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Assessment of the Antibacterial activities against Gram-negative bacteria of selected fruit peels.

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ABSTRACT
Antibacterial activity of extracts was investigated by Agar well diffusion assay. The effect of different solvents on the antibacterial activity of orange, lemon and grapefruit peels collected from three different geographical regions i.e Burewala, Rahim Yar Khan, Lahore was estimated. Results showed that solvents have significant effect on antibacterial activity of fruit peels. Sample extracted in methanol showed more antibacterial activity than ethanol extracts. We also detected climate effects on antibacterial activity. Lahore samples showed more activity as compare to other cities

CHAPTER-1

1. Introduction

Fruits and vegetables have huge amounts of biologically active contents that are responsible for their massive health care advantages. Functions responsible for these benefits are anticancer, immunity development, antiviral, detoxification, and antioxidant properties [1].

Citrus fruit tree is a small tree with alternate leaves, mostly evergreen, with a strong sweet fragrance of flowers, round or egg-shaped berries. Their fruits contain 8-14 juicy parts having large seeds that are greenish or white. They are conventionally used to cure sore throats, indigestion, alleviate intestinal gas and bloating, minimize phlegm, and as a food flavoring additive. Citrus limon, Citrus sinensis, Citrus paradisi, and Citrus reticulata crude extracts are mainly used to relieve high blood pressure, respiratory disorders, rheumatism, etc [2].

Citrus fruits are among the most common tree species in the world and their consumption and production have increased considerably since the 1980s. Oranges, tangerines, lemons, and limes are all increasingly growing in demand. Many different species are very likely to occur in the genus Citrus, including Citrus paradisi(grapefruit), Citrus sinensis (orange), Citrus deliciosa (mandarin), Citrus aurantifolia (lime), and Citrus limon (lemon). Owing to their various applications, citrus fruits are also of great economic importance[3]. Dried leaves have been commonly used in flavorings, care products, and medical synthesis as ingredients [4].

Citrus fruits are of worldwide importance and are among the most used fruits in the world after bananas, tomatoes, and mangoes. they provide a sufficient amount of minerals, pectin, vitamins ad dietary fibers. They are highly suggested due to their great bioactive activities like antibacterial, antioxidant, anti-inflammatory, strong anti-atherogenic properties, inhibition of blood coagulation, anti-tumor activities citrus fruits are used fresh and juice whereas the peels are thrown off as a waste. Studies have suggested that the peel waste of the citrus fruits contains huge amounts of the bioactive phytochemicals, that are being discarded as waste. From USD national nutrient database, the peels of the citrus fruits and some other like gava contains an appreciable amount of vitamins and minerals [5]. Citrus (lemon) is the most economic fruit in the world. It has a huge diversity in its species and almost 40 species are present worldwide. Studies have already been done to examine the antibacterial activity in the peels of the lemon. That showed that lemon peels are rich in antibacterial activity and a huge source of bioactive compounds. Thus we can also use lemon peels to enhance our immunity against microorganisms causing damage to our body [6].

1.1 Production stats

According to FAOSTAT estimates, in more than 140 countries, citrus species are grown globally, with more than 8.7 million hectares and around 131 million tons of fruit produced in 2012. And the world's leading citrus fruit-producing countries are

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China, Brazil, the U.S.A., India, Mexico, and Spain, comprising nearly two-thirds of global production. Citrus cultivation has traditionally existed in China, and the citrus varieties were naturally selected [7].In Pakistan, the annual production of citrus fruits is about 2.0 million metric tons approximately [8].

1.2 In Pakistan, massive citrus growing areas

Table 1:Massive citrus growing areas in Pakistan [8]

Province	Major districts
Punjab	Rahim Yar Khan, Lahore, Jhang, Sahiwal, Mianwali, Sialkot, Multan, Gujranwala
Khyber Pakhtunkhwa	Peshawar, Swabi, Mardan, Hazzara, Nowshera, Swat
Sindh	Sukkur, Khairpur, Nwabshaw
Baluchistan	Sibbi, Makran, Kech

1.3 Nutritional value

Citrus genera consist of such members that have naturally important nutritional value contains vital nutrients in ample amount. Fruits are a necessary part of the human's diet due to their vital nutritional and medicinal uses. They are naturally fat and cholesterol-free but they contain several other nutrients like calcium, potassium, and phosphorus, etc. There is a major source of vitamins, enzymes, and minerals. they are very light to our digestive system as they possess fiber quantity. These fruits are rich in ascorbic acid, fiber, and folic acid thus reduce cancer, cardio, and Cerebral diseases [9].

Citrus fruits are the commonly known rich source of ascorbic acid, besides this, they also contain several secondary metabolites which include flavonoids, alkaloids, carotenoids, and essential oils. These secondary metabolites perform many essential bioactivities of huge importance for our health. these activities include anticancer, anti-inflammatory, antibiotic activities [10].

1.4 Phytochemicals

Phytochemicals are also known as phytonutrients, naturally existing bioactive compounds. Fruits, vegetables, nuts, and seeds are rich in these phytochemicals. There are hundreds of phytochemicals, we had yet isolated a few from plants. commonly-known phytochemicals are Vitamin A, Vitamin C, Vitamin E, flavonoids, phenolic acid [11].

Fruits are the rich and main source of nutritional needs, whereas plants also produce some other chemicals that are not involved in nutritional values but are used by plants for their defense. When humans ingest these plants, they get nutritional benefits along with these phytochemical compounds. Many of the plants are used in medicinal fields. Many of them are used in home remedies. These compounds are present in a safe amount thus not toxic and help in the prevention of severe diseases [12].

1.5 Antibacterial activity and its importance

Antibacterial is the component that seizes and kill microbial growth like bacteria. And those substances that are produced from other organisms that can kill the bacteria are known as antibiotics. These antibiotics are used for human welfare and to cure disease but now the focus is to produce antibiotics through natural sources [6].

In the food industry controlling bacterial growth is a great experience it also important for preventing food from contamination and health care problems. In the last few years, many drug-resistant strains have been developed in our environment, one of the most important strains is the gram-positive bacteria Staphylococcus aureus [13].

Nowadays there is growing pressure of reducing chemical substances added in food products for the prevention of bacterial growth. Hence the directions are more diverted towards the natural sources i.e., plants or animals. Thus plant derives or plant-based substances are now preferably used as antibacterial activity [14].

1.6 Antibacterial activity in citrus fruit peels

Waste of citrus fruits like lemon, orange, and grapefruits are rich in bioactive compounds like

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flavonoids, vitamin C, polyphenols. All these substances are less in other plants while citrus fruit peels are rich in them. Antibacterial activity is directly related to these component [15].

Bacterial infections are a leading source of health issues, physical impairments, and death all across the world. Antimicrobials abundance in medicinal plants, rendering them a safer and more cost-effective option to treat bacterial infections. Natural compounds derived from medicinal plants have antibacterial properties that can be used to treat bacterial, fungal, and viral infections. In the previous three decades, pharmaceutical companies have developed a variety of novel antibiotics, yet microorganism resistance to these medications has increased. Plant antimicrobial action has been linked to secondary metabolites or phytochemicals such as phenols, flavonoids, alkaloids, terpenoids, and essential oil. The phenol and polyphenol groups of substances are made up of hundreds of different molecules with a varied structure but with a common phenol ring [16].

1.6.1 Compounds responsible for antibacterial activity

Citrus fruit peels contain flavonoids, phenolic acids, several vitamins in ample amount. These compounds have many bioactivities. These plants are rich in vitamins like Vitamin C, which shows antibacterial activity, and intake of them also reduces several diseases i.e scurvy. Antibacterial activity is directly related to the concentration of these compounds [15].

1.6.2 Structures of antibacterial compounds

Vitamin A

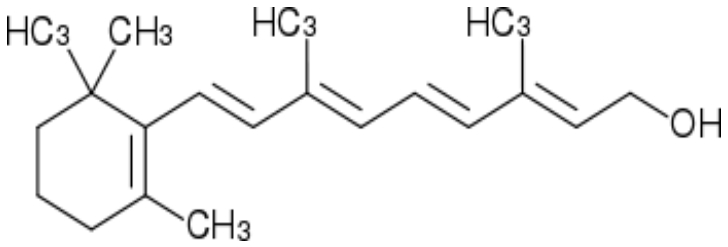

Figure 1:Chemical structure of vitamin A

Vitamin E

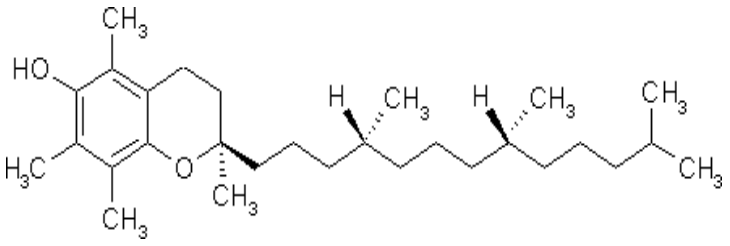

Figure 2:Chemical structure of vitamin E

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Vitamin C

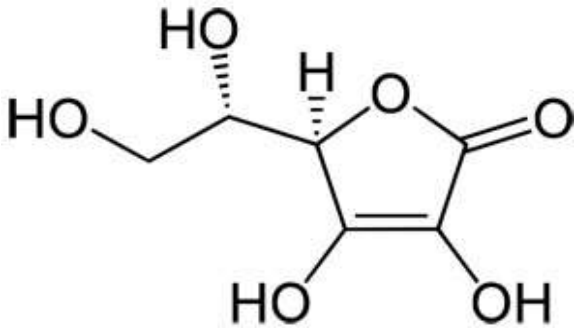

Figure 3:Chemical structure of vitamin C

Flavonoids

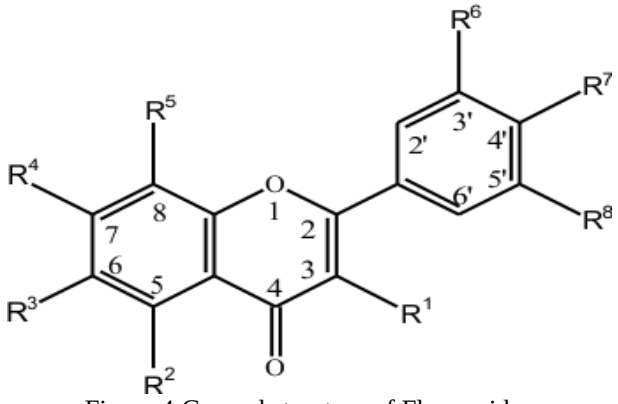

Figure 4:General structure of Flavonoids

Table 2: Substitution in the general structure for some of the main citrus flavonoids [17]

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
Flavanones								
Hesperidin	H	OH	H	O-rut	H	OH	OCH_3	H
Naringin	H	OH	H	O-nh	H	H	OH	H
Neohesperidin	H	OH	H	O-nh	H	OH	OCH_3	H
Narirutin	H	OH	H	O-rut	H	H	OH	H
Flavones								
Hesperetin	H	OH	H	OH	H	OH	OCH_3	H
Naringenin	H	OH	H	OH	H	H	OH	H
Flavone Aglycon								
Luteolin	H	OH	H	OH	H	OH	OH	H
Polymethoxyflavones								
Nobiletin	H	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	H
Tangeretin	H	OCH_3	OCH_3	OCH_3	OCH_3	H	OCH_3	H

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Phenolic acids

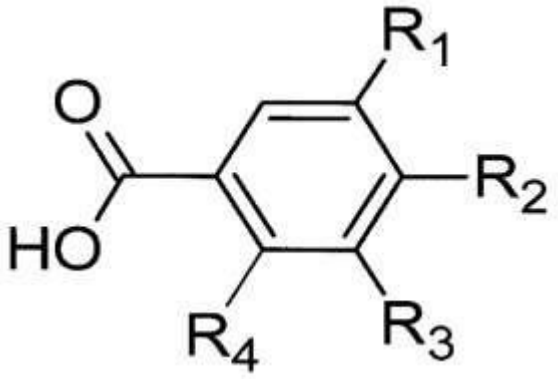

Figure 5:General structure of phenolic acids

Table 3: Substitution in the general structure for some of the main citrus phenolic acid.[18].

	R_1	R_2	R_3	R_4
Salicylic acid	H	H	H	OH
Gentisic acid	OH	H	OH	H
p-Hydroxybenzoic acid	H	OH	H	H
Protocatechuic acid	OH	OH	H	H
Vanillic acid	OCH_3	OH	H	H
Gallic acid	OH	OH	OH	H
Syringic acid	OCH_3	OH	OCH_3	H

1.7 Mechanism of antibacterial activity

Antibacterial compounds act in two ways i.e by killing the bacteria (bactericide) or by inhibiting the bacterial growth (bacteriostatic).These actions are performed by either any of these functions, by cell wall disturbance, ATP production, protein synthesis, pH disturbance, intracytoplasmic changes and several other functions like discussed above [19].

1.7.1 Vitamin C

E.coli bacteria are killed via Fenton reaction which produces highly reactive hydroxyl radicals. These radicals are generated by bactericidal antibiotics. Vitamin C is also a similar compound that regulates the Fenton reaction. Hydroxyl radicals cause bacterial death by DNA damage by oxidizing guanine nucleotide.

In aerobic conditions, bacterial cells metabolize vitamin C as a carbon source, as a result of which the cell undergoes oxidative stress, due to the formation of harmful products like acetate and lactate causing the bacterial cell to die [20].

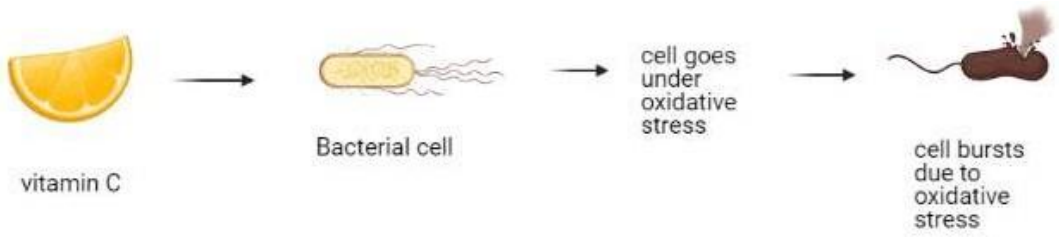

Figure 6:Mode of action of vitamin C

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1.7.2 Flavonoids

Mode of action of Flavonoids is by inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism [21].

Flavonoids show antibacterial activity mainly in three ways.

1) Nucleic acid formation is disturbed or inhibited for example in V. harvevi cells show inhibitory activity of DNA and RNA activity after the addition of genistein.
2) Cell membrane function inhibition for example galangin increases potassium loss from a bacterial cell membrane causing damage to it.
3) Energy metabolism is altered or inhibited when the outer and inner cytoplasmic membrane is disrupted causing a disturbance in the exchange of metabolites and nutrients causing inhibition of energy supply. Cell membrane synthesis inhibition is also a possible mechanism of antibacterial activity [22].

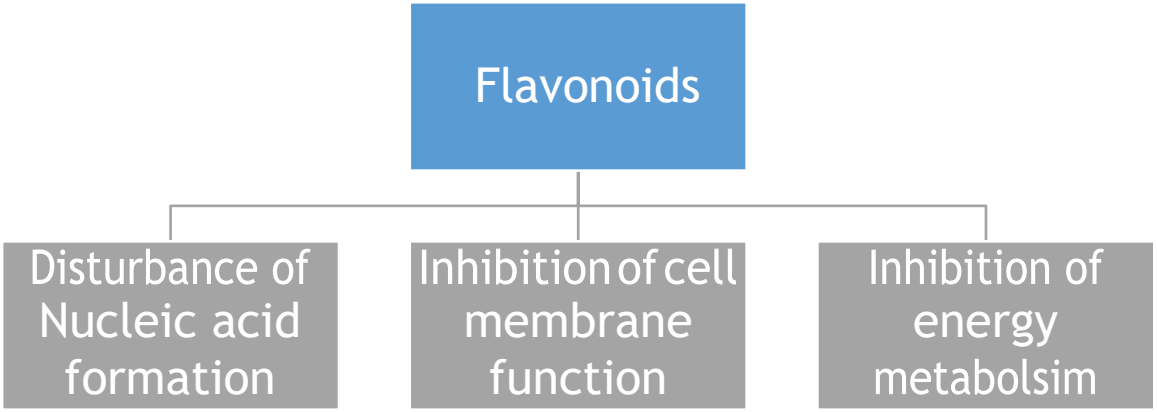

Figure 7:Modes of action of Flavonoids

1.7.3 Tannins

Tannic acid has good iron-binding capacity. In those bacteria which exists in aerobic conditions need oxygen for various function. It shows a strong inhibitory effect due to its strong iron-binding capacity.

Antibacterial mechanism of tannins is summarized as.

1) Tannins have the property to undergo complexation with substrates and enzymes. Thus, many enzymes when added with tannins get inhibited.
2) Tannins acts on membranes of bacteria causing tannins toxicity. Tannic acid shows inhibition in the growth of many intestinal bacteria such as Escherichia coli. Tannic acid binds to iron more efficiently than other acids such as gallic acid. It becomes chelate with iron and makes it unavailable to bacteria. Aerobic bacteria need iron for their growth and performing various functions such as heam formation and many other essential functions. This is due to the iron- binding capacity of tannic acid [23].

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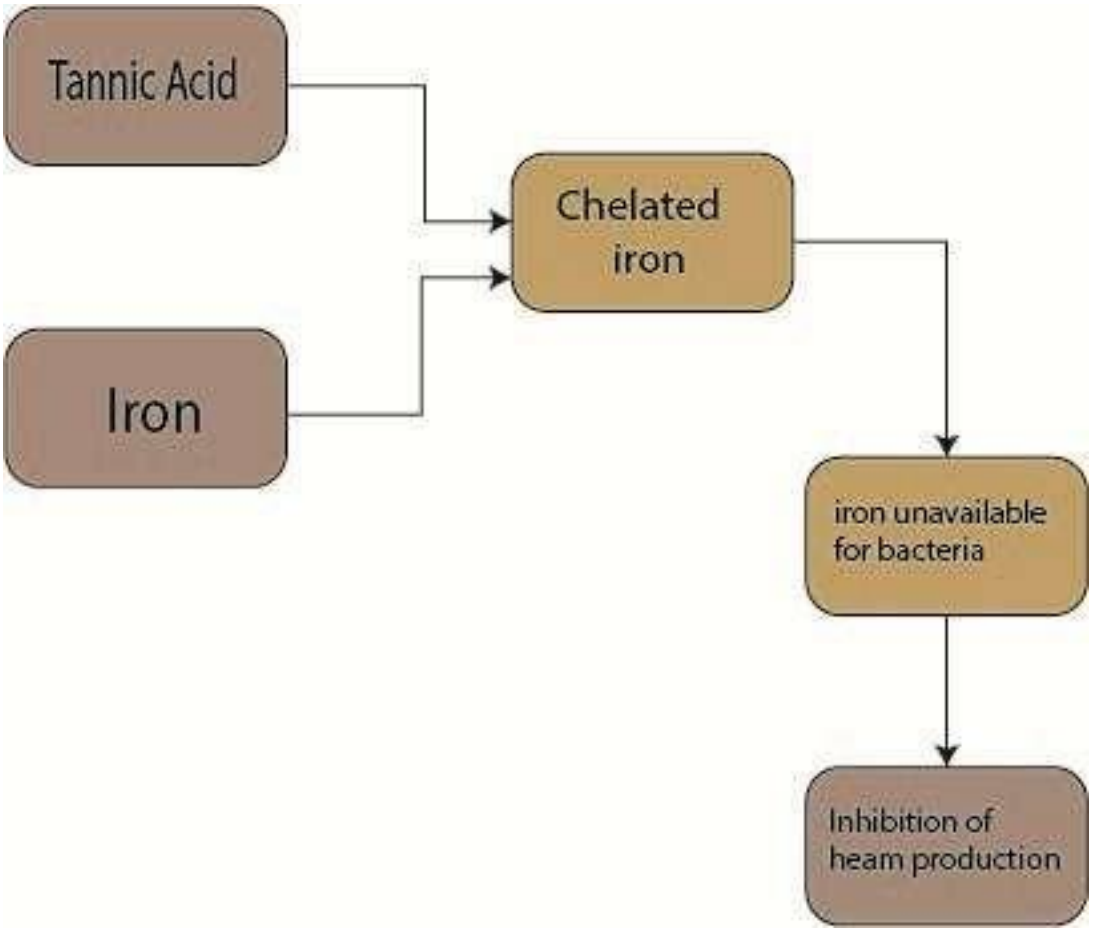

Figure 8:Mode of action of tannins

Table 4:Mode of action of antibacterial compounds.

	Mode of action	References
Vitamin C	It regulates Fenton's reaction as a result, hydroxyl radicals are produced which causes bacterial death by oxidizing guanine nucleotide.	[20]
Flavonoids	Mode of action of Flavonoids is by inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism	[22]
Tannins	Tannic acid has a strong iron-binding capacity, in aerobic bacteria, it shows strong inhibitory activity. As iron is essential for various functions like heam production.	[23]

1.8 Gram-Negative bacteria

It is generally known that both Gram-positive and Gram-negative bacterial strains present a significant public health concern. Antibiotics have controlled infections arising from both community and hospital environments over the years [24]. Pseudomonas aeruginosa, Escherichia coli, Proteus species, and Klebsiella pneumoniae are very common disease-causing bacteria separated from wounds [25].

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1.8.1 E.coli

Escherichia coli is the primary facultative anaerobe of the colonic human flora. Typically, within hours of birth, the organism colonizes the infant gastrointestinal tract and, after that, The shared advantage of E.coli and the host. However, also common "nonpathogenic" strains of E.coli remain harmlessly restricted to the intestinal lumen. In the damaged or immunosuppressed host, E.coli is present, or when gastrointestinal barriers are broken. E.coli can cause infections. In addition, one of the highly adapted E.coli may be vulnerable to infection even by the most robust members of our genus.The E.coli have evolved the ability to cause a large range of diseases in humans. Infections that emerge from pathogenic E.coli can be confined to the mucosal surfaces or can spread across the body. Infection with potentially pathogenic E.coli occurs in three general medical syndromes:

1) inflammation of the urinary tract,
2) sepsis and
3) diarrheal disease [26].

The morbidity and mortality linked with several recent major outbreaks of Shiga toxin-producing Escherichia coli (STEC) gastrointestinal disease has highlighted the serious harm to public health posed by these species. In countries with advanced health care systems, such diseases can overwhelm critical care services [27].

1.8.2 Pseudomonas aeruginosa

Common inhabitants of soil, freshwater, and aquatic environments are Gram-negative bacilli of the Pseudomonas genus. It is an opportunistic pathogen that causes human diseases, Pseudomonas aeruginosa attracts more attention. Bacteriocins are synthesized by most strains. The interest in this bacterium is attested by the full genome sequencing carried out in 2000 [28].

In burn victims, urinary tract infections in infected individuals, and hospital-acquired pneumonia in patients on respirators, P.aeruginosa is now a frequent cause of bacteraemia. It is also the major cause of mortality in patients having cystic fibrosis, whose irregular airway epithelia allows P.aeruginosa to colonize the lungs in the long term. These infections cannot be eliminated, mainly because of the bacterium's natural resistance to antibiotics, eventually leading to respiratory failure and death [29].

A common nosocomial pathogen that causes infections with a high mortality rate is Pseudomonas aeruginosa. This latter is partly due to the intrinsically high resistance of the organism to many antimicrobials and the growth of increased resistance, particularly multidrug resistance, in healthcare facilities [30].

1.9 Purpose of current study

The main objective of this research article is follows

- Evaluation of antibacterial activity in fruit peels by well diffusion method
- Effects of climate on antibacterial actvity
- Effects of different solvent on extraction processes of antibacterial compounds
- Development of cheap antibacterial compounds source, from fruit wastes and it will also reduce the environmental pollution.
- Uses of fruits wastes in beneficial ways

1.10 Literature Review

NAIR et al.,2005 invested antibacterial activity in nine plants. Both aqueous and organic solvents were utilized to test antibacterial activity. Sapindus emarginatus, Hibiscus rosa-sinensis, Mirabilis jalapa, Rheo discolor, Nyctanthes arbortristis, Colocasia esculenta, Gracilaria corticata, Dictyota spp., and Pulicaria wightiana were among the plants examined. Pseudomonas testosteroni, Staphylococcus epidermidis, Klebsiella pneumoniae, Bacillus subtilis, Proteus morganii, and Micrococcus flavus were examined for antibacterial activity. The antibacterial activity of all of these plants was investigated using two methods: agar disk diffusion and agar ditch diffusion. The germs Pseudomonas testosteroni and Klebsiella pneumoniae were proved the most resistant strains.S. emarginatus has a high level of activity against the germs that were tested. As a result, it can be chosen for further research to see if it has medicinal promise. Its leaf extract can also be employed as a lead molecule in the fight against illnesses caused by the bacteria strains investigated [31].

Negi et al., 2001 screened antibacterial activity of Citrus paradise in hexane, chloroform, acetone, and methanol. Hexane and chloroform extracts exhibited three spots with varying concentrations on thin layer chromatography (TLC); hence, both extracts were combined and separated into alcohol soluble and insoluble fractions.These fractions' antibacterial activity was tested against a variety of microorganisms. The most active fraction was determined to be the alcohol soluble fraction, followed by hexane extract. Gram-positive bacteria

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were more resistant to extracts than Gram-negative bacteria [32].

Rana and Dixit, 2017 carried this study for antibacterial activity analysis in lemon peel extract.Lemon peel extracts were discovered to include carbohydrates, alkaloids, flavonoids, steroids, tannic acids, and phenolic compounds, according to phytochemical analysis and bioactive compound analysis. Disc diffusion assay was used to study the antibacterial activity. They concluded that Lemon peel extracts are high in phytochemicals, bioactive substances including antioxidants, and antibacterial activity, therefore they may be utilized for a variety of medicinal and therapeutic applications to boost the body's defenses against disease-causing microbes [33].

Manandhar et al.,2019 screened antimicrobial activity against four different plant extracts against twelve microorganisms. Agar well diffusion method was used to screen antibacterial activity.The majority of the extracts had antibacterial capabilities, according to the results. With zones of inhibition (ZOI) of 17 mm, 13 mm, 16 mm, 11 mm, and 12 mm, respectively, the extract of

O. corniculata had the maximum potential against Escherichia coli, Salmonella Typhi, MDR Salmonella Typhi, Klebsiella pneumoniae, and Citrobacter koseri[34].

CHAPTER 2

2.Material and method

2.1 Sample collection:

The fresh fruits viz Citrus X sinensis (Orange), Citrus paradisi (Grapefruit), Citrus Limonwere collected from different geographical regions of Pakistan. These regions include Burewala, Lahore, Rahim Yar Khan. Fruits were washed thoroughly under fresh tap water. Then fruit peels were separated and again washed properly. We got 2kg fruit peels of each fruit. These fruit peels were dried under shade and then in a hot air oven to remove any moisture content. Dried peels of Citrus sinensis, Citrus paradisi, and Citrus Limon were then ground to powder using a grinder for extraction. This powder was labeled and sealed in airtight bags. These bags were stored at 4^{o}C till further experimentation.

2.2 Ultrasonic assisted Extraction:

An ultrasonic bath apparatus with an rectangular container whose volume was 16.4 cm × 13.3 cm×10.2 cm was used for extraction. This apparatus was annealed with a transducer (38.5 kHz). It was used at 50.93W ultrasonic power.

We extracted our sample peel powder in two solvents i.e methanol and acetone.5 gram of ground peel sample was added into 40ml of solvents (1:8 w/v)in a 100 ml glass beaker. Beaker was then kept in the ultrasonic bath container at temperature of 30°C for 10 minutes. This process was repeated for all samples collected from Lahore, Borewala and Rahim Yar Khan. When the extraction was done, the supernatant was filtered using Whatman filter paper.

2.3 Test microorganisms and preparation of inocula

In this study, we used two gram-negative bacteria as test microorganisms. These gram-negative bacteria include E.coli and Pseudomonas aeruginosa. Nutrient broth medium was prepared by dissolving 1.3g of nutrient broth powder in 100ml of distilled water and mixed it gently by shacking the flask. In a 500ml flask 100ml of media was dispensed and then autoclaved at 121°C for 15 minutes. The medium was then poured into the petri plates. Bacterial cultures were inoculated with the help of inoculating loop and the loop was sterilized by flame sterilization. The petri plates were stored in an incubator at 37°C overnight.

2.4 Agar plates preparation

For the preparation of nutrient agar medium, 14g of nutrient agar powder was weighed at a Digital weighing balance. The powder was dissolved in 500ml of distilled water. Then shacked and dissolved it properly with the help of a glass spatula. Then media was dispensed in a 500ml flask and then autoclaved at 121°C for 15 minutes. The petri plates were labeled by using a permanent marker. The medium was poured onto labeled petri plates and then allowed to solidify.

2.5 Determination of antibacterial activity

Petri plates with nutrient agar were seeded with prepared inocula. This inoculum was spread on the agar gel properly and uniformly. Bacterial cells were then allowed to grow and form colonies at 37^{o}C over approximately 12hour. Then the required number of wells were formed in these plates with help of a sterile cork borer. The depth of these wells was 8mm. These wells were sealed with 20µL of liquid nutrient agar. 100µL peel extract was poured into respective wells using a micropipette. After this, the plates were incubated for 24hour at 37^{o}C. after the incubation period, with the help of Vernier caliper diameter of the clear zone around these wells was

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measured. This experiment was done three times for each extract. Mean zone of inhibition value was calculated by using the standard deviation.

CHAPTER 3

3. Results and discussion

3.1 Antibacterial activity of Citrus X sinensis

The zone of inhibition of C.sinensis methanol extract of Lahore was 12.9±0.1mm, Rahim Yar Khan was 15.0±0.1mm and Borewala was 18±0.08mm. Whereas the zone of inhibition of acetone extract of Lahore was 11.8±0.1mm, Rhim Yar Khan was 13.96 ±0.2mm and Borewala was 14±0.1mm. Figure 7 represents that methanol extract of all C.sinensis is more efficient than acetone extract against E.coli. Methanol extract of C. sinensis from Borewala showed maximum zone of inhibition (18±0.08mm), C.sinensis methanol extract of Rahim Yar Khan showed second highest ZOI (15.0±0.1mm), third-highest ZOI(14±0.1mm) is of Borewala acetone extract, fourth-highest ZOI(13.9±0.2mm) was showed by acetone extract of C.sinensis from Rahim Yar Khan, second-lowest ZOI(12.96±0.1mm) was found to be of C.sinensis methanol extract from Lahore. The lowest ZOI (11.8±0.1mm) was found to be of acetone extract of C.sinensis from Lahore. Thus methanol extract of C.sinensis is more efficient against the gram-negative bacteria E.coli than acetone extracts. The Borewala methanol extract has maximum antibacterial activity and the acetone extract of C.sinensis sample from Lahore has minimum antibacterial activity against the E.coli. Bacterial strains of E.coli were less resistant toward methanol extracts and relatively more resistant toward acetone extracts.

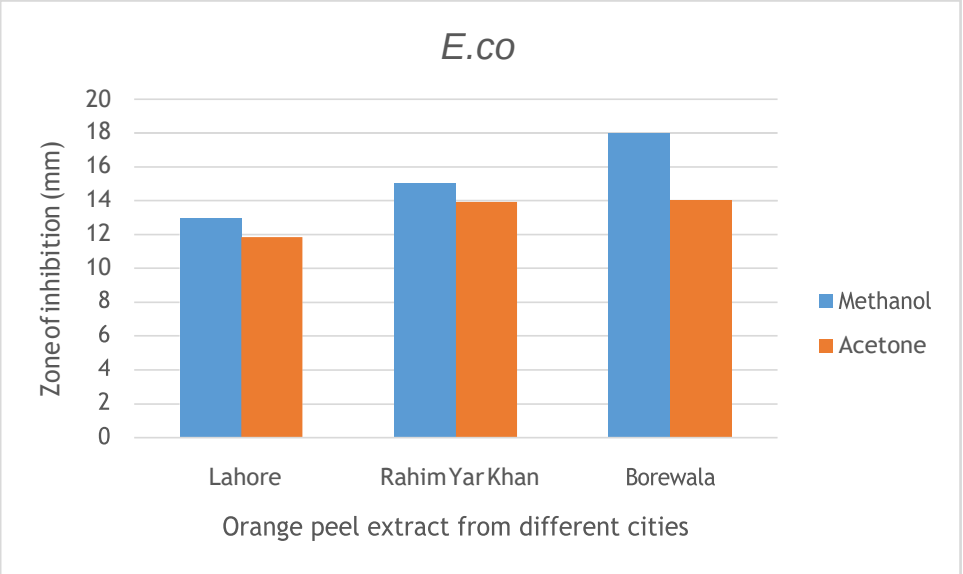

Figure 9:Graph showing antibacterial activity of Citrus X sinensis peel extract against E.coli.

The zone of inhibition of C.sinensis methanol extract of Lahore was 8.1±0.08mm, Rahim Yar Khan was 13.1±0.08mm and Borewala was 15.0±0.1mm. Whereas the zone of inhibition of acetone extract of Lahore was 13±0.1mm, Rhim Yar Khan was 17.93 ±0.1mm and Borewala was 13.0±0.1mm. Figure 8 represents that methanol extract of two C.sinensis samples, from Lahore and Borewala, are more efficient than acetone extract. Methanol extract of C. sinensis from Lahore showed maximum zone of inhibition (18±0.08mm), C.sinensis acetone extract of Rahim Yar Khan showed second highest ZOI (17.9±0.1mm), third-

highest ZOI(15.0±0.1mm) is of Borewala methanol extract, fourth-highest ZOI(13.1±0.08mm) was showed by methanol extract of C.sinensis from Rahim Yar Khan, second-lowest ZOI(13.06±0.1mm) was found to be of C.sinensis acetone extract from Borewala. The lowest ZOI (13±0.1mm) was found to be of acetone extract of C.sinensis from Lahore. Thus methanol extract of C.sinensis is relatively more efficient against the gram-negative bacteria P.aeuroginosa as compare to acetone extracts. Methanol extract from Borewala is most efficient against P.aeuroginosa while the acetone extract of C.sinensis sample from Lahore is least efficient

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against the P.aeuroginosa. Bacterial strains of P.aeuroginosa were less resistant toward methanol extracts and relatively more resistant toward acetone extracts.

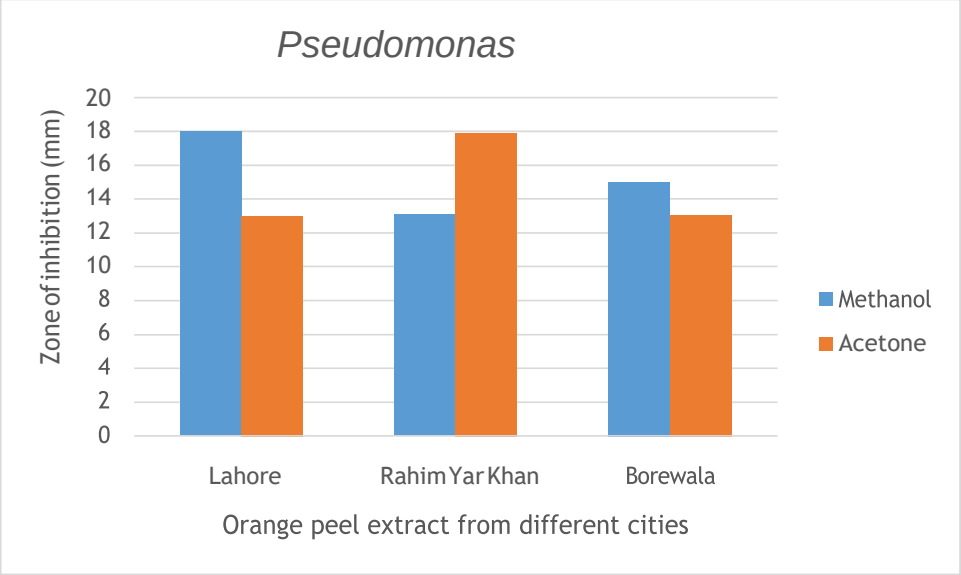

Figure 10: Graph showing antibacterial activity of Citrus X sinensis peel against P.aeuroginosa.

3.2 Agar plates after 24hour incubation

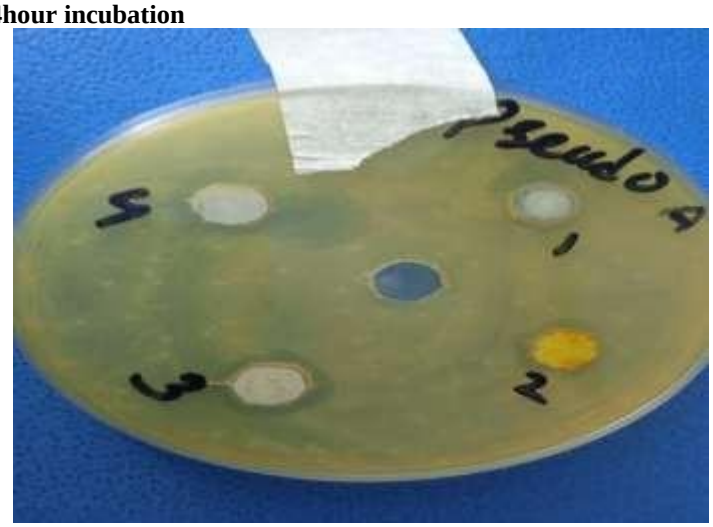

Figure 11:Zone of inhibition by acetone extracts in P.A inoculated plate.

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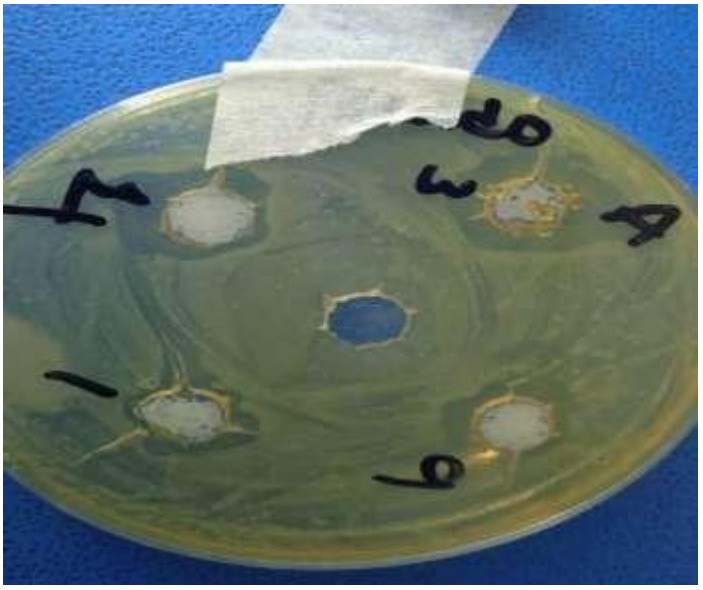

Figure 12: Zone of inhibition by acetone extracts in P.A inoculated plate.

Figure 13: Zone of inhibition by acetone extracts in P.A inoculated plate.

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Figure 14: Zone of inhibition by methanol extracts in P.A inoculated plate.

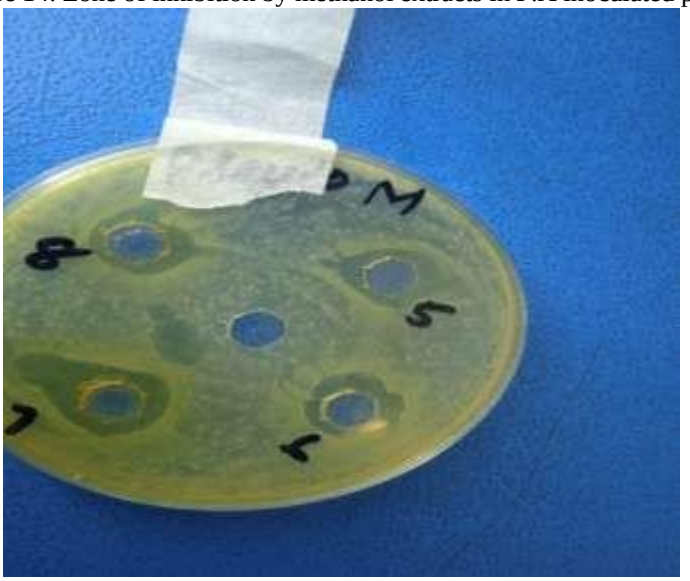

Figure 15: Zone of inhibition by methanol extracts in P.A inoculated plate.

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Figure 16: Zone of inhibition by methanol extracts in E.coli inoculated plate.

Figure 17: Zone of inhibition by acetone extracts in E.coli inoculated plate.

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Figure 18 : Zone of inhibition by methanol extracts in E.coli inoculated plate.

Figure 19: Zone of inhibition by methanol extracts in E.coli inoculated plate.

3.3 Antibacterial activity of Citrus limon

The zone of inhibition of C.limon methanol extract of Lahore was 14.03±0.1mm, Rahim Yar Khan was 14.06±0.1mm and Borewala was 15.0±0.1mm. Whereas the zone of inhibition of acetone extract of Lahore was 11.9±0.1mm, Rhim Yar Khan was 12.±0.1mm and Borewala was 11.03±0.1mm. Figure 9 represents that methanol extract of C.limon samplesis more efficient than acetone extracts. Methanol extract of C.limon from

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Borewala showed maximum zone of inhibition (15.0±0.1mm), C.limon methanol extract of Rahim Yar Khan showed second highest ZOI (14.06±0.1mm), third-highest ZOI(14.03±0.1mm) is of Lahore methanol extract, fourth-highest ZOI(12±0.1mm) was showed by acetone extract of C.limon from Rahim Yar Khan, second-lowest ZOI(11.9±0.1mm) was found to be of C.limon acetone extract from Lahore. The lowest ZOI (11.0±0.1mm) was found to be of acetone extract of C.limon from Borewala. Thus methanol extract of C.limon from is relatively more efficient against the gram-negative bacteria E.coli as compare to acetone extracts. Methanol extract from Borewala is most efficient against E.coli while the acetone extract of C.limon sample from Borewala is least efficient against E.coli. Bacterial strains of E.coli were less resistant toward methanol extracts and relatively more resistant toward acetone extracts.

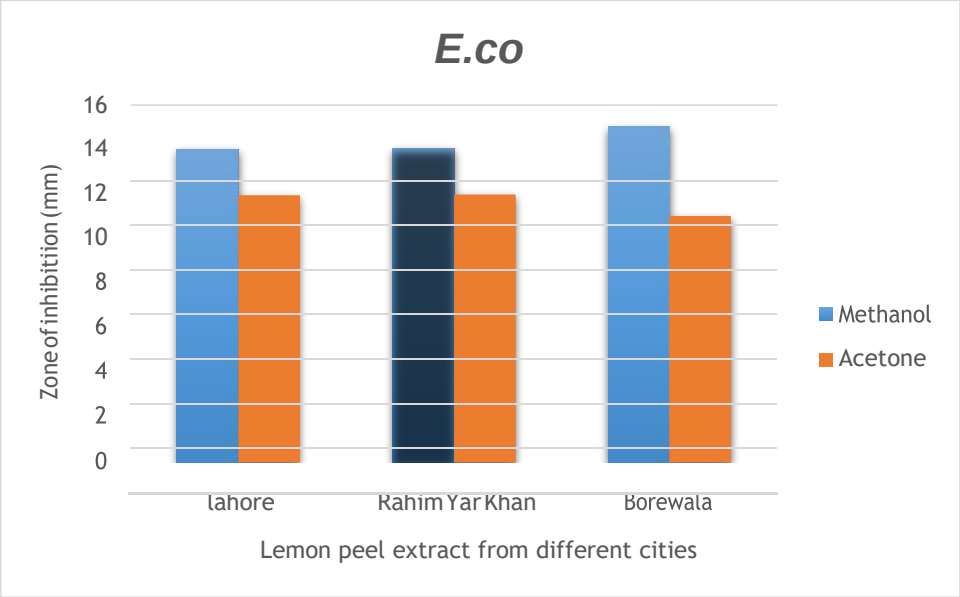

Figure 20: Graph showing antibacterial activity of Citrus limon peel extract against E.coli.

The zone of inhibition of C.limon methanol extract of Lahore was 14.03±0.1mm, Rahim Yar Khan was 15.03±0.1mm and Borewala was 13.9±0.1mm. Whereas the zone of inhibition of acetone extract of Lahore was 12.1±0.08mm, Rhim Yar Khan was 17.0±0.1mm and Borewala was 14.03±0.2mm. Figure 10 represents that methanol extract of C.limon sample from Lahoreis more efficient than its acetone extract while acetone extract Rahim Yar Khan is more efficient than its methanol extract. Acetone extract of C.limon from Rahim Yar Khan showed maximum zone of inhibition (17.0±0.1mm), C.limon methanol extract of Rahim Yar Khan showed second highest ZOI (15.0±0.1mm), third-highest ZOI(14.0±0.1mm) is of Lahore methanol extract and acetone extract of Borewala sample(14.0±0.2), second-lowest ZOI(13.9±0.1mm) was found to be of C.limon methanol extract from Borewala. The lowest ZOI (12.1±0.08mm) was found to be of acetone extract of C.limon from Lahore. P.aeuroginosa is the least resistant toward acetone extract of Rahim Yar Khan sample and most resistant toward acetone sample of Lahore.

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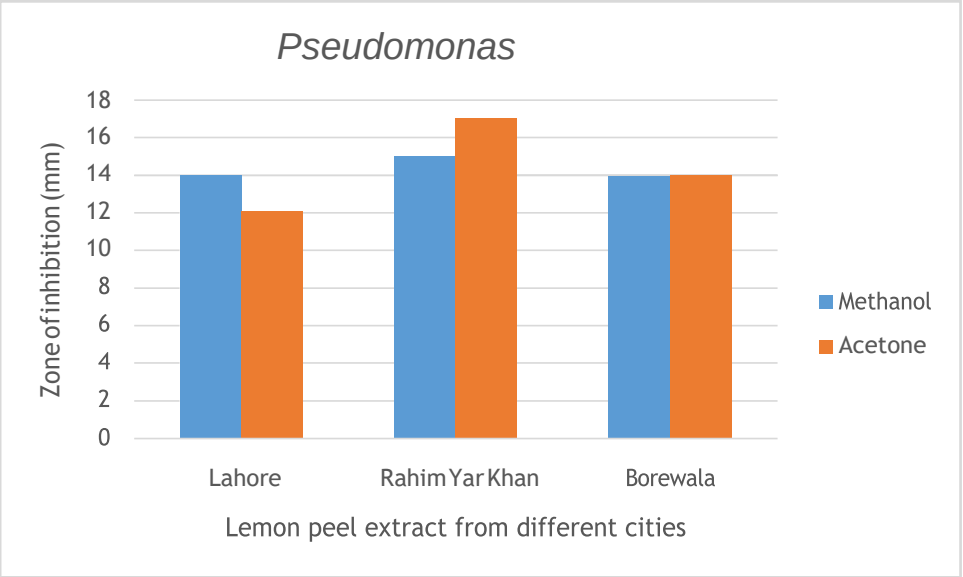

Figure 21:Graph showing antibacterial activity of Citrus limon peel extract against P.aeuroginosa

3.4 Antibacterial activity of Citrus paradisi

The zone of inhibition of C.paradisi methanol extract of Lahore was 13.0±0.1mm, Rahim Yar Khan was 13.1±0.08mm and Borewala was 15.0±0.1mm. Whereas the zone of inhibition of acetone extract of Lahore was 12.0±0.1mm, Rhim Yar Khan was 15.9±0.1mm and Borewala was 15.8±0.1mm. Figure 11 represents that acetone extract of C.paradisi samplesis more efficient than acetone extracts for Rahim Yar Khan and Borewala. Acetone extract of C.paradisi from Rahim Yar Khan showed maximum zone of inhibition (15.9±0.1mm), C.paradisi acetone extract of Borewala showed second highest ZOI (15.8±0.1mm), third-highest ZOI(15.0±0.1mm) is of Borewala methanol extract, fourth-highest ZOI(13.1±0.08mm) was showed by methanol extract of C.paradisi from Rahim Yar Khan, second-lowest ZOI(13.0±0.1mm) was found to be of C.paradisi methanol extract from Lahore. The lowest ZOI (12.0±0.1mm) was found to be of acetone extract of C.paradisi from Lahore. Thus acetone extract of C.paradisi from Rahim Yar Khan and Borewala are more efficient against the gram- negative bacteria E.coli as compare to their methanol extracts. Acetone extract from Rahim Yar Khan is most efficient against E.coli while the acetone extract of C.paradisi sample from Lahore is least efficient against E.coli.

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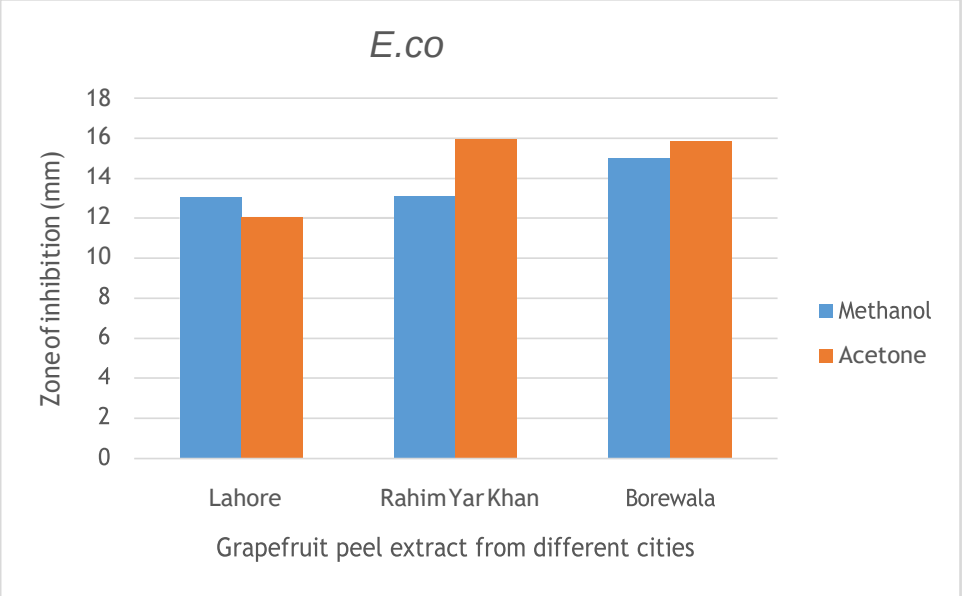

Figure 22: Graph showing antibacterial activity of Citrus paradisi peel extract against E.coli.

The zone of inhibition of C.paradisi methanol extract of Lahore was 16.9±0.1mm, Rahim Yar Khan was 14.0±0.1mm and Borewala was 12.0±0.1mm. Whereas the zone of inhibition of acetone extract of Lahore was 18±0.1mm, Rhim Yar Khan was 13±0.2mm and Borewala was 15.0±0.2mm. Figure 12 represents that acetone extract of C.paradisi samples from Lahore and Borewalaare more efficient than acetone extracts. Acetone extract of C.paradisifrom Lahore showed maximum zone of inhibition (18±0.1mm), C.paradisi methanol extract of sample from Lahore showed second highest ZOI (16.9±0.1mm), third-highest ZOI(15.0±0.1mm) is of Borewala sample acetone extract, fourth-highest ZOI(14.0±0.1mm) was shown by methanol extract of C.paradisi from Rahim Yar Khan, second-lowest ZOI(13±0.2mm) was found to be of C.paradisi acetone extract from Rahim Yar Khan. The lowest ZOI (12.0±0.1mm) was found to be of methanol extract of C.paradisi from Borewala. Thus acetone extract of C.paradisi from Lahore and Borewala are more efficient against the gram- negative bacteria P.aeuroginosa as compare to their methanol extracts. Acetone extract from Lahore is most efficient against P.aeuroginosa while the methanol extract of C.paradisi sample from Borewala is least efficient against P.aeuroginosa.

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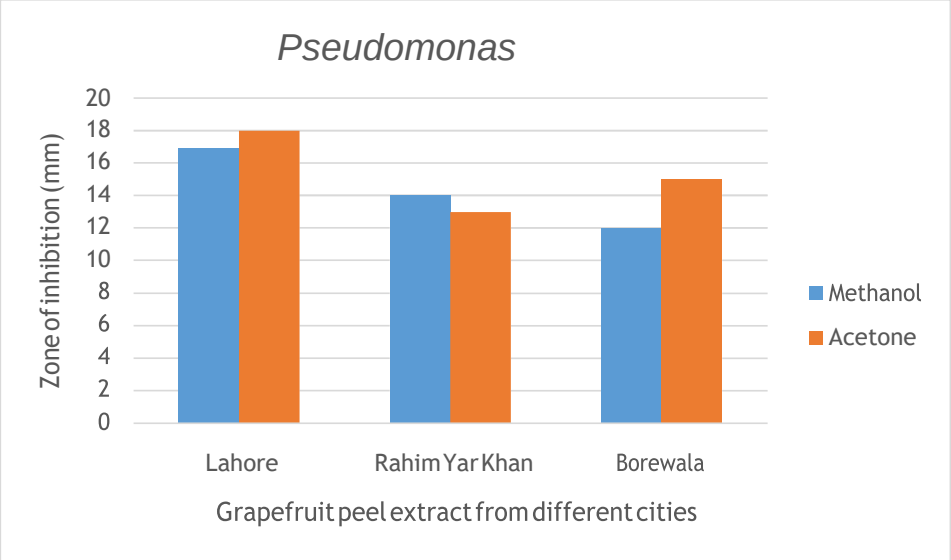

Figure 23:Graph showing antibacterial activity of Citrus paradisi peel extract against P.aeuroginosa.

Table 5:Zone of inhibition (mean±S.D) of samples from different regions of Pakistan

Test		C.sinensis			C.limon			C.paradisi		
Organism	ents	Lahore	Rahim Yar Khan	Burewala	Lahore	Rahim Yar Khan	Burewala	Lahore	Rahim Yar Khan	Burewala
E.coli	Methanol	12.9±0.1	15.0±0.1	18±0.08	14.0±0.1	14.0±0.1	15.0±0.1	13.0±0.1	13.1±0.08	19.3±0.1
P.aeuroginosa		18±0.08	13.1 ±0.08	15.0 ±0.1	14.0±0.2	15.0±0.1	13.9±0.1	16.9±0.1	14.0±0.1	12.0±0.1
E.coli	Acetone	11.8±0.1	13.9±0.2	14±0.1	11.9±0.1	12±0.1	11.0±0.1	12.0±0.1	15.9±0.1	15.8±0.1
P.aeuroginosa		13±0.1	17.9±0.16	13.0±0.1	12.1±0.08	17.0±0.12	14.0±0.2	18±0.1	13±0.2	15.0±0.2

3.4 Conclusion

In this study, C.paradisi methanol extract from Lahore was proved most efficient for P.aeuroginosa with the highest zone of inhibition. Acetone extract of Rahim Yar Khan C.paradisi sample was proved the most effective for P.aeuroginosa strains among all other peel samples. Methanol extract of C.sinensis sample from Lahore is most efficient against P.aeuroginosawith the highest zone of inhibition. Acetone extract of C.sinensis sample from Rahim Yar Khan was most efficient against P.aeuroginosa than all other acetone extracts. Methanol extract of C.sinensis from Borewala showed maximum zone of inhibition against E.coli among all other methanol extracts while in acetone extracts again the Borewala sample showed maximum inhibition.C.limon methanol extract of Rahim Yar Khan was proved most efficient against P.aeuroginosa while acetone extract of Rahim Yar Khan also proved most efficicent.Methanol extract of Borewala showed maximum zone of inhibition against E.coli while acetone extract of Rahim Yar Khan showed maximum zone of inhibition.

CHAPTER 4

4. References

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