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Research

Antimicrobial Activity of Citrus Fruits Peels Juice on Different Pathogen

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Abstract

The aim of the project is to carry out antimicrobial activity of different citrus fruit peel extracts on pathogenic bacteria such as *Staphylococcus aureus, Proteus mirabilis, Bacillus cereus* and *Escherichia coli* and *Micrococcus.* The *Citrus reticulate, Citrus limetta, Citrus limon, Citrus aurantifolia.* were collected from local market of Karad. The antimicrobial activity of peel extract of *Citrus reticulate, Citrus limetta, Citrus limon, Citrus aurantifolia*. The sample powder take and then make the 95% ethanol. Then the powder dissolved in the 95% ethanol for 24 hrs. After 24 hrs the extract was filtered using Whatmann filter paper no. 1 and this ethanolic extract are ready for further use. Inhibitory zones of peel extracts of *Citrus reticulate, Citrus limetta, Citrus limon, Citrus aurantifolia* against laboratory strains of microorganisms.

Keywords: Citrus peels, Antimicrobial activity, Ethanolic extract, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Proteus mirabilis, Disc diffusion method, Natural preservatives, Phytochemicals.

INTRODUCTION

Citrus lemon is a conventional fruit which belong to plant family Rutaceae and is commercially know as sweet orange. Citrus species is a spreading evergreen, sometimes spiny trees which could be 12m tall with oral elliptic leaves and rounded fruits that are up to 12cm in diameter investigated that citrus trees are evergreen trees that produce fruits of different forms and sizes (from round to oblong), which are full of fragrance, flavor and juice. Chanthaphon et al. (2008) reported that citrus fruit belong to six genera (Fortunella, Eremocitrus, clymendia, poncirus, Microcitrus and citrus), which are native to the tropical and subtropical regions of Asia, but the major commercial fruits such as oranges, mandarins, lime, lemons and grape fruits. Mandalari et al. (2006) reported that orange constitute about 60% of the total citrus world production. Adode (2002) observed that fruits contain 80 to 90% sugar and acids, citric acid are abundant acid in the sap. Roger (2002) reported that the internal constitutes the pulp which is rich in soluble sugars, ascorbic acid, pectin, fibers, different organic acids and

potassium salt that gives the fruit its characteristics citrine flavor. Hasija et al. (2015) reported that citrus peel oil can be used as natural preservative to minimize the ill effects of these synthetic preservatives and protect consumer health[1].

The emergence of multidrug resistance bacterial strains are also becoming a global concern, with particular emphasis on *E. coli, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Streptococcus pyogen.* The increasing occurrence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectra of untreatable bacterial infections and adds urgency to the search for new infection-fighting and safe strategies investigated that Citrus fruit are highly nutritious medicinal plant and found to be commonly in cultivation throughout the tropic. Hindi and Chabuck (2013) have demonstrated the antimicrobial effects of aqueous extracts of peel and juice from fresh and dried citrus and sweet lemon against gram-positive and gram-negative bacteria and yeast isolates, including *Staphylococcus aureus, Enterococcus faecalis, Salmonella typhi, E.coli* and *Candida albicans*. So aim of present study is to evaluate antimicrobial activity of citrus fruits on certain pathogenic microorganism[2].

Citrus sinensis (Orange), Citrus paradis (Grapefruit), Citrus limon (Lemon), Citrus (Tangerine), Citrus grandis (Shaddock), Citrus aurantium (Sour orange), Citrus medica (Citron), and Citrus aurantifolia (Lime) are some important fruit of genus Citrus. Citrus are well known as one of the world's major fruit crops that are produced in many countries with tropical or subtropical climate. Brazil, Japan, China, Mexico, Pakistan, and countries of the Mediterranean region, are the major Citrus producers. The Citrus fruits and their by–products are of high economic and medicinal value because of their multiple uses, such as in food industry, cosmetics and folk medicine. In additions to large scale consumption as fresh fruits, the fruits are mainly processed to produce juice. The waste of Citrus processing industry left after juice extraction, such as peels, seeds and pulps, corresponding to about 50% of the raw processed fruit, can be used as a potential source of valuable by products. Specifically, the Citrus peels, commonly treated as agro-industrial waste, are a potential source of valuable secondary plant metabolites and essential oils[3].

Herbal medicine has been widely used as an integral part of primary health care in many countries. Medicinal plants may constitute a reservoir of new antimicrobial substances to be discovered. The World Health Organization (WHO) that the 4 billion people use herbal medicines for same aspect of primary healthcare[4]. In developing countries 80% of people use the traditional medicines which are based on plants products. Thus many studies have been conducted on medicinal plants. Currently 80% of the world population depends on the plants derived medicines for the first line of primary health care for human alleviation because it has no side effects[5].

All citrus fruits including the lemon are excellent for the treatment of cases of putrefaction, particularly when it concerns disorders in the liver. Citruses in many cases really help in stirring up any of the latent toxins accumulated inside the body which cannot be eliminated by any other means. Consuming lemon juice is of great value when it is necessary to be rid of the impurities and the fermentative effects of an impaired liver (D'Aoust 1997). Citric acid rich diets have often been employed with excellent results in treating many such disorders. It is important to remind oneself that the elimination diet is only a part of a correct lifestyle, since excessive citrus juices can actually thin the blood - they must not be used in large amounts for prolonged periods of time (Lachowicz et al 1998)[6].

MATERIALS AND METHODS

Collection of the samples:

Four different types of citrus fruits such as *Citrus reticulate, Citrus limetta, Citrus limon, Citrus aurantifolia* were collected from local market Karad, Dist. Satara.

Preparation and extraction of the sample:

The fruits were collected and washed thouroughly with distilled water and peels were separated and oven dried at 55^0C for 1 hrs. and then ground into coarse powder and preserved at room temperature for further use. The sample was extracted by using methanol or ethanol and the extract was then filtered through Whatmann filter paper no.1.

Preparation of ethanolic extraction:

The sample powder take and then make the 95% ethanol. Then the powder dissolved in the 95% ethanol for 24 hrs. After 24 hrs the extract was filtered using Whatmann filter paper no. 1 and this ethanolic extract are ready for further use.

Preparation of test organisms:

Preparation of standard bacterial suspension:

The 18 hours old bacterial culture of *Staphylococcus aureus, Proteus mirabilis, Bacillus subtilis, and Escherichia coli* were obtained and checked for their purity and Gram nature by carrying out Gram staining and other confirmations of its morphological characters and purity and the test organisms were inoculated into sterile saline tubes. The turbidity of bacterial culture adjusted with 0.5 MacFarland standard (10^8 CFU/ml.).

Determination of antimicrobial activity of peel extract of *Citrus reticulate, Citrus limetta, Citrus lemon, Citrus aurantifolia*

Preparation of paper discs:

The discs were prepared by punching the Whatmann filter paper no.1 with the help of punching machine. These discs is then taken into screw capped tube and sterilized in autoclaved at 121^0 C for 20 minutes to ensure the sterilization.

Determination of antimicrobial activity

Disc diffusion method was used to study the antimicrobial activity of the different citrus fruit peels viz. citrus reticulate, citrus limetta, citrus lemon, citrus aurantifolia. The nutrient agar medium was prepared and autoclaved at 121^0C for 20 minutes. Then medium was poured In sterile petri-plates and allowed to solidify. 0.1 ml of standardized bacterial culture suspension was spread on sterile Nutrient agar plates with the help of spreader aseptically. These plates were allowed to diffuse for five minutes before applying discs. Then discs were soaked into different peel extracts of *Citrus reticulate, Citrus limetta, Citrus lemon, Citrus aurantifolia*. Then disc were aseptically kept on the agar medium plates by using sterile forceps. The plates were incubated at 37^0C for 24 hours. After incubation plates were observed for zone of inhibition and zone of inhibition were measured and results were noted down.

RESULTS AND DISCUSSIONS

Results of antimicrobial activity of different peel extracts of citrus fruits, such as *Citrus reticulata, Citrus limetta, Citrus limon, Citrus aurantifolia* against laboratory pathogens. Result of antimicrobial activity of peel extracts of *Citrus reticulata, Citrus limetta, Citrus limon, Citrus aurantifolia* is shown in table 1.

Table 1: Results of antimicrobial activity of peel extracts of *Citrus reticulate, Citrus limetta, Citrus limon, Citrus aurantifolia*

Name of test organism	Ethanol extracts of *Citrus reticulate*	Ethanol extracts of *Citrus limatta*	Ethanol extracts of *Citrus limon*	Ethanol extracts of *Citrus aurantifolia*
Bacillus subtilis	1mm	3mm	2mm	3mm
Proteus mirabilis	-	-	3mm	2mm
Staphylococcus aureus	3mm	10mm	5mm	5mm
Escherichia coli	5mm	5mm	3mm	-

indicate no zone of inhibition observed.

The ethanolic extracts of *Citrus reticulata* showed antimicrobial activity against *Bacillus subtilis (1mm), Staphylococcus aureus (3mm), Escherichia coli (5mm).*

The ethanolic extracts of *Citrus limatta* showed antimicrobial activity against *Bacillus subtilis (3mm), Staphylococcus aureus (10mm), Escherichia coli (5mm).*

The ethanolic extracts of *Citrus limon*showed antimicrobial activity against *Bacillus subtilis (2mm), Proteus mirabilis (3mm), Staphylococcus aureus(5mm), Escherichia coli (3mm).*

The ethanolic extracts of *Citrus aurantifolia* showed antimicrobial activity against *Bacillus cereus (3mm), Proteus mirabilis (2nm), Staphylococcus aureus(5mm).*

The ethanolic extract of *Citrus reticulate* and *Citrus* limatta is does not showed antimicrobial activity against Proteus mirabilis. While the ethanolic extract of citrus aurantifolia does not showed antimicrobial activity against test organisam *Escherichia coli.*

Fig 1: Zone of Inhibition Showing Antimicrobial Activity of Citrus Reticulata and Citrus Limon Peel Extracts Against Bacillus subtilis and Staphylococcus aureus

The *Citrus reticulate, Citrus limetta, Citrus limon, Citrus aurantifolia.* were collected from local market of Karad. Antimicrobial potential of all the peel extracts were tested against the laboratory strains of *Bacillus subtilis, Proteus mirsbilis, Staphylococcus aureus and Escherichiacoli* by disc diffusion method. From this present study showed that the ethanolic extracts of *Citrus reticulate, Citrus limetta, Citrus lemon, Citrus aurantifolia* was found to be having good antimicrobial activity against *Bacillus subtilis, Proteus mirabilis, Staphylococcus aureus and Escherichia coli*. Thus the peel extracts of *Citrus reticulate, Citrus limetta, Citrus lemon, Citrus aurantifolia.* was generally used for medicinal purpose.

The findings of this study clearly indicate that the ethanolic extracts of citrus fruit peels possess notable antimicrobial activity against selected pathogenic bacteria. The tested fruits Citrus reticulata, Citrus limetta, Citrus limon, and Citrus aurantifolia demonstrated varying degrees of inhibition against Bacillus subtilis, Proteus mirabilis, Staphylococcus aureus, and Escherichia coli using the disc diffusion method.

Among the tested extracts, Citrus limetta showed the highest inhibition zone (10 mm) against Staphylococcus aureus, suggesting a strong antibacterial potential, which may be attributed to the presence of flavonoids, limonoids, essential oils, and ascorbic acid in its peel. Similarly, Citrus aurantifolia and Citrus limon also showed moderate to good activity against Staphylococcus aureus and Proteus mirabilis, which reinforces the traditional use of citrus in folk medicine for treating infections.

Interestingly, Citrus reticulata and Citrus limetta exhibited no activity against Proteus mirabilis, and Citrus aurantifolia showed no inhibition against Escherichia coli, indicating that the antimicrobial efficacy is species-specific and pathogen-dependent. The observed differences might be due to variations in phytochemical composition, extraction efficiency, or resistance mechanisms of the bacteria.

The study supports previous research highlighting the medicinal value of citrus peels, often considered waste, and underscores their potential in the development of eco-friendly antimicrobial agents. Given the rise of antibiotic-resistant pathogens, such natural alternatives could play a vital role in future pharmaceutical applications. Further studies including phytochemical profiling, MIC determination, and compound isolation are recommended to better understand the active principles and their mechanisms of action.

CONCLUSION

The present study demonstrated that the ethanolic peel extracts of various citrus fruits—Citrus reticulata, Citrus limetta, Citrus limon, and Citrus aurantifolia—exhibit antimicrobial activity against several pathogenic bacteria including Bacillus subtilis, Proteus mirabilis, Staphylococcus aureus, and Escherichia coli. Among them, Citrus limetta showed the strongest inhibitory effect, particularly against Staphylococcus aureus (10 mm zone of inhibition). The findings suggest that citrus peel waste, often discarded, possesses bioactive compounds with potential for medicinal and antimicrobial applications. Thus, citrus peel extracts can be considered a promising natural alternative to synthetic antimicrobial agents, especially in the context of rising antibiotic resistance.

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