Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Research Article

Received on: 22-12-2016
Accepted on: 29-12-2016
Published on: 15-02-2017

Corresponding Author:

*** Dr. Suneeta Panicker,**
Department of Microbiology, Dr. D. Y. Patil Arts, Commerce & Science College, Sant Tukaram Nagar, Pimpri, Pune-411018, Maharshtra, India.
Telephone: +91 9970924233

*Email Id-
suneetapanicker@gmail.com

Antimicrobial Activity of Citrus Fruit Pulp, Whole Citrus Fruit Juice and Silver Nano Particles Synthesized Using Citrus Fruit Pulp

Suneeta Panicker, Pawan Chavan, Amit Sharma, Laxmi Singh, Nivedita Khandve

ABSTRACT

Citrus fruits are suggested as antimicrobial agents from ancient times. Several microorganism derived antibiotics currently in use to treat a variety of human diseases, but the problem of antibiotic resistance is upcoming rapidly due to various factors. The demand for novel antimicrobial agents from natural resources has been increased worldwide. The present study was carried out to find the antimicrobial activity of pulp juice, whole fruit juice & the combination of citrus fruits (lemon, orange, sweet lime). Antibacterial analysis showed that lemon juice was highly active against all the tested organisms, whereas the orange and sweet lime showed very less & no activity respectively. The combinations of the three citrus juices were active against all the tested organisms but the Gram positive bacteria were more sensitive than Gram negative. The lemon pulp juice also showed a high antifungal activity against *Candida albicans*. Furthermore, Nanoparticles with antimicrobial property, biosynthesized using different sources are of significance in therapeutic applications of nanotechnology. We also synthesized Silver Nano particles were from fruit pulp juices. It was found that the antibacterial activity of lemon was enhanced whereas orange and sweet lime silver Nano particles were also active against all test organisms.

Key-words: Citrus fruits, antimicrobial activity, silver Nano particles.

Cite this article as:
Suneeta Panicker, Pawan Chavan, Amit Sharma, Laxmi Singh, Nivedita Khandve, Antimicrobial Activity of Citrus Fruit Pulp, Whole Citrus Fruit Juice and Silver Nano Particles Synthesized Using Citrus Fruit Pulp, Asian Journal of Pharmaceutical Technology & Innovation, 05 (22);06-15 , 2017. www.asianpharmtech.com

Department of Microbiology, Dr. D. Y. Patil Arts, Commerce & Science College, Pimpri, Pune-411018

Introduction

Citrus fruits are suggested as antimicrobial agents from ancient times. Several microorganism derived antibiotics currently in use to treat a variety of human disease, but the problem of antibiotic resistance is upcoming rapidly due to various factors [1, 2]. The demand for novel antimicrobial agents from natural resources has been increased worldwide. These herbal products have fewer side effects and are easily available at affordable costs. The citrus plants are easily available, cheap, less side effecters as compared to allopathic & chemical drug. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities [3, 4]. In addition, the fibre of citrus fruit also contains bioactive compounds, such as polyphenols, the most important being vitamin C (or ascorbic acid), and they certainly prevent and cure vitamin C deficiency-the cause of scurvy [5].

The present study was carried out to find the antimicrobial activity of pulp juice, whole fruit juice & the combination of citrus fruit juices (lemon, orange, sweet lime). Antibacterial analysis showed that lemon juice was highly active against all the tested organisms, whereas the orange and sweet lime had very less / no activity. The combinations of the three citrus juices were active against all the tested organisms but the Gram positive bacteria were more sensitive than the Gram negative. The lemon pulp juice also showed a high antifungal activity against *Candida albicans*.

The use of nanoparticles is gaining impetus in the present century as they possess defined chemical, optical and mechanical properties [6]. The metallic nanoparticles are most promising as they show good antibacterial properties due to their large surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains [7]. Silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms [7, 8]. Furthermore, in the present study, silver Nano Particles (NPs) were also synthesized from these citrus fruit juices and their antimicrobial activity was determined. It was found that the NPs synthesized from orange and sweet lime juice were active against all test organisms.

Methodology

Sample collection

We collected organic citrus fruits such as sweet lime, lemon & orange from local market, washed with distilled water and dried at room temperature.

Preparation of pulp juice

The fruit pulp was harvested by removing the skin and the seeds. This pulp was crushed mechanically and the juice obtained was filtered through sterile muslin cloth. Aliquots of 2 ml were frozen until further use.

Preparation of whole fruit juice

For whole fruit juice, whole fruits (peel, pulp and seeds) were crushed mechanically and filtered by sterile muslin cloth. This whole fruit juice was concentrated to 1/4th volume by using lyophilizer and 2 ml aliquots of this juice were frozen until further use.

Antibacterial activity of pulp juice, whole fruit juice, combinations of different pulp juice and combinations of different whole fruit juice

The antibacterial activity was checked by well diffusion method [9]. Briefly, overnight grown bacterial cultures were spread on sterile nutrient agar plates, wells were bored and 100µl of each juice was added to the respective well. Plates were incubated for 15min at 4^{0}C for pre-diffusion and then were incubated at 37^{0}C for 18-20 hr. Zone of inhibition of the test organism indicated the antimicrobial activity of the juices.

Antifungal activity of pulp juice, whole fruit juice, combinations of different pulp juice and combinations of different whole fruit juice

The antifungal activity was checked by agar over layer method [10]. Briefly, fungal cultures (*Fusarium oxysporum, Aspergillus niger and Candida albicans*) were inoculated in Sabouraud's broth and incubated for 48hr. Sterile Whatmann filter paper discs dipped in pulp juice were placed on the base agar. 100µl of 48hr old culture was mixed with molten agar and was poured over the base agar. Plates were incubated at 30^{0}C for 48hrs. Zone of inhibition of the test organism indicated the antifungal activity of the juice.

Combinations of pulp extract and whole fruit extract

Combinations of pulp juice and whole fruit juice were prepared by mixing different concentrations of each fruit juice.

Table 1: Composition of Combinations

	Combination 1	Combination 2	Combination 3	Combination 4
Lemon	700 µl	150 µl	150 µl	300 µl
Orange	150 µl	700 µl	150 µl	350 µl
Sweet lime	150 µl	150 µl	700 µl	350 µl

Study of antibacterial activity on antibiotic resistant pathogens

Imipenem, Monocef and Taxin resistant *S. aureus, Pseudomonas spp., Salmonella spp., E. coli* and *Hafnia alvei* isolated from contaminated drinking water and burn wound in our laboratory. The antibacterial activity of the pulp juice and their combinations against these resistant pathogens was checked by well diffusion method as described above [9].

Temporal study of antibacterial activity of pulp juice

For the temporal study, the antibacterial activity of the pulp juice was determined for each hour after preparing it for 4 hours i.e.; freshly prepared juice, 1 hr after juice preparation and 2hr & 3hr after juice preparation. Juices were kept at 4^0C during the intervals.

Synthesis and Antimicrobial activity of silver Nano particles (AgNPs) synthesized from pulp juices

To 5ml pulp juices, a final concentration of 1mM silver nitrate solution was added and incubated on shaker. Hourly antibacterial activity of the synthesized AgNPs was checked by well diffusion methods as described [9].

Results

Antibacterial activity of pulp juice and combinations of pulp juices against *E. coli*

Lemon pulp juice showed highest antibacterial activity against *E. coli* (1.9 cm zone of inhibition) whereas; sweet lime did not show any activity. Combination 1 containing more of lemon juice showed higher activity against *E. coli* (1.9 cm) (Fig 2). Minimum activity (1.0 cm zone) was shown by combination 3.

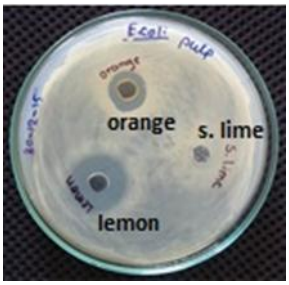


Figure 1: Pulp activity on *E. coli*

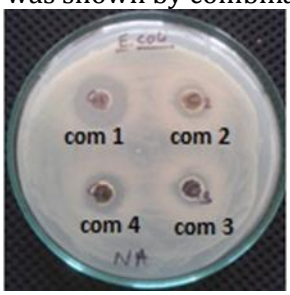
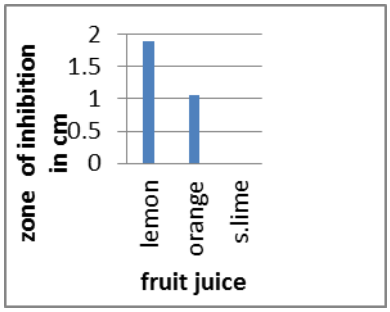
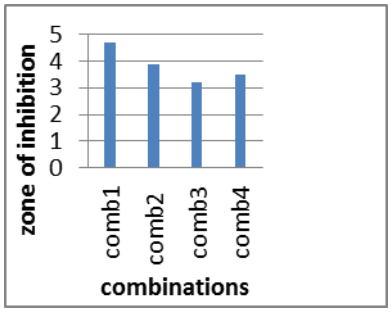


Figure 2: Pulp combinations activity on *E. coli*



Graph 1: Pulp juice activity on *E. coli*



Graph 2: Pulp combination activity on *E. coli*

Antibacterial activity of pulp and combinations of pulp juices against *S. aureus*

The lemon pulp juice showed highest (3.13 cm zone of inhibition) antibacterial activity against *S. aureus* (Fig 3). Whereas, sweet lime pulp juice showed the least 2.3 cm zone of inhibition.

Also, combination 1, which contains more lemon juice, showed higher (4.7 cm zone of inhibition) antibacterial activity against *S. aureus* and minimum activity was shown by combination 3 (Fig 4). The activity seen in the combinations can be mainly due the synergistic activity among the juices.

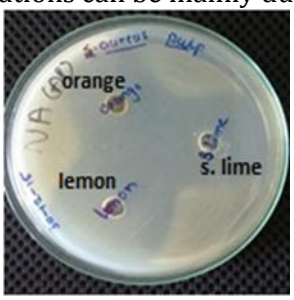
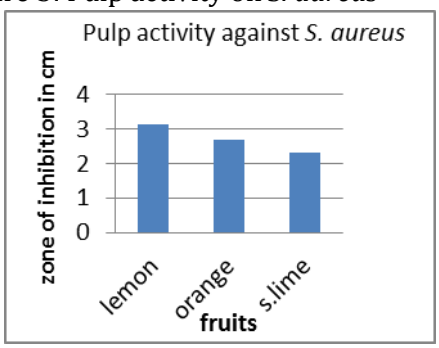
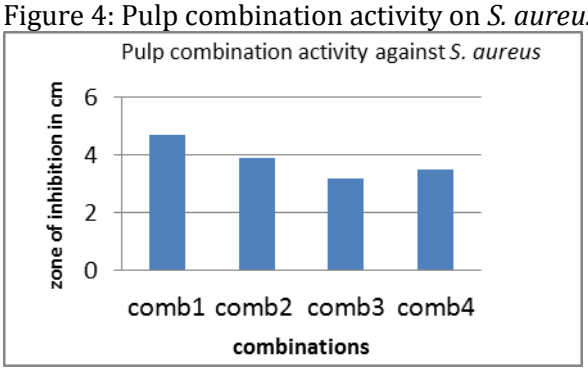


Figure 3: Pulp activity on *S. aureus*

Figure 4: Pulp combination activity on *S. aureus*



Graph 3: pulp activity on *S. aureus*



Graph 4: combination activity on *S. aureus*

Antibacterial activity of pulp and combinations on other bacteria

Antibacterial activity of pulp juice against other bacteria like ***Bacillus, S. epidermidis, Shigella, Pseudomonas*** was determined. *S. epidermidis* appeared to be more sensitive and showed a maximum (3.8 cm) zone of inhibition for lemon. Sweet lime, which did not show any activity against *E. coli*, *Bacillus, Shigella & Pseudomonas,* was active against *S. epidermidis* (2.8 cm) (Fig.5).

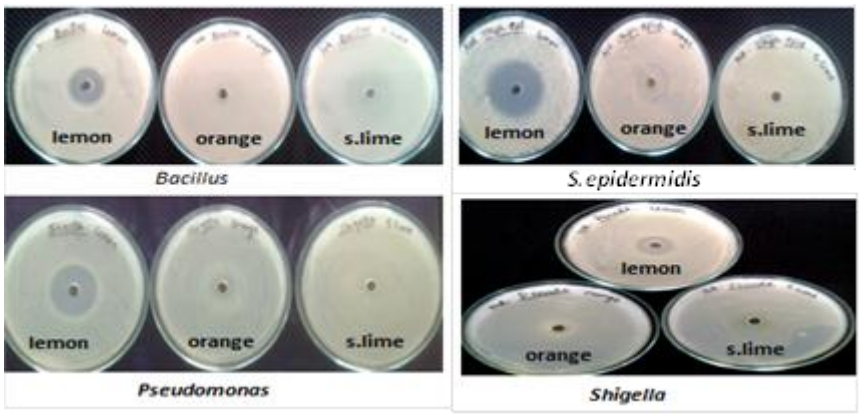
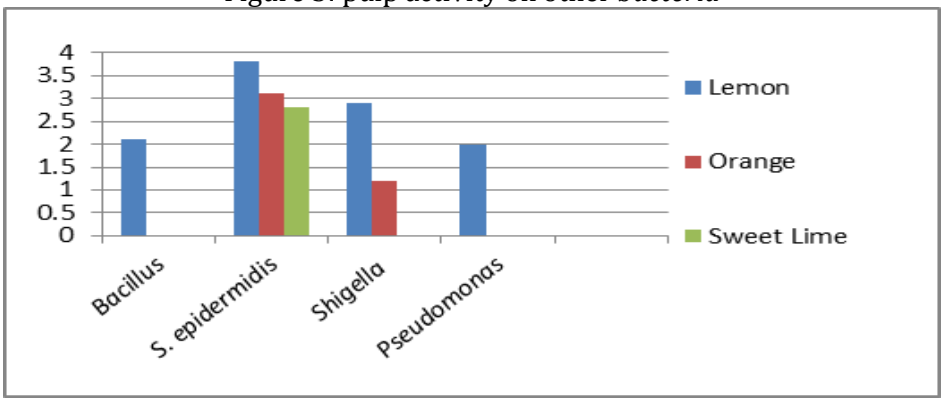


Figure 5: pulp activity on other *bacteria*



Graph 5: Pulp juice activity on other bacteria

In pulp combination activity, *S. epidermidis* again, showed more sensitivity for combination 1 and less for combination 3. *Bacillus* and *Shigella* also showed considerable sensitivity to pulp combinations. *Pseudomonas* showed less sensitivity to pulp combinations (Fig 6).

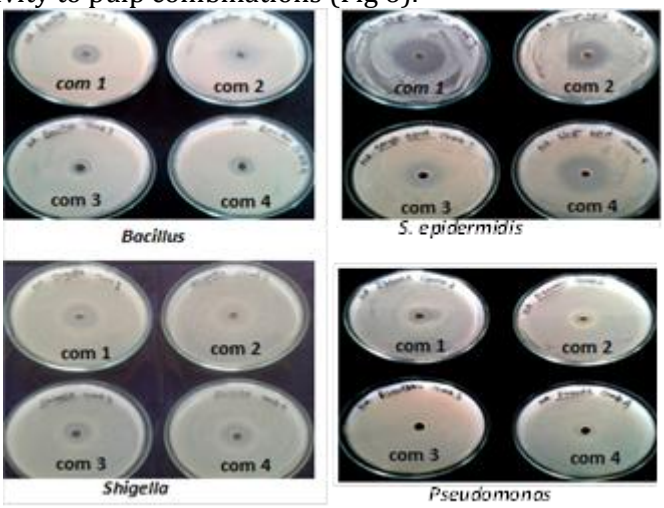
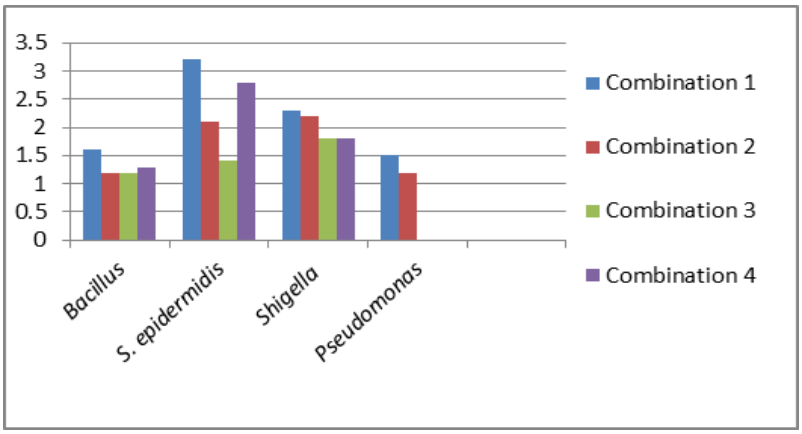


Figure 6: Pulp combination activity on other bacteria



Graph 6: Combinations activity on other bacteria

Antibacterial assay of Whole fruit juices for *E. coli and S. aureus*

Only lemon whole fruit juice showed antimicrobial activity against *E. coli* and *S. aureus. E. coli* showed 1.9 cm zone of inhibition when the assay was performed separately on separate plates for each citrus whole fruit juice and 2.1cm when all the three citrus whole fruit juices were assayed on the same plate. Similarly, *S. aureus* showed 2.2 cm and 2.3 cm zone of inhibition respectively (Fig 7).

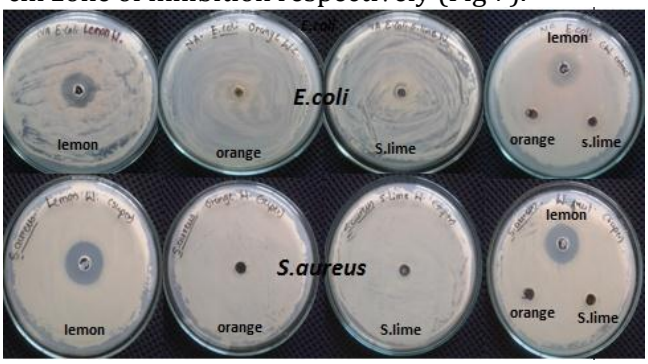
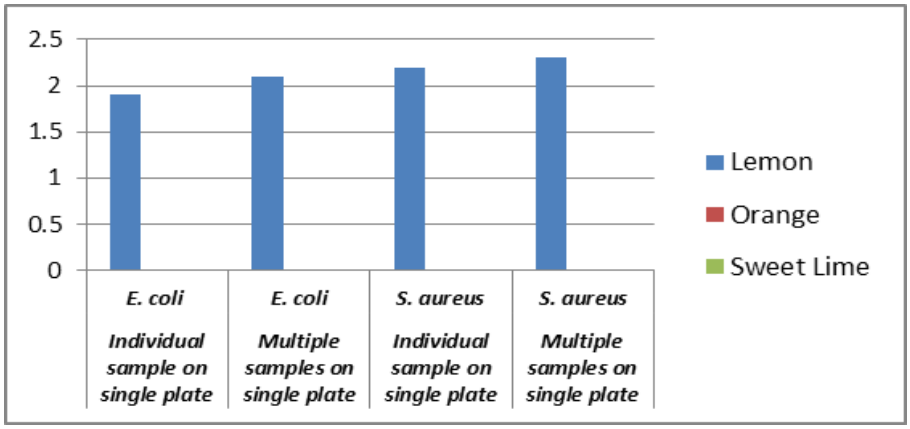


Figure 7: Whole fruit juices assay for *E. coli and S. aureus*



Graph 7: Whole fruit juices assay for *E. coli and S. aureus*

Antibacterial activity of whole fruit juice combinations against *E. coli and Staph aureus*

In combinations of whole fruit juices, combination 1 showed more antibacterial activity than the other combinations. *E. coli* showed 1.7 cm zone of inhibition for combination 1 whereas *S. aureus* showed 1.2 cm zone of inhibition (Fig 8).

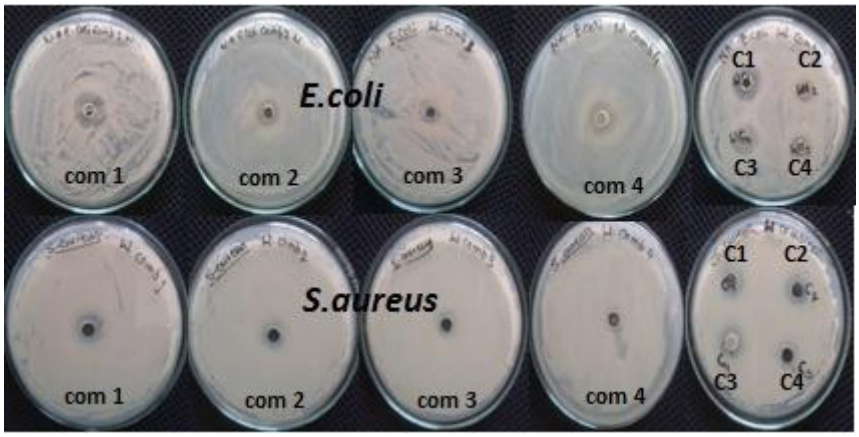
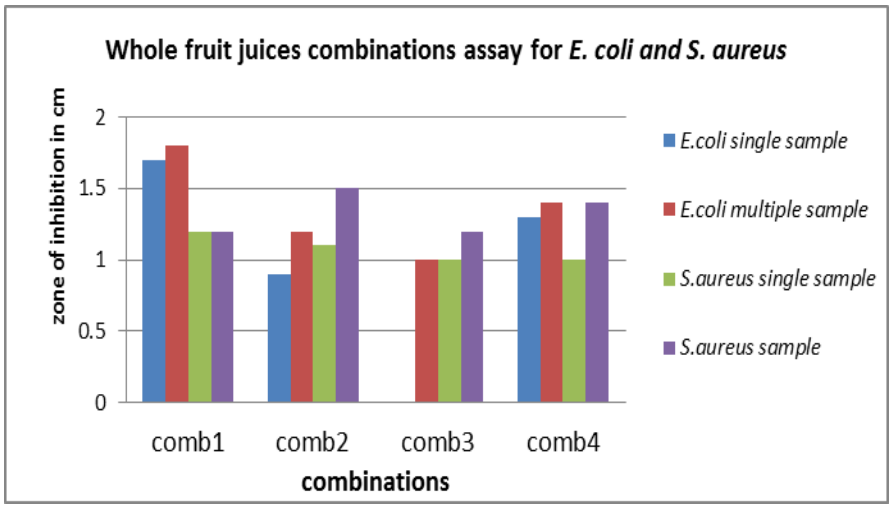


Figure 8: Whole fruit juices combination assay for *E. coli and S. aureus*



Graph 8: Whole fruit juices combination assay for *E. coli and S. aureus*

Study of antifungal activity of pulp juice

It was observed that lemon pulp juice and lemon pulp juice synthesized NPs possess more antifungal activity for *Candida albicans* than the other two citrus fruits (Fig 9). Neither orange and sweet lime pulp juices nor the Nano particles synthesized from them showed any antifungal activity against *Aspergillus niger* and *Fusarium oxysporum*.

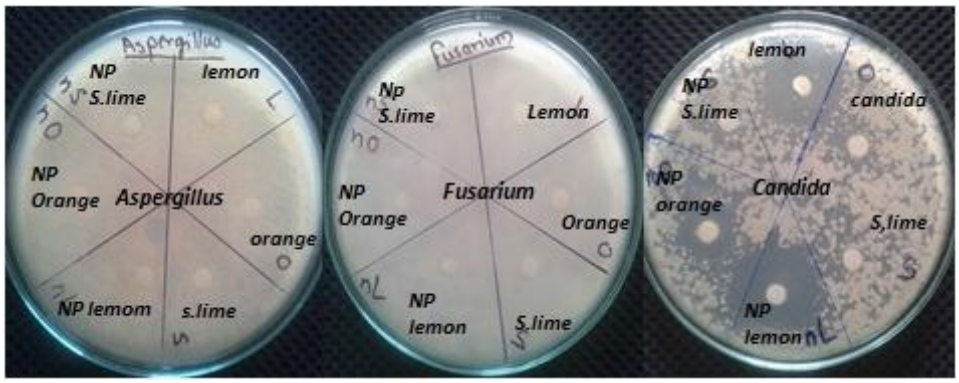
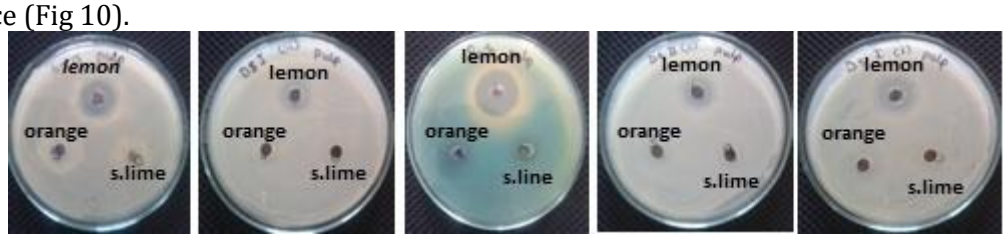


Figure 9: Antifungal activity of pulp juice by agar over layer method

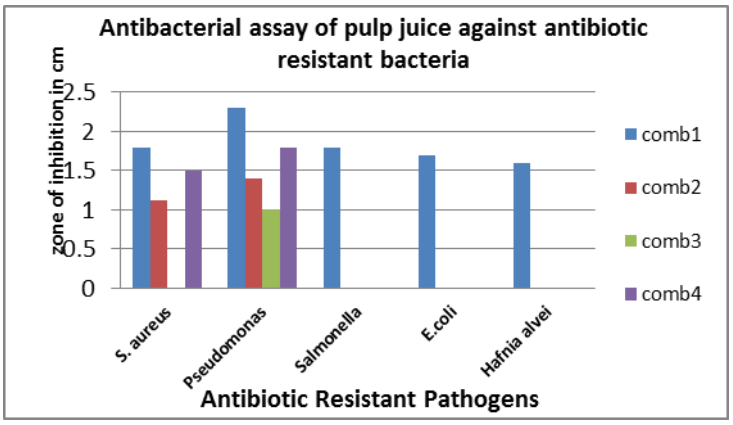
Antibacterial assay of pulp juice against antibiotic resistant bacteria

Imipenem, Monocef and Taxin resistant *S. aureus, Pseudomonas, Salmonella, E. coli* and *Hafnia alvei* isolated in our laboratory from contaminated drinking water and burn wound, were found to be sensitive to lemon pulp juice but not to the orange and sweet lime. It was observed that all these resistant organisms were sensitivity lemon pulp juice (Fig 10).



S. aureus *E. coli* *Pseudomonas* *Hafnia alvei* *Salmonella*

Figure 10: Antibiotic resistant bacteria sensitive to pulp juice



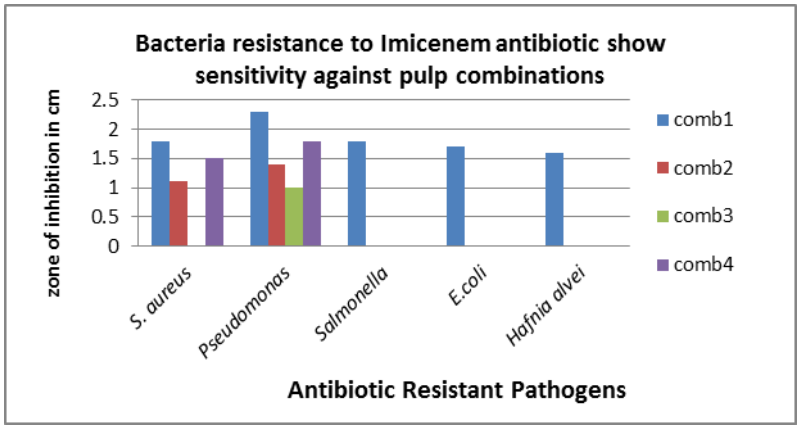
Graph 9: Antibiotic resistant bacteria sensitive to pulp juice

Antibacterial assay of pulp combinations juice against antibiotic resistant bacteria

Imipenem, monocef and taxin antibiotic resistant organisms isolated in our laboratory were used as test organisms. Combination 1 showed more activity. Of all the tested organisms *Pseudomonas* showed more sensitivity (2.3cm) than others (Fig 11).

S. aureus *Pseudomonas* *Salmonella* *Hafnia alvei* *E.coli*

Figure 11: Antibiotic resistant Bacteria sensitivity to pulp combinations



Graph 10: Bacteria resistance to antibiotic, show sensitivity against pulp combinations

Temporal activity for pulp juice and its combinations to *E. coli*

We observed that the activity of lemon pulp juice and its combination decreases by increase in time period. Fresh lemon pulp showed 2.4 cm zone of inhibition which decreased to 1.8 cm after 3hrs. Similarly fresh combination 1 showed 2.4 cm zone of inhibition and decreased to 1.7 cm after 3hr.

Pulp	Fresh	1hr	2hr	3hr
Lemon	**2.4cm**	2.2cm	2cm	1.8cm
Orange	0cm	0cm	0cm	0cm
S.lime	0cm	0cm	0cm	0cm

Combination	Fresh	1hr	2hr	3hr
Comb1	**2.4cm**	2.3cm	1.8cm	1.7cm
Comb2	0cm	0cm	1.4cm	1.3cm
Comb3	0cm	1.4cm	1.4cm	1.3cm
Comb4	1.9cm	1.5cm	1.4cm	1.3cm

Table 2: Temporal activity for pulp juice and its combinations against *E. coli*

Antimicrobial activity of silver Nano particles synthesized from pulp juice

It was observed that the antimicrobial activity of pulp juice AgNPs increased by increase in time period. It was also observed that *S. aureus* is more sensitive to the pulp juice AgNPs than other bacteria.

Table 3: Antimicrobial activity of pulp juice silver NPs

	After 4 hrs			After 6 hrs			After 24 hrs		
	Lemon	Orange	S.lime	Lemon	Orange	S.lime	Lemon	Orange	S.lime
E.coli	2.4cm	1.4cm	1.2cm	2.6cm	1.7cm	1.5cm	3.0 c m	1.9cm	1.7cm
S.aureus	2.1cm	0.9cm		2.4cm	1.1cm	0.9cm	2.7cm	1.3cm	0.9cm
S.epidermis	3.0cm	2.2cm	1.6cm	3.3cm	2.5cm	1.9cm	3.5cm	2.5cm	2.1cm
Pseudomonas	2.0cm	1.6cm	1.1cm	2.2cm	1.7cm	1.3cm	2.5cm	1.9cm	1.5cm
Shigella	2.1cm	1.5cm	1.1cm	2.4cm	1.7cm	1.2cm	2.5cm	1.8cm	1.3cm
Bacillus	1.9cm	0.8cm	0.9cm	2.1cm	1.0cm	1.1cm	2.3cm	1.1cm	1.4cm

Discussion

In this study it was observed that the lemon juice was more active against the Gram positive than Gram negative ones. Our findings, also agree with an earlier report (11, 12), that Gram negative organisms are mostly resistant to bioactive agents (due to the nature of their cell wall) than the Gram positive. Similarly in another study, essential oils from citrus fruits showed good activity against Gram positive pathogens [13, 14].

Fusariun oxysporum did not show any sensitivity against any pulp juice but in a different study it was showed to be sensitivity against oils synthesized from citrus fruits [15].

Temporal studies showed that the antibacterial activity of lemon juice decreased with time, but after 3 hrs all combinations showed inhibitory activity, which can be attributed to the time requirement for the components of the juices to interact and result into compounds that show the synergistic effect. Synergistic interaction between two agents means that their joint effect is stronger than the sum of effects of the individual agents [16]. Investigation of the antimicrobial activity of lime extract alone and in combination with other substance mixture or herbs has been investigated [17]. Similarly, in the present study we observed that when the lemon juice was combined with different citrus fruit juices, its antibacterial activity was enhanced. Interestingly, the synergistic effect was also observed when all three juices were assayed on the same plate. Also, according to most researchers, lime extract which is known to be very potent in treatment of infectious diseases and ailments when used alone and in combination with other herbs / solvent / extract, ranks fifth in antimicrobial potency when compared with the other forms and type of solvent in which, it is used locally [18]. In Sub-Saharan Africa, especially West Africa, including Ghana and Nigeria, herbal medicines are believed to be more effective when taken in combination [14].

The present study also made an approach of biosynthesis of silver nanoparticles using fruit juices as reducing agents. It was observed that lemon juice which was effective against all test organisms; NPs of it were more effective. Also, the sweet lime and orange juices which had no / less activity, NPs of them showed activity for all test organisms. Contradictorily, a similar study showed that Gram positive bacteria were relatively resistant to the bactericidal action of the NPs synthesized from Lemon, orange and sweet lime, than Gram negative bacteria and orange juice NPs were more effective in their antibacterial activity than those from other fruit juices [19]. The antibacterial activity of the nanoparticles may be centred on permeability of bacterial cell wall layers or its charges [20]. Studies have shown that nanoparticles may infiltrate the cells causing intracellular loss leading to cell death and this inhibition depends on the concentration of AgNPs [21].

Conclusion

The approach of use of citrus fruit juices for biosynthesis of nanoparticles can be adapted to serve as a good pharmaceutical tool in the production of drugs that could be used to combat infections. The method is cost effective and environmental friendly. To conclude, we suggest inexpensive, nontoxic, ecofriendly and abundantly available citrus fruits to be used in combinations against most human pathogens.

Acknowledgement

Authors are thankful to Dr. D. Y. Patil Arts, Commerce & Science College, Pimpri, Pune for providing facilities available at their laboratory.

References

1. Abhishek Mathur , Satish K. Verma, Reena Purohit, Vinay Gupta, VK Dua, GBKS Prasad, Deepika Mathur, Santosh K. Singh and Shivsaran Singh.. Evaluation of *in vitro* antimicrobial and antioxidant activity of peel and pulp of some citrus fruits. *Journal of Biotechnology and Bio therapeutics*. (2011); **1**: 1-17.
2. Akhilesh Khushwaha, Raghvendra Pratap Singh, Vikas Gupta, Madhulika Singh Antimicrobial properties of peels of citrus fruits. *International Journal of Universal Pharmacy and Life Sciences*. (2012); 2(2): 24-38.
3. Amita Tomar, Mridula Mall and Pragya Rai. Pharmacological importance of citrus fruit. *International journal of pharmaceutical science and research.* (2012); **4**: 156- 160.
4. Amiya kumar Prusty, Prasanna parida. Green Synthesis of Silver Nanoparticle Using *Eichhornia crassipes* and Study of in-vitro Antimicrobial Activity. *Scholars Academic Journal of Pharmacy.* (2014); 3(6): 504-509.
5. Donatus Ebere Okwu. Citrus fruits: a rich source of phytochemicals and their roles in human health. *Int. J. Chem. Sci*. (2008); 6(2): 451-471.
6. Susheela Sharma, Sunil Kumar, B.D. Bulchandani, Shalini Taneja and Shelza Banyal, Green Synthesis of Silver Nanoparticles and their Antimicrobial Activity against Gram Positive and Gram Negative Bacteria. *International Journal of Biotechnology and Bioengineering Research.* (2013); 4 (7): 711-714.
7. Gong, P.; Li, H.; He, X.; Wang, K.; Hu, J.; Zhang, S. & Yang, X. Preparation and antibacterial activity of Fe_3O4 Ag nanoparticles. *Nanotechnology*. (2007); 18(28): 604-611.

8. Shreshtha Verma, S. Abirami and V. Mahalakshmi, Anticancer and antibacterial activity of silver nanoparticles biosynthesized by *Penicillium spp.* and its synergistic effect with antibiotics. *J. Microbiol. Biotech. Res*. (2013); 3(3): 54-71.
9. Bansode D. S and Chavan M. D. Study on antimicrobial activity and phytochemical analysis of citrus fruit juices against selected enteric pathogen. *International research Journal of Pharmacy.* (2012); **3**: 122-126.
10. Themis Collares Antunes1, Marcela Proença Borba1, Cristina de Castro Spadari, Ana Lúcia Antunes, Ana Paula Guedes Frazzon, José Carlos Germani, Sueli Van Der Sand. Screening of actinomycetes with activity against clinical isolates of gram positive cocci with multiresistant profile. *Journal of Advanced Scientific Research.* (2014); **5**(1): 13-17.
11. Gordana R. Dimic, Suncica D. Kocic-Tanackov, Olivera O. Jovanov, Dragoljub D. Cvetkovic, Sinisa L. Markov and Aleksandra S. Velicansk. Antibacterial activity of lemon, caraway and basil extracts on *Listeria* spp. *Acta* Periodica Technologica, (2012); 43: 239-246.
12. Hugo, W.B., Russel, A.D., and Brown, S.S. *Pharmaceutical microbiology*: Black well scientific publication, Oxford. (1991); 265-274.
13. Shalu Hasija, Geeta Ibrahim, Ashok Wadia Antimicrobial Activity of *Citrus Sinensis* (Orange), *Citrus Limetta* (Sweet Lime) and *Citrus Limon* (Lemon) Peel Oil on Selected Food Borne Pathogens. *International Journal of Life Sciences Research*. (2015); 3(3): 35-39.
14. Praphulla Rao, Chandraprasad M S, Lakshmi YN, Jahnavi Rao, Aishwarya P, Srinidhi Shetty. Biosynthesis of Silver Nanoparticles using Lemon Extract and its Antibacterial Activity. *International Journal of Multidisciplinary and Current Research*. (2014); 2: 165-169.
15. Amita Tomar, Mridula Mall and Pragya Rai, Pharmacological importance of citrus fruits. *International Journal of Pharmaceutical Science and Research*. (2013); 4 (1): 156-160.
16. Herodez S.S., Hadolin M., Skerget M., Knez Z. Solvent extraction study of antioxidant from Balm (*Melissa officinalis* L.) leaves. *Food Chem*. (2003); 80: 275-282.
17. Lourdes Rodriguez-Fragoso, Jorge Reyes Esparza, Scott W. Burchiel, Dea Herrera Ruiz, Eliseo Torres, Risks and benefits of commonly used herbal medicines in Mexico. *Toxicology and Applied Pharmacology*. (2008); 227(1): 123-135.
18. Hungund BS, Dhulappanavar GR, Ayachit NH. Comparative Evaluation of Antibacterial Activity of Silver Nanoparticles Biosynthesized Using Fruit Juices. *J Nanomed Nanotechnol*. (2015); 6: 271. doi:10.4172/2157-7439.1000271
19. Nwanko, I. U., Osaro-Matthew, R C and Ekpe, Synergistic Antibacterial Potentials of *Citrus aurantifolia* (Lime) and honey against some bacteria isolated from sputum of patients attending federal Medical Center Umuahi, *Int. J. Curr. Microbiol. App. Sci*. (2015); 4(4): 534-544.
20. Chamakura K, Perez-Ballestero R, Luo Z, Bashir S, Liu J Comparison of bactericidal activities of silver nanoparticles with common chemical disinfectants. *Colloids Surf B Biointerfaces*. (2011); 84: 88-96.
21. Carmen Losasso, Simone Belluco,Veronica Cibin, PaolaZavagnin, Ivan Miceti, Federica Gallocchio, Michela Zanella , LisaBregoli, Giancarlo Biancotto and Antonia Ricci Antibacterial activity of silver nanoparticles: sensitivity of different Salmonella serovars. *Front. Microbiol*. (2014); 5: 227.