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ANTIMICROBIAL ACTIVITY OF GRAPEFRUIT'S (*CITRUS PARADISI*) ESSENTIAL OIL AND METHANOLIC EXTRACTS OF PULP, PEEL, AND SEED

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ABSTRACT

The essential oil from the fresh grapefruit peels was extracted by the hydrodistillation method. The methanolic extracts of dried and powdered grapefruit pulp, peel, and seed were prepared by using methanol as a solvent. The antimicrobial activity of essential oil and methanolic extracts of grapefruit's pulp, peel and seed were determined by using the disc diffusion method against bacteria (*Salmonella Typhi, Escherichia coli, Staphylococcus aureus*) using streptomycin as standard and against fungi (*Aspergillus niger, Aspergillus terreus, Alternaria alternata*) using 1% fluconazole as standard. The results showed that the essential oil and the methanolic extracts of grapefruit's pulp, peel, and seed exhibited zones of inhibition against bacteria *Salmonella Typhi* were 27mm, 25mm, 8mm, and 5mm, *Escherichia coli* were 24, 20mm, 7mm, and 9mm, *Staphylococcus aureus* were 19mm, 17mm, 5mm, and 6mm and against fungi *Aspergillus niger* were 40mm, 39mm, 16mm, and 18mm, *Aspergillus terreus* were 29mm, 17mm, 22mm, and 23mm and *Alternaria alternata* were 23mm, 18mm, 17mm, and 22mm respectively.

KEYWORDS: *Citrus paradisi*, Grapefruit, Essential oil, Methanolic extract, Antimicrobial activity, disc diffusion method.

INTRODUCTION

Plants possess numerous phytochemicals that serve as a way to overcome microbial resistance. Because they provide a potential solution by providing alternative ways to combat infections that no longer respond effectively to conventional antibiotic treatments (Control & Prevention, 2019; Khameneh *et al.,* 2016). The reliance on herbal medications has arisen because of the tremendous expense of significant antimicrobial supplements that were used to treat specific diseases and the limited availability of modern medical facilities. Their widespread use is due to their ease of access, lower cost, and minimal adverse side effects. These phytochemicals are similar to those found in commercial antibiotics and can be used as traditional remedies to treat numerous disorders. These medicinal plants may serve as a source of new antimicrobial agents that must be discovered. The increasing demand for innovative antibiotics is linked to increased germs resistant to traditional drugs, making plants a safer alternative source with antimicrobial properties (Ezeigbo *et al.,* 2016).

Citrus fruits have been used for several years for their herbal, medicinal, and agricultural properties. Grapefruit (*Citrus paradisi*) belongs to the *Rutaceae* family, commonly known as the citrus fruit family which includes 158 genera and approximately 2100 species (Appelhans *et al.,* 2021). It is a subtropical citrus fruit, recognized for its considerable size having a sweet-tart taste. The trees of grapefruit have a height ranging from 16 to 20 feet while leaves are elongated, slender, and dark green, reaching a maximum length of 14 to 15cm (Morton, 1987). The flowers are white, having four or five petals, and reach 5 cm in size. The fruit has a yellow-orange skin and is typically shaped like a flattened sphere, with a diameter ranging from 11 - 15cm having a segmented flesh that comprises a wide range of bioactive components (Cristóbal-Luna *et al.,* 2018; Ranganna *et al.,* 1983). In Pakistan, the Punjab province accounts for 95% of the entire citrus fruit production from which grapefruit contributes only 0.3%. It is largely cultivated in the subtropical areas of central Punjab, specifically in Sargodha, Sahiwal, Khanewal, Layyah, and Toba Tak Singh (Siddique & Sharif; Usman *et al.,* 2020).

Upon consumption of the grapefruit, 50% of the fruit remains as inedible waste, including its pulp, peel, seed, and other remnants (El-Adawy *et al.,* 1999; Mahato *et al.,* 2018). The lack of treatment of this waste poses a significant risk to ecosystems as well as the environment

because of its significant biological oxygen demand (BOD). Nevertheless, it also harbors substantial quantities of bioactive components (Andrea *et al.*, 2003; Safdar *et al.*, 2017). The bioactive components include carotenoids, limonoids, ascorbic acid, phenolic acids, limonene, and flavonoids that serve as an effective remedy due to their anti-fungal, antibacterial, antiviral, preservative, and astringent attributes. So, grapefruit is consumed for several purposes such as cell regeneration, cholesterol reduction, cancer prevention, detoxification, cleansing, weight loss promotion, arthritis treatment, and heart health maintenance. The grapefruit's wide array of noteworthy and favorable qualities makes it a valuable resource for researchers in developing innovative treatments that can perform multiple functions in a single formulation. (Imran *et al.*, 2013).

Multiple studies have shown that terpenes found in citrus essential oils can hinder the growth of numerous microorganisms such as bacteria, viruses, and fungi. Terpenes possess distinct chemical characteristics that enable them to disrupt the cell membranes of microbes, impede their enzyme function, and interfere with crucial biological processes within the microorganisms. They have demonstrated wide-ranging antibacterial properties, which makes them highly intriguing contenders for treating drug-resistant infections (Khameneh *et al.*, 2019).

Research has been conducted on extracting essential oil from tangerine fruit (*Citrus reticulata*) peel and evaluating its antimicrobial activity (Shahzad *et al.*, 2009). Additionally, the antimicrobial activity of ethanolic extracts from various parts of grapefruit was investigated by (Anyiam *et al.*, 2024) and (Cvetnic & Vladimir-Knezevic, 2004). The antibacterial activity of different citrus fruits has also been studied by (Afroja *et al.*, 2017). This study aims to investigate the antimicrobial activity of essential oil as well as the methanolic extracts of grapefruit in order to evaluate their potential as natural antimicrobial agents and explore their applicability in preventing microbial infections or preserving food.

MATERIALS AND METHODS

Fresh Grapefruits were gathered from the fruit market of Sheikhupura District, Punjab Pakistan. The grapefruit’s pulp, peels, and seeds were separated manually from the fruit and were dried for 28 days at ambient conditions.

Extraction of Essential Oil from Grapefruit's Peels

The grapefruit peels were separated manually from the fruit and weighed approximately 3 kg. Peels were diced into smaller fragments by using a knife and then subjected to hydrodistillation using a Dean-Stark apparatus for 12 hours (Shahzad *et al.*, 2009). The extracted essential oil was separated in a separating funnel and subsequently dried by using anhydrous sodium sulphate. It yields a light yellow-colored oil which was stored in a hermetically sealed container at a temperature of 4°C in the refrigerator for further research.

Methanolic Extraction of Grapefruit

The pulp, peels, and seeds of grapefruit were manually separated, dried for 28 days in the shade, and then processed using a grinder machine to get a powder. 100 grams of dried and grounded grapefruit samples, combined with 500 milliliters of methanol, were stored in a 1000ml sealed container. The mixtures were left to macerate for 15 days, with regular agitation. Subsequently, the combinations underwent filtration to eliminate any residuals present in the liquid extract. Then, the solvent was recovered by the distillation process, and the resulting concentrates were stored in hermetically sealed sample bottles at ambient conditions (Mokbel & Suganuma, 2006).

Figure 1, Essential oil and Methanolic Extracts of Grapefruit.

Antimicrobial Activity of Grapefruit

The antimicrobial activity including antibacterial and antifungal activities of essential oil, grapefruit pulp, peel, and seed methanolic extracts were assessed using the agar disc diffusion method described by (Baydar *et al.*, 2004). The antibacterial and antifungal activity were assessed using a range of pathogens, including *Salmonella Typhi*, *Escherichia coli*, and *Staphylococcus aureus* bacteria, as well as *Aspergillus niger*, *Aspergillus terreus*, and

Alternaria alternata fungus. The fungi were cultured using standard Potato Dextrose Agar (PDA) media, while the bacteria were cultured using the standard Nutrient Agar (NA) media at the appropriate temperatures. The pathogens utilized for antimicrobial activity were derived from many sources, including *Salmonella Typhi* from contaminated food fruit, *Escherichia coli* from aloe vera gel, and *Staphylococcus aureus* from milk. *Aspergillus niger* was obtained from guava while *Aspergillus terreus* and *Alternaria alternata* were isolated from soil.

The growth media were prepared, sterilized by autoclaving, and then moved to the sterilized petri dishes. The microbial colonies from test tube slants were transferred to their relevant media Petri dishes. 6 mm paper discs that were sterilized and dried were impregnated with 20 µL of essential oil and methanolic extracts of grapefruit pulp, peel, and seed. The discs, including one control, were placed on recently produced microbial cultures, with five discs on each plate. Streptomycin, in a volume of 20 µL per disc, served as the standard for bacteria, while 1 % fluconazole, also at a volume of 20 µL per disc, served as the standard for fungi. The Petri dishes were incubated at their designated temperatures, and the zones of inhibition were measured in millimeters (mm) against the tested pathogens after 24 hours for bacteria and after 48 hours for other fungi.

RESULTS AND DISCUSSION

Antibacterial Activities of Grapefruit

The antibacterial activity of essential oil and methanolic extracts of grapefruit were evaluated by disc diffusion method against *Salmonella Typhi*, *Escherichia coli*, and *Staphylococcus aureus* bacteria that have been shown in Figure 2.

Figure 2: Antibacterial activity against Salmonella Typhi, Escherichia coli, Staphylococcus aureus bacteria.

In Figure 2, different numbers showed samples employed for evaluating antibacterial activities with zones of inhibition. Among these number, 1 showed zone of inhibition of essential oil, 2 showed methanolic extract of grapefruit peel, 3 showed methanolic extract of grapefruit seed, 4 showed methanolic extract of grapefruit pulp and center showed zone of inhibition for standard (Streptomycin). These results are recorded in Table 1 as shown below.

Table 1: Zone of Inhibition (mm) of Various Parts of Grapefruit against Bacteria.

Tested Organisms	Standard (Streptomycin)	Essential Oil	Methanolic Extract of Grapefruit Pulp	Methanolic Extract of Grapefruit Peel	Methanolic Extract of Grapefruit Seed
Salmonella typhi	27	34	25	8	5
Escherichia coli	24	17	20	7	9
Staphylococcus aureus	19	11	17	5	6

Results showed that the essential oil extracted from the peels of grapefruit possess higher antibacterial activity against *Salmonella Typhi*, *Escherichia coli*, and *Staphylococcus aureus* bacteria as compared to the methanolic extracts of grapefruit. After 24 hours of incubation, essential oil showed maximum inhibition zone of 34mm for *Salmonella Typhi*, 17mm for *Escherichia coli* and 11mm for *Staphylococcus aureus.* While the methanolic extract of grapefruit pulp showed inhibition zone of 25mm for *Salmonella Typhi,* 20mm for *Escherichia coli* and 17mm for *Staphylococcus aureus.* The minimum inhibition zones were observed for the methanolic extracts of grapefruit seed and peel of 8mm and 5mm for *Salmonella Typhi,* 7mm and 9mm for *Escherichia coli,* 5mm and 6mm for *Staphylococcus aureus* respectively. Their antibacterial activities were represented by order as follows: essential oil > pulp > seed > peel. These results are similar to those (Cvetnic & Vladimir-Knezevic, 2004) who reported that the ethanolic extracts of pulp showed higher antimicrobial activity than seed. The comparison of these results were also illustrated in Figure 3.

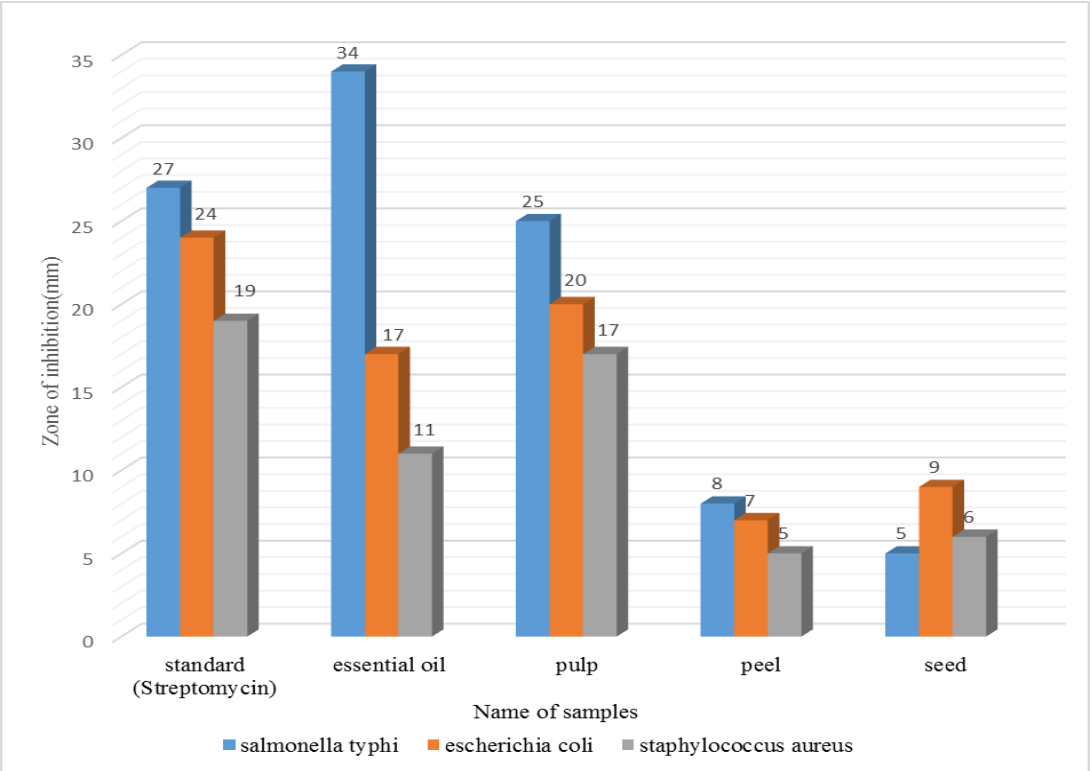

Figure 3: Antibacterial Activities of Different Parts of Grapefruit.

Antifungal Activities of Grapefruit

The antifungal activity of essential oil and methanolic extracts of grapefruit were also evaluated by disc diffusion method against *Aspergillus niger*, *Aspergillus terreus* and *Alternaria alternata* fungi that have been shown in Figure 4.

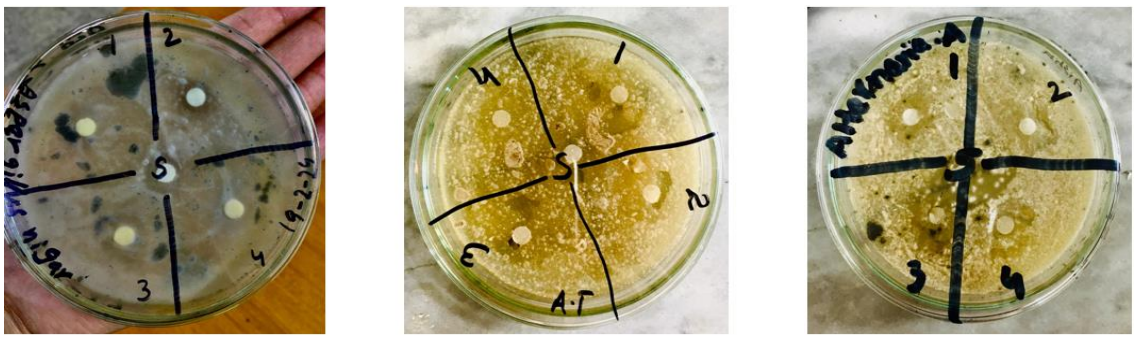

Figure 4: Antifungal Activities of Grapefruit against Aspergillus niger, Aspergillus terreus, Alternaria alternata fungi.

In Figure 4, different numbers represented the samples employed for conducting antifungal activities with zones of inhibition. Among these numbers, 1 showed zone of inhibition of essential oil, 2 showed methanolic extract of grapefruit peel, 3 showed methanolic extract of

grapefruit seed, 4 showed the methanolic extract of grapefruit pulp and S showed zone for standard (1% Fluconazole).

The results were recorded in Table 2 as shown below.

Table 1: Zone of Inhibition (mm) of various parts of Grapefruit against Fungi.

Tested Organisms	Standard (Streptomycin)	Essential Oil	Methanolic Extract of Grapefruit Pulp	Methanolic Extract of Grapefruit Peel	Methanolic Extract of Grapefruit Seed
Aspergillus niger	18	40	39	16	18
Aspergillus terreus	18	29	17	22	23
Alternaria alternata	17	23	18	17	22

Results showed that the essential oil extracted from the peels of grapefruit possess higher antifungal activity against *Aspergillus niger*, *Aspergillus terreus* and *Alternaria alternata* fungi as compared to the methanolic extracts of grapefruit. After 48 hours of incubation, essential oil showed maximum inhibition zone of 40mm for *Aspergillus niger*, 29mm for *Aspergillus terreus* and 23mm for *Alternaria alternata.* While the methanolic extract of grapefruit pulp showed inhibition zone of 39mm for *Aspergillus niger*, 17mm for *Aspergillus terreus* and 18mm for *Alternaria alternata.* Meanwhile, the methanolic extracts of grapefruit seed and peel exhibited inhibition zones of 16mm and 18mm for *Aspergillus terreus,* 22mm and 23mm for *Aspergillus terreus*, 17mm and 22mm for *Alternaria alternata* respectively. Their antifungal activities were represented by order as follows: essential oil > pulp > seed > peel. These results are similar to those (Cvetnic & Vladimir-Knezevic, 2004) who reported that the ethanolic extracts of pulp showed higher antimicrobial activity than seed. The comparison of these results were also illustrated in Figure 5.

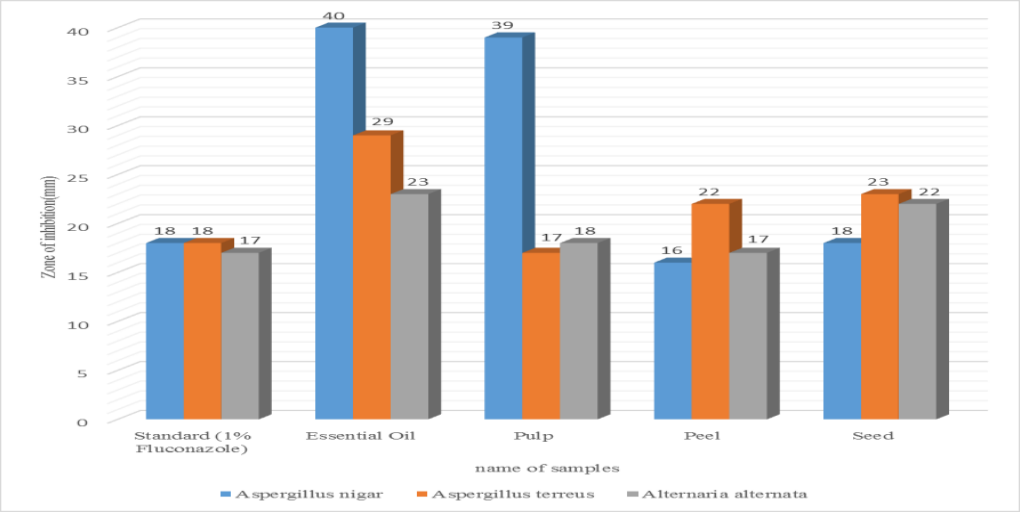

Figure 5: Antifungal Activities of Various Parts of Grapefruit.

DISCUSSION

Various published studies have examined the antimicrobial activity of essential oil extracted from grapefruit peels as well as grapefruit pulp, seed, and peel extracts (Akroum *et al.,* 2009; Anyiam *et al.,* 2024; Cvetnic & Vladimir-Knezevic, 2004; Uysal *et al.,* 2011). The results showed that the essential oil and the methanolic extracts of grapefruit inhibited all the tested bacterial and fungal strains when compared with the standard. Literature showed that the essential oil from grapefruit peels and some other citrus fruits inhibited *Escherichia coli, Salmonella Typhi, Bacillus cereus, Staphylococcus aureus, Pseudomonas sp., M. luteus, M. smegmatis, Aspergillus niger, Aspergillus terreus, Aspergillus falvis, Aspergillus ficcum, Aspergillus fumigatus, Fusarium oxysporium, Aspergillus fumigatus* (Dabbah *et al.,* 1970; Javed *et al.,* 2013; Kirbaşlar *et al.,* 2009; Shahzad *et al.,* 2009) that were similar to the present findings.

(Uysal *et al.,* 2011) also investigated the antimicrobial activity of the essential oil of grapefruit by hydrodistillation method. He showed that the essential oil exhibited inhibition zones of 41mm for *Staphylococcus aureus* and 28mm for *Escherichia coli* which was higher than the present results of 11mm for *Staphylococcus aureus* and 17mm for *Escherichia coli.*
The antimicrobial activity of grapefruit is because of the presence of bioactive components. The grapefruit seed extract possesses polyphenolic compounds like catechin, epicatechin, dimeric, trimeric, and tetrameric procyanidins (Saito *et al.,* 1998). The grapefruit peel extract possesses polyphenolic compounds, aldehydes, organic acids, and limonoids, etc. The grapefruit pulp possesses β-carotene, sugars, organic acids, volatile components, d-limonene, caryophyllene, sterols, minerals, and limonoids (Bennett *et al.,* 1989; Braddock & Bryan, 2001; Ohta *et al.,* 1993; Tushishvili *et al.,* 1982; Zheng *et al.,* 2016).

The essential oil extracted from grapefruit peels possesses higher levels of phenolic components that are responsible for showing higher antimicrobial activity (Tp, 2005; Tumane *et al.,* 2014). (Johann *et al.,* 2007) also showed that the phenolic components in the peels of citrus fruits showed antimicrobial activity against a range of microorganisms. (Sikkema *et al.,* 1995) showed that monoterpenes in the essential oil have an antimicrobial potential that allows them to diffuse through the damaged cell membrane structures of microorganisms. Hence, it has been demonstrated that they are also abundant in terpenes, and exhibit significant antimicrobial properties (Afolayan & Ashafa, 2009).

It is also attributed to its active components, which affect specific metabolic operations of microbial cells (Methawiriyasilp *et al.*, 2003). Furthermore, certain components present in smaller quantities may also contribute to the antimicrobial properties of the oil, maybe through a synergistic interaction with other active chemicals (Matasyoh e*t al.*, 2007).

CONCLUSION

The essential oil was extracted from grapefruit peels by hydrodistillation method and methanolic extracts of grapefruit pulp, peel, and seed were prepared. Their antimicrobial activities including both antibacterial and antifungal activity were determined by using the agar disc diffusion method. After incubation at their respective time intervals inhibition zones were formed. The essential oil, as well as the methanolic extracts of grapefruit pulp, peel, and seed, showed antibacterial efficacy against *Salmonella Typhi, Escherichia coli,* and *Staphylococcus aureus* bacteria and antifungal efficacy against *Aspergillus niger, Aspergillus terreus,* and *Alternaria alternata* fungi. Their effectiveness showed their potential for application as natural ingredients in numerous medications and food products.

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