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Antimicrobial Activity of *Citrus Sinensis* (Orange), *Citrus Limetta* (Sweet Lime) and *Citrus Limon* (Lemon) Peel Oil on Selected Food Borne Pathogens

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Abstract: **Due to rapid growth of the food processing industry and the consumption of processed foods, the demand for natural antimicrobial agents is on the rise. Consumers have become more aware about the health effects of the synthetic preservatives used in food. Hence natural preservatives are developed to meet the demand of consumers. These natural antimicrobials are developed either from plants or their parts, animals or even micro organisms. Even the waste generated from the food industries is being considered as an alternative to produce natural antimicrobials. The aim of the present study was to utilize the waste generated from the citrus fruit processing (peel) industry. The essential oil was extracted from the peel by hydro distillation process and was checked further for antimicrobial activity against food borne pathogens like *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shigella species and Candida albicans* using agar disc diffusion assay .The essential oil from the citrus (orange, sweet lime and lemon) peel was found to show inhibitory effect against all the organisms tested and thus showed broad spectrum activity. Comparison between the inhibitory activities of 3 essential oils was carried out. Lemon peel oil was found to show the most inhibitory effect as compared to orange peel oil and sweet lime peel oil. The results suggest that citrus peel oil can be used as natural preservative over other synthetic preservatives to minimize the ill effects of these synthetic preservatives and protect consumer health.**

***Keywords*: citrus peel oil, natural antimicrobial agent, food borne pathogens, citrus essential oil.**

1. INTRODUCTION

The genus *Citrus* is an important group of fruits rich in phenolic compounds as well as vitamins, minerals, dietary fibres, essential oils and carotenoids. They have a strong commercial value for fresh product market and food industry. Moreover, citrus productive generates large quantity of wastes and by-products that constitute an important source of bioactive compounds.

The demand for novel antimicrobial agents from natural resources has been increased worldwide for preservation of food (Velu S. *et al*., 2014). The antimicrobial agents are the agents that kill or inhibit the growth of other micro organisms. These have potential benefits over synthetic antimicrobials (Tagoe *et al.*, 2010). Natural antimicrobials received popularity from a series of issues related to microorganisms control and as a source of pharmaceutical active compounds (Amrita *et al.*, 2009). Alternatively as the safety aspects of chemical or synthetic food additives are dubious, the demand for naturally occurring preservatives is on the rise worldwide (Chanthaphon *et al.*, 2008). The exploration of novel antimicrobial agents from natural resources such as plant or plant products and others has been used mainly for treating diseases, food safety and food preservation purpose (Hammer *et al.*, 1999). Citrus is mainly used in the food industry for its fresh juice or citrus based drinks (Speigel P. *et al*., 1996). Citrus processing by-products are representative of a rich source of natural flavonoids, owing to the large amount of peel produced, and that citrus peels contain a high concentration of phenolic compounds (Rouseff *et al*., 2009).

The potential antimicrobial components in citrus peel are D-limonene, terpenes, sesquiterpene, oxygenated monoterpene, linalool, acid esters, aliphatic hydrocarbons and other unidentified hydrocarbons. The antimicrobial activity of citrus peel extract can be assessed on various food borne pathogens (Wahab O *et al.* 2013). Food borne pathogens include *Escherichia coli*, *Salmonella species*, *Shigella species*, *Klebsiella pneumonia*, *Vibrio species*, *Clostridium botulinum*, *Enterococcus species* and more. Food borne illnesses are on a rapid increase. The causative agents of these illnesses can be traced back by their presence in food either due to food contamination, food spoilage or mishandling of food. Use of natural antimicrobial agents may prevent or extend the time duration required for spoilage of food (Desrosier *et al*., 1997).

The present study aims at checking the antimicrobial activity of citrus peel on selected food borne pathogens. This essential oil of citrus peel can be used in food industries as a natural preservation method mainly in foods that require minimum heat processing or are required to be marketed without much cooking processes.

2. METHODS AND MATERIALS

2.1 Essential oil extraction of citrus peel:

The peel of fresh citrus fruits (orange, sweet lime and lemon) was removed and 250 g of the peel was pulverised to puree in a blender. The peel puree was subjected to hydro distillation in a 1 litre round bottom flask over a heating mantle with anti bumping granules. As the distillation progressed the oil and water layer separated in a collecting flask. The oil was separated using a dropper and was stored in amber coloured vials.

2.2 Microbial culture collection:

The microbial cultures used in the study were food borne organisms and were obtained from department of microbiology, Jai Hind College, Mumbai, India. The cultures include: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella* and *Candida albicans*. The cultures were maintained on sterile nutrient agar slant. The culture used was 24 hours old suspended in sterile saline.

2.3 Antimicrobial assay by disc diffusion method:

Nutrient agar was used as the growth medium in the present study. The antimicrobial agent to be tested was the essential oil extracted from the citrus peel (orange, sweet lime and lemon). The oil was impregnated on a sterile Whatman no. 1 filter paper disc (4mm) and was placed on the previously surface inoculated agar by surface spread method. The plates were incubated at 37˚C for 24 hours. Results were recorded by measuring the diameter of zone of inhibition. Clear inhibition zones around the disc indicated the presence of antimicrobial activity. The assay was performed in triplicates.

3. RESULTS AND DISCUSSION

Antimicrobial activity of orange peel oil was assessed against the above mentioned food borne pathogens using disc diffusion method. The results are presented in TABLE 1, TABLE 2 and TABLE 3 in terms of test organisms used and the diameter of zone of inhibition.

Table 1: Table showing diameter of zone of inhibition (in mm) with respect to orange peel oil

Organisms	Diameter of Zone of inhibition with respect to orange peel oil (in mm)			Mean± S.D (in mm)
Escherichia coli	11	12	11	11.33±0.57
Staphylococcus aureus	10	8	11	9.67±1.527
Salmonella typhi	10	10	11	10.33±0.57
Shigella species	8	9	9	8.67±0.57
Candida albicans	13	10	12	12.33±0.5

Orange peel oil showed inhibition against all the test organisms (Gram positive, Gram negative and yeast) with a maximum zone of inhibition against *Candida albicnas* and minimum zone of inhibition against *Shigella species.* Orange peel oil thus showed a broad spectrum activity against all the Gram positive, Gram negative and yeast used in the present study.

Table 2: Table showing diameter of zone of inhibition (in mm) with respect to sweet lime peel oil

Organisms	Diameter of Zone of inhibition with respect to sweet lime peel oil (in mm)			Mean± S.D (in mm)
Escherichia coli	9	10	9	9.33±0.57
Staphylococcus aureus	6	7	7	6.66±0.57
Salmonella typhi	8	10	7	8.33±1.52
Shigella species	10	11	11	10.66±0.57
Candida albicans	13	14	12	13.0±1.0

Sweet lime peel oil showed inhibition against all the test organisms (Gram positive, Gram negative and yeast) with a maximum zone of inhibition against *Candida albicans* and minimum zone of inhibition against *Staphylococcus aureus. Candida albicans* was found to be the most susceptible organism. Sweet lime peel oil thus showed a broad spectrum activity against all the Gram positive, Gram negative and yeast used in the present study.

Table 3: Table showing diameter of zone of inhibition (in mm) with respect to lemon peel oil

Organisms	Diameter of Zone of inhibition with respect to lemon peel oil (in mm)			Mean± S.D (in mm)
Escherichia coli	12	15	14	13.66±1.52
Staphylococcus aureus	20	21	20	20.33±0.57
Salmonella typhi	18	17	17	17.33±0.57
Shigella species	16	17	16	16.33±0.57
Candida albicans	20	22	22	21.33±1.154

Lemon peel oil showed inhibition against all the test organisms (Gram positive, Gram negative and yeast) with a maximum zone of inhibition against *Candida albicans* and minimum zone of inhibition against *Escherichia coli.* Lemon peel oil thus showed a broad spectrum activity against all the Gram positive, Gram negative and yeast used in the present study.

The reason for the different sensitivity of the Gram-negative bacteria compared to that of Gram-positive bacteria could be due to differences in their cell wall composition. Gram-positive bacteria contain an outer peptidoglycan layer, which acts as a permeability barrier, whereas Gram-negative bacteria have an outer phospholipid membrane (Samarakoon *et al., 2012*).

Table 4: Comparison of inhibitory activity of the 3 essential oils against the test organisms

Organisms	Diameter of Zone of inhibition (in mm) (mean±S.D)		
	orange peel oil	sweet lime peel oil	Lemon peel oil
Escherichia coli	11.33±0.57	9.33±0.57	13.66±1.52
Staphylococcus aureus	9.67±1.527	6.66±0.57	20.33±0.57
Salmonella typhi	10.33±0.57	8.33±1.52	17.33±0.57
Shigella species	8.67±0.57	10.66±0.57	16.33±0.57
Candida albicans	12.33±0.5	13.0±1.0	21.33±1.154

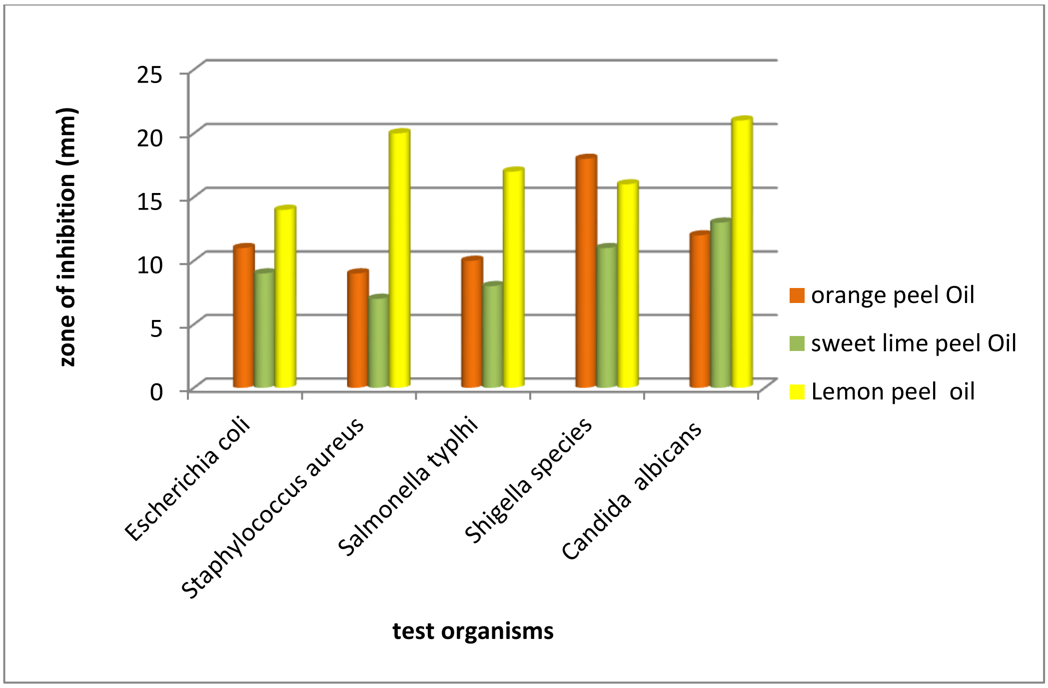

Figure1: Graph showing diameter of zone of inhibition (in mm) with respect to the 3 essential oils against the test organisms

The graph clearly indicates that all the 3 essential oils showed a broad spectrum activity against the test organisms used in the study. However it was found that lemon peel oil had maximum inhibitory action against all the test organisms except *Shigella species*, which was maximally inhibited by orange peel oil. Also it was found that sweet lime peel oil had minimum inhibition against all the test organisms except *Candida albicans*, which was minimally inhibited by orange peel oil.

The inhibitory activity of the essential oil may be a cumulative effect of D-limonene and some other unidentified components or flavonoids and phenolic compound present. Unal *et al.*also demonstrated the antifungal and inhibitory effect of D-limonene on a variety of yeast strains. For instance, flavonoids are known as antimicrobial agents and some phenolic compounds have be shown to inhibit the growth of *Staphylococcus aureus* (Cushnie and Lamb, 2005).

4. CONCLUSION

The aim of the present study was to utilize the waste generated from the citrus fruit processing (peel) industry. The essential oil was extracted from the peel by hydro distillation process and was checked further for antimicrobial activity against food borne pathogens like *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shigella species and Candida albicans* .The essential oil from the citrus (orange, sweet lime and lemon) peel was found to show inhibitory effect against all the organisms tested and thus can be concluded that it showed broad spectrum activity. Lemon peel oil was found to show the most inhibitory effect than orange peel oil and sweet lime peel oil.

Citrus peel oil can find a variety of applications in food industry. Citrus essential oils could represent good candidates to improve the shelf life and the safety of minimally processed fruits, skim milk and low-fat milk (Lanciotti *et al*., 2004). Plant essential oils show great promise as natural preservatives due to their low bacteriostatic and bactericidal concentrations against some of the most important food-borne pathogens and also the growing demand for natural alternatives of artificial preservatives (Fazlara *et al*., 2008). Citrus oils have been commonly used as flavouring agent in food industries and are classified as GRAS (generally recognised as safe), therefore they may be potentially ideal alternatives as starting point for the use of essential oils for antimicrobial preservation of foods. Citrus oils showed notable results in preventing spoilage in different type of food such as fish, meat, chicken, fruit and vegetables, dairy products and confectionery (Fisher and Phillip, 2008).

However, further studies with pure compounds of citrus peel oil have to be performed to evaluate and get a definite conclusion of the antimicrobial activity of citrus oil.

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