International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2023; 11(2): 44-50
Received: 18-12-2022
Accepted: 23-01-2023

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Alternative medicine for urinary tract infections (UTI) against *E. coli* and other bacterial population

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DOI: https://doi.org/10.22271/flora.2023.v11.i2a.859

Abstract
Urinary tract infection (UTI) is one of the major diseases faced by females. The main causative organism is *E. coli*. Though this bacterium is harmless and present in large intestine as symbiotic relationship but when it comes in contact with vagina it causes severe infection and warrants use antibiotics. As use of antibiotics leads to antibiotics resistant bacteria there is need to develop alternative medicines those may be used to fight this menace, there are several traditional medicines being used by native people and present huge scope for new drug discovery. This project work aimed at such minor research. Leaves of guava has been used traditionally to treat common fever, skin infections and others. To validate such traditional claims, it was put under the study whether this could work against *E. coli* bacteria. The aqueous leaf extracts of chosen guava plants *Psidium guajava* L. were prepared. These were studied for their antibacterial property against *E. coli. P. guajava* L. leaf extract showed satisfactorily results and zone of inhibition was shown in case of *E. coli.* 500µl *P. guajava* extract concentration gave best results on *E. coli* culture plate, showing no growth at all. For comparison with other microbes, when leaf extract was tested, no live microbes were seen in *S. aureus* culture broth, when *P. guajava* extract was added and incubated. This culture also showed decrease OD level each day, showing low microbial count. *E. coli* culture broth showed a little growth with the addition of extract. *Pseudomonas* and *Klebsiella* did not respond at all.

Keywords: Antimicrobial, guava *Psidium guajava L.*, *E. coli*, MIC, traditional medicines, antibiotics, UTI

Introduction
Urinary tract infections are a serious public health problem caused by a variety of pathogens, the most common of which are *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis*, and *Staphylococcus saprophyticus*. Urinary tract infections (UTIs) are one of the most common bacterial infections, affects an estimated 150 million people worldwide each year [1]. Female gender, a previous UTI, vaginal infection, sexual activity, diabetes, obesity, and genetic susceptibility are risk factors for cystitis [2]. Uropathogenic Escherichia coli (UPEC) is the common causative agent of both simple and complicated UTIs (UPEC). For the agents involved in uncomplicated UTIs, UPEC is followed in prevalence by *Klebsiella pneumoniae*, *Staphylococcus, saprophyticus, Enterococcus faecalis*, Group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp [3].

Some of the other factors that can contribute to the risk of UTIs are
Sexual activities, Spermicides, Diabetes, Congenital disabilities and/or pathological defects (Example: Prostrate enlargement) in the structure of UTS, blocking proper urine flow, Pregnancy, Age of the patient (both aged population and younger kids are more likely to Contract UTIs than the middle age groups), Congenital disabilities and/or pathological defects (Example: Prostrate enlargement) in the structure of UTS, blocking proper urine flow, Adults and infants both have poor hygiene practices (who are still potty-training), UTIs have a history of recurring, Changes in vaginal microflora can be caused by menopause or by using certain medications, Advanced aged groups who are in nursing homes, Patients experiencing urinary retention, Medical conditions that necessitate the use of a urinary catheter, Incontinence of the bowel, Kidney calcification, Staying immobile for an extended period of time, as well as following surgery or a fracture recovery (Example: Immobile state due to hip fracture recovery) Surgery or other UTS-related procedures.

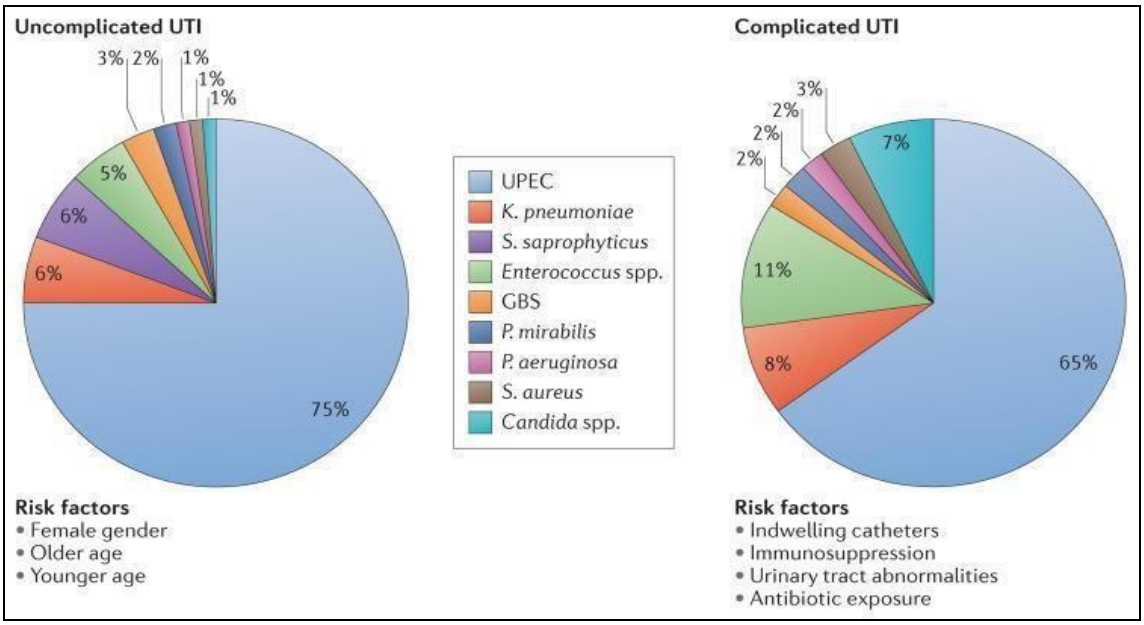

Fig 1: Uncomplicated v/s Complicated UTI (Ana L. Flores-Mireles *et al*., 2015) [11]

Doctors currently recommend a variety of antibiotics for UTI patients, including Cefixime, Amoxicillin, Ciprofloxacin, Cefprozil, and others. However, frequent or long-term use of these can lead to microbial resistance as well as a variety of side effects in patients [4, 5]. *Psidium guajava* (commonly known as guava) is a well-known tropical tree that is widely grown for its fruit. *Psidium guajava* and its constituents have a long history of medicinal use [6]. It contains a high concentration of antibacterial and antimicrobial compounds [7]. Ethanolic extracts of the stem have potent anti-diabetic properties [8]. Guava is high in antioxidants and phytochemicals, such as essential oils, polysaccharides, vitamins, minerals, enzymes, and alkaloids, triterpenoid acid, glycosides, steroids, tannins, flavonoids, and saponins [9]. Saponin, lyxopyranoside, Guaijavarin oleanolic acid, arabopyranoside, quercetin and flavonoids are found in the fruit [10].

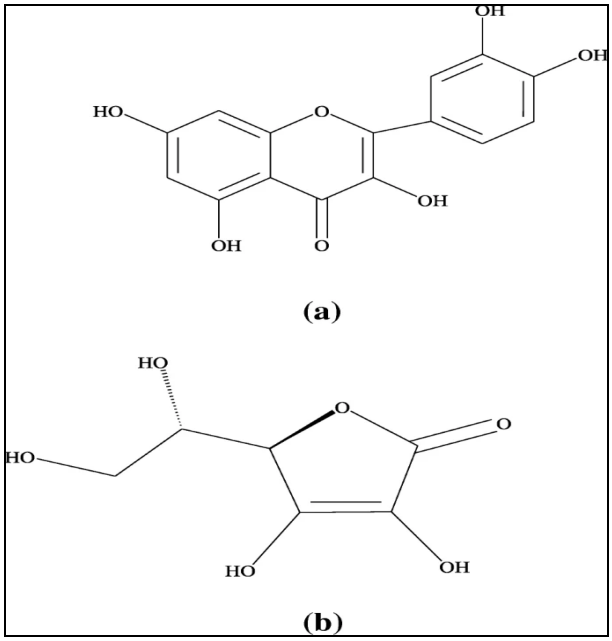

Fig 2: a) Chemical structure of quercetin **b)** Chemical structure of ascorbic acid

Keeping in mind the historical context, important ingredients, and common applications of Psidium guajava (guava), current research focuses on the phytochemistry and medicinal value of this useful plant.

Material and Methods

Following media and microbes were used: Culture media and sources: Nutrient broth (HiMedia laboratories, India) Agar (HiMedia laboratories, India); Distilled water made from ELGA Purelab Option, India; Luria Bertanni (HiMedia laboratories, India); Nutrient Agar (HiMedia laboratories, India) Antibiotic Discs used were: Streptomycin, 25 mcg / disc (HiMedia laboratories, India) Neomycin, 30 mcg / disc (HiMedia laboratories, India) Kanamycin, 30 mcg / disc (HiMedia laboratories, India) Gentamycin, 10 mcg / disc (HiMedia laboratories, India). Microbes used were: Escherichia coli ATCC25922, Staphylococcus aureus ATCC29313, Pseudomonas aeruginosa ATCC27853, and Klebsiella pneumonia ATCC 13883. Following methods were used for the studies: All processes were done under standard

microbiological practices. Bactericidal activities of plant extract were studied against *E. coli* microbes. Guava plant leaves was used. Extract and LB (Luria Bertani) agar plates were prepared. Microbes were cultured and tested against these known microbes. Different concentration and combination were studied. Optical density was also checked for suitable results.

a) Preparation of Leaves Extracts

Leaves of guava plants were taken and dried in the oven at 40 degrees Celsius for 2-3 days. These were grinded in the pestle and mortar, powdered and weighed. 50g of this dried powder was taken in one flask and 1000ml of Distilled Water (D/W) was added. These mixtures were boiled at 100°C for 4hours. After boiling, the extract was filtered through Whatman filter paper. The extract was then concentrated up to 100ml. volume. Half of the extracts were autoclaved and half were used as boiled to see effect of high temperature on activity of extracts. Their antimicrobial activities were checked on *E. coli* and other bacterial population and were compared with that of different antibiotic discs.

b) Preparation of LB broth

In one-liter distilled water, 10gm peptone, 5gm sodium chloride, 5gm yeast extract was added in a conical flask. It was autoclaved at 121 degrees Celsius for 30 minutes at 15lbs pressure. Broth was stored in refrigerator till used.

c) Preparation of LB agar

In one liter D/W 10gm peptone, 5gm sodium chloride, 5gm yeast extract, 10 gm agar was added, boiled to melt agar, poured into petri plates and plates were autoclaved at 121 degrees Celsius for 30 minutes.

d) Preparation of cultures

Inoculated the *E. coli* bacteria in agar plates, streaked properly and incubated for the 24 hours at 37 degrees Celsius.

3. Results and Discussion

Effect of Plant extracts on *E. coli*:

Here are the figures of the nutrient agar plates cultured with *E. coli* along with plant extract and antimicrobial discs in wells:

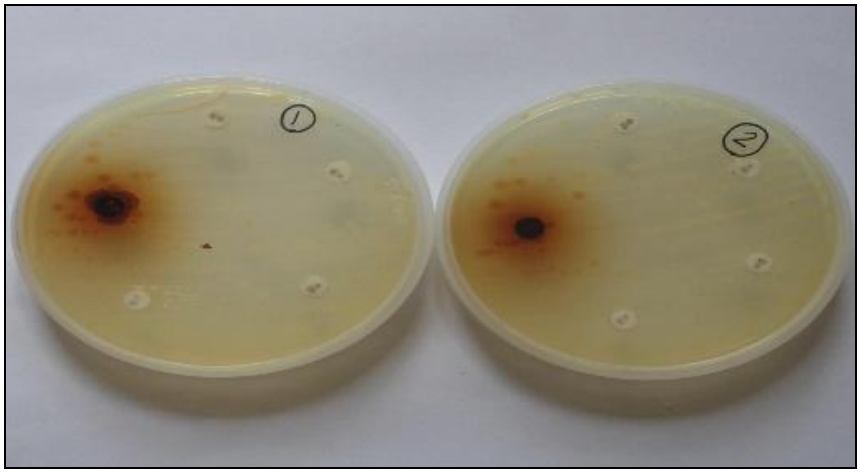

Fig 3: Effect of mixture of *P. guajava* extract on *E. coli*. Autoclaved extract (left, plate 1) and Boiled extract (right, Plate 2) both showing same effect.

In this case, *P. guajava* extract showed very good result as there were excellent zone of inhibition up-to 5mm (Fig. 3). Zone of inhibitions shown by extracts was better than in case of antibiotic discs. Plant aqueous extracts were used in the concentration of 0.5g/ml and each well contained 50µl of extract. By calculations, the amount of dried plant extract in each well is 25mg. Thus, it was observed that 25mg/well of *P. guajava* is active against *E. coli*, as compared to 10mcg Gentamycin, 30mcg Neomycin, 30mcg Kanamycin and 25mcg Streptomycin. This explains that by using more concentration of extracts (mainly *P. guajava*), the effect can be increased. Also, there was no difference found in autoclaved and boiled plant extract used, which clear cut indicates that the compounds in plant leaves taken were heat resistance and did not get destroyed by boiling or autoclaving.

***E. coli* culture plates with different concentrations of *P. guajava* extract:**

Four *E. coli* culture plates were prepared by spreading 200µl culture on NA plates to generate *E. coli* mat on agar plates. After incubating them for 24hrs, *P. guajava* extract was poured in different concentrations (100µl, 500µl and 1000µl per plate). Growth was observed and compared with the control. Following were the results:

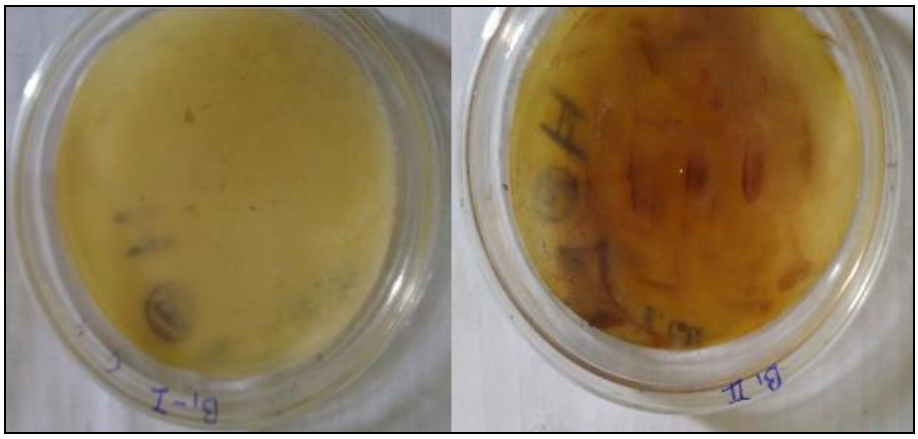

Fig 4: *E. coli* culture plate, as control (left) and with 100µl of *P. guajava* extract (right).

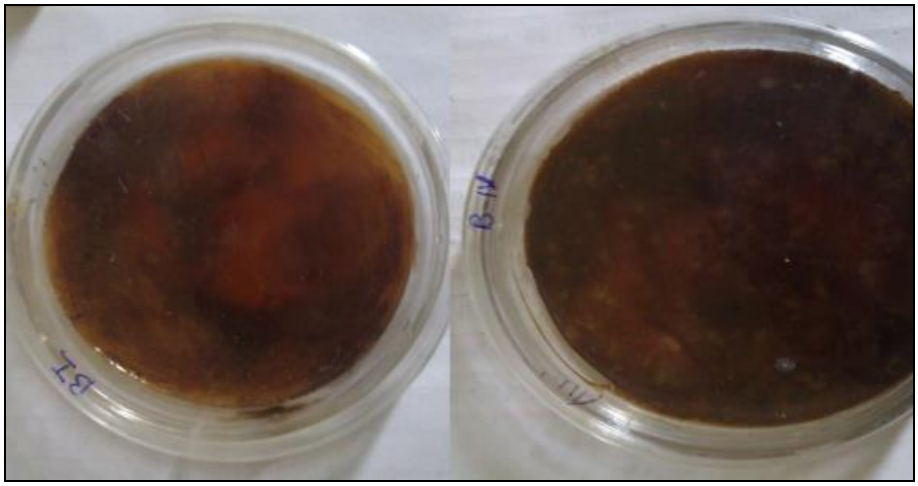

Fig 5: *E. coli* culture plate with 500µl (left) and 1000µl of *P. guajava* extract (right).

After comparing the microbial growth on plates with control, it was seen that plate with 100µl showed some growth of *E. coli* (Fig. 4 right) whereas, plates with 500µl and 1000µl showed no growth at all (Fig. 5). This explains that 500µl and more volume of *P. guajava* extract is sufficient to kill the *E. coli* growth. This data can be further specified by using different concentrations and volumes of extract.

NA plates with different concentration of *E. coli* and *P. guajava* extract:

NA plates were prepared in sterile conditions. Mixture of *P. guajava* extract and *E. coli* culture broth were spread in different volumes (nil: 600µl; 100µl: 500µl; 200µl: 400µl; 500µl: 100µl), respectively. After incubation for 24hrs microbial growth was observed and compared with that of control. Here are the results:

Fig 6: NA plate, without any extract and 600µl *E. coli* broth (left), with 100µl extract and 500µl *E. coli* broth (right)

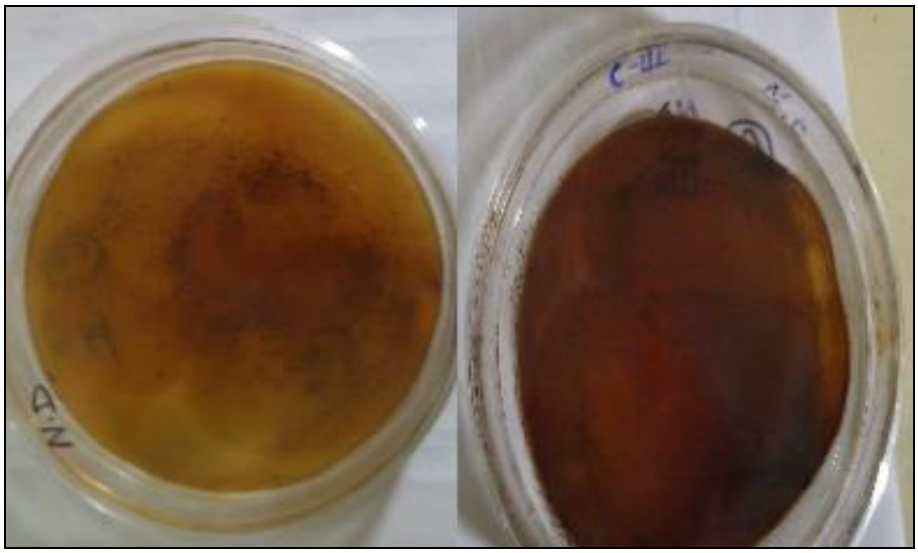

Fig 7: NA plate, with 200µl extract and 400µl *E. coli* broth (left), with 500µl extract and 100µl *E. coli* broth.

Comparing microbial growth with control plate, it was seen that plate with only 100µl of extract and 500µl *E. coli* showed more microbial growth (Fig. 6 right). On the other hand, plate having 200µl extract and 400µl *E. coli* showed less growth (Fig. 6 left). Also, plate with 500µl extract and 100µl *E. coli* showed no growth at all (Fig. 7 right). This indicates that increasing doses of *P. guajava* extract were more effective against *E. coli*. Combining the above two results in case of *E. coli*, it can be said that 500µl of aqueous *P. guajava* extract, prepared from the original concentration of 0.5g/ml extract, sufficiently killed 200µl of *E. coli* culture. Further optimization is required. After, observing positive results of guava leaf extracts on *E. coli*, three other microbes were also tested for comparing the results though it was not the part proposed project work. So, *P. guajava* was further studied for its bactericidal property on known pathogens *viz*, *E. coli*, *Klebsiella*, *Pseudomonas* and *Staphylococcus aureus*. Different experiments those were done are given below along with their results:

Nutrient broth with different concentrations of known pathogens and *P. guajava* extract

Conical flasks with different combinations of four known pathogenic microbial culture and *P. guajava* extract were prepared in 50ml of NB each. O.D of broth was taken daily for four days. The results of O.D at 600nm are given below in the Table 1:

Table 1: Effect of *P. guajava* extract on four different microbes in nutrient broth and O.D at different time intervals.

Time Of Incubatio n At 37˚C	5ml GE + 50ml NB	1ml *E.col I* + 50ml NB	1ml *Pseudomona s* + 50ml NB	1ml *E.col i* + 5ml GE + 50ml NB	1ml *Pseudomona s* + 5ml GE + 50ml NB	1ml *Klebseila* + 5ml GE + 50ml NB	1ml *S.aureus* + 5ml GE + 50ml NB
24 hrs	3.36	0.548	0.32	3.527	3.098	3.745	3.49
48 hrs	3.28	0.605	0.339	3.67	3.25	3.68	3.28
72 hrs	3.27	0.612	0.334	3.459	3.27	3.85	2.764
96 hrs	3.21	0.619	0.337	3.389	3.269	3.912	2.748

GE- *P. guajava* extract; NB- Nutrient broth. O.D. of flasks containing *S. aureus* and *E. coli* along with *P. guajava* extract decreased each day. This shows the decrease in microbial content due to the presence of *P. guajava* extract. *S. aureus* showed the best result. Debris of dead microbe was seen at the bottom of flask in its case after 48 hrs. No live microbes were apparent. To confirm this, 5ml of this broth treated with *P. guajava* extract and *S. aureus*, was inoculated in sterile Nutrient broth and was incubated for 72hrs at 37 degrees Celsius. There was no turbidity or growth of *S. aureus* in sterile broth. *Pseudomonas* and *Klebsiella* did not show any decrease in O.D. and hence showed no effect of *P. guajava* extract on them.

Streaking of NB after 96hrs on NA plates

To confirm effect of *P. guajava* after 96hrs of incubation, all treated broth (*P. guajava* extract with four known microbes) were streaked on NA plates and incubated for 24hrs at 37 degrees Celsius. The growth and survival of microbes was compared with their respective controls. Below are given the figures of those plates:

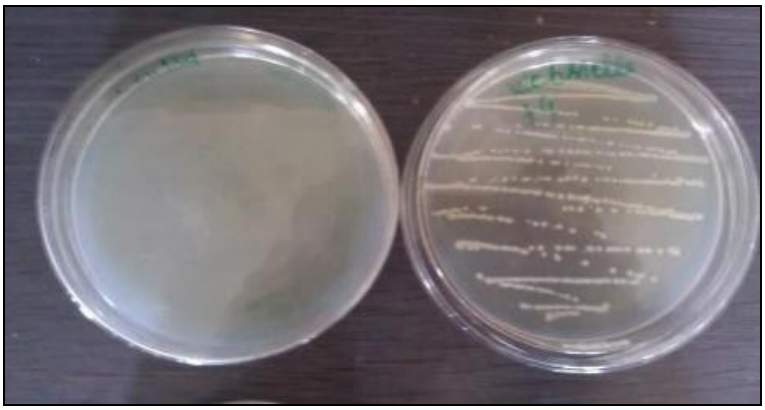

Fig 8: NA plate, as a control (left) and with *Klebsiella* and *P. guajava* extract (right)

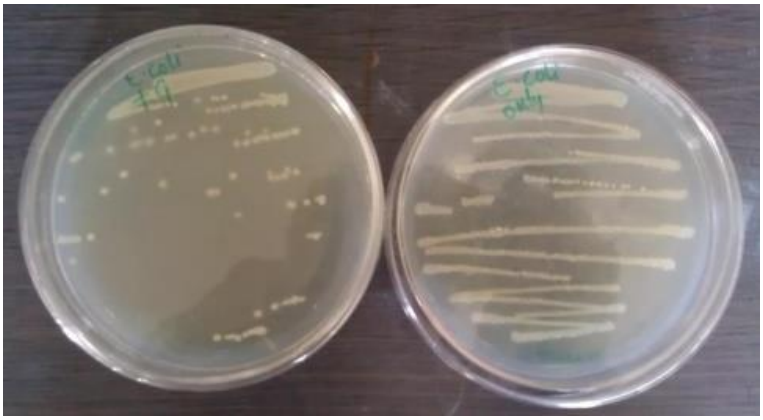

Fig 9: NA plate, with *E. coli* and *P. guajava* extract (left) and with only *E. coli* (right).

Fig 10: NA plate, with *Pseudomonas* and *P. guajava* extract (left), and with only *Pseudomonas* (right).

Fig 11: NA plate, with only *S. aureus* (left), and with *S. aureus* and *P. guajava* extract (right).

NA plate streaked with broth containing *P. guajava* extract and *S. aureus* did not show any growth of *S. aureus* (Fig. 11). Also, *E. coli* plate along with extract showed comparatively less growth (Fig. 9). However, there was no effect seen on

Klebsiella and *Pseudomonas* (Fig. 8 and Fig. 10). This may be due to high resistance of these pathogens towards *P. guajava* extract. The above results clearly explain strong antimicrobial effect of *P. guajava* leaf extract on *S. aureus*.

Conclusion

The aqueous leaf extracts of chosen guava plants *Psidium guajava* were prepared. These were studied for their antibacterial property against *E. coli. P. guajava* leaf extract showed satisfactorily results and zone of inhibition was shown in case of *E. coli*. 500µl *P. guajava* extract concentration gave best results on *E. coli* culture plate, showing no growth at all. For comparison with other microbes, when leaf extract was tested, no live microbes were seen in *S. aureus* culture broth, when *P. guajava* extract was added and incubated. This culture also showed decrease OD level each day, showing low microbial count. *E. coli* culture broth showed a little growth with the addition of extract. *Pseudomonas* and *Klebsiella* did not respond at all.

Acknowledgement

I duly acknowledge generous support of Mr. Ramesh Kumar (my father), Mrs. Kunti (my mother), Dr. Puran Chand (Grandfather), Sh. Mahendra Kumar (uncle), Mr. Karan Kumar (my elder brother), Dr. Anil Sharma (HOD, Biotech department), Dr. Ankush (my friend). Above all, my project supervisor Prof. Ajay Gupta's all-round help and vital supports, at my most critical time, are most deeply acknowledged and shall always be remembered.

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