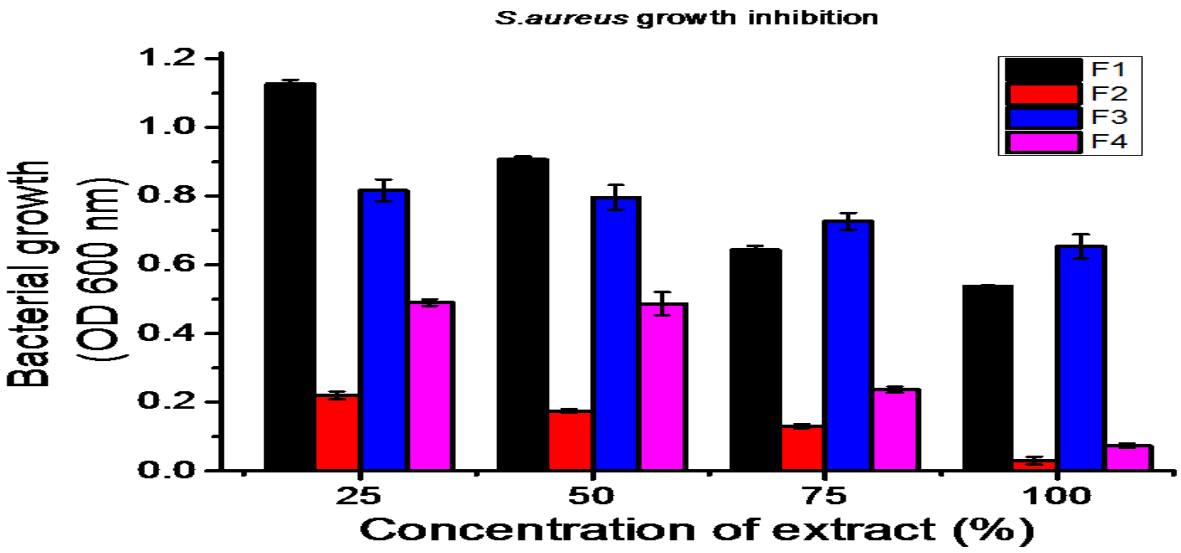

Figure 5: Minimum inhibitory concentration of different juice sample against *S.auresus*.

IV. CONCLUSION

The study shows that the peel of citrus is not only a mordant but also excellent antimicrobial source. Simple use of lemon juice can prevent such types of infections and could help in keeping a good and healthy skin. Citrus plant extracts have an important role as antimicrobial agents against different microorganisms. Citrus plants are natural, cheap and increase antibiotic resistance among bacteria.

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