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Effects of citrus essential oils on growth kinetics of foodborne pathogens: In silico ADMET studies of major compounds

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Abstract

The peels of citrus fruit are always discarded as waste and cause environmental pollution. The waste citrus peels can be used to extract essential oils which are environmental friendly, cost effective and natural alternative to chemical agents to inhibit foodborne pathogens. Essential oils are mixture numerous biologically active compounds which has many biological properties like antimicrobial, antioxidant and often use as food preservative. In current study the effects of essential oils of citrus varieties mainly PL1, PL2, PL3 and PL4 were evaluated against *Bacillus cereus* MTCC430 and *Yersinia enterocolitica* MTCC859 and found that oils were affecting the growth kinetics of bacterial strains at MIC, 1/2MIC and 1/4MIC concentrations. The yields of essential oils were found to be PL1: *Citrus jambhiri* followed by PL4: *Citrus aurantifolia* (rough lemon), PL3: *Citrus limon* (elongated) and PL2: *Citrus medica* that is 7.5%, 6.2%, 5.3% and 3.5% respectively. The ADMET profiling showed safety of the selected compounds with better bioavailability score and good brain penetration. The vast possibilities of bioresources of North East India need to be explored.

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Introduction

The large number foodborne diseases are caused by different bacteria which affect human health and economy. The diseases are caused by foodborne pathogens occurred when pathogens entered in human body and multiply or pathogens colonise food products and produce toxins which are then consumed by human beings. More than 200 foodborne diseases were identified that affect individuals with compromised immune systems. The most common microorganisms that are responsible for foodborne diseases are *Bacillus cereus* and *Yersinia enterocolitica* which results complications like vomiting, abdominal cramps, fever and diarrhoea etc. (Bintsis *et al.*, 2017; Tauxe *et al.*, 2007; Akarca *et al.*, 2019). The recognition of new foodborne pathogens and new challenges like antimicrobial resistance is affecting large part of the world population and new pathogens are mainly emerging due to changing global environmental conditions with time (Gourama *et al.*, 2020). The microorganisms like bacteria, fungi and viruses cause food contamination in different stages and some of the pathogens and toxins produced by them are resistant to heat. So, the controlling of these pathogens is very complex and cannot be prevented by conventional methods like cooking and freezing etc. (Martinović *et al.*, 2016). Essential oils are mixture of secondary metabolites which possess numerous biological activities like antioxidant, anti-inflammatory and antimicrobial activities etc. The numerous essential oils are used as food preservative and main barriers of using essential oils as preservative are their organoleptic effects on the food products. There is a negative notion about synthetic food preservatives due to increased health consciousness among peoples. Therefore, essential oils which are natural and safe can be used as preservative in food industries (Falleh *et al.*, 2020; Hyldgaard *et al.*, 2012; Özogul *et al.*, 2022). The essential oils are effective against both gram positive and gram-negative bacteria and this may be attributed to the presence of high content of oxygenated compounds of the oils. The essential oils derived from plants prevent deterioration of fats in foodstuffs and delay onset of diseases (Tongnuanchan *et al.*, 2014; Adelakun *et al.*, 2016; Loying *et al.*, 2023). The current study focuses on the growth inhibiting potential of Citrus essential oils against foodborne pathogens by affecting growth patterns and ADMET (Absorption, distribution, metabolism, excretion and toxicity) profiling revealed that selected compounds of the essential oils have safe profiles and could be natural antimicrobial agents to inhibit foodborne pathogens in foodstuffs.

Materials and methods

Extraction of essential oils and determination of yield

Four indigenous citrus varieties of North East India PL1: *Citrus jambhiri*, PL2: *Citrus medica,* PL3*: Citrus limon* (elongated) and PL4: *Citrus aurantifolia* (rough lemon) were collected from different part of North East India, and then surface sterilized with 70% of ethanol followed with distilled water washing and the collected peels were used to extract essential oils using Clevenger apparatus method described by Thien Hien Tran *et al.*, 2019 and yield of the EOs were determined by method mentioned by Elyemni *et al.*, 2019 and then kept in storage at -20°C until needed.

Bacterial strains and their growth conditions

Bacterial strains *Bacillus cereus* MTCC430 and *Yersinia enterocolitica* MTCC859 were obtained from Microbial Type Culture Collection (MTTC), IMTECH Chandigarh and grown in LB medium at 37°C at shaker incubator.

Determination of the effects of essential oils on growth patterns of the bacterial strains

The effects of the oils on bacterial growth curve were determined by the method described by Wang *et al.* (2020) with slight modifications. Briefly 200 µl of bacterial strains with 10^6cfu/ml was inoculated in 200 ml of LB medium and then essential oils with concentrations 1/2MIC and MIC were treated into the medium inoculated with bacteria. Then oil treated bacterial culture were incubated in shaker incubator at 35°C with 150 rpm and absorbance of the bacterial cultures were monitored for every 2 hours intervals at 600 nm.

In-silico toxicity assessment of the oils

The compositions of the essential oils were reported by surjya *et al.*, 2023. To assess the insilico toxicity of the EOs, the canonical smiles formats of the compounds identified in the EOs by GCMS were downloaded from the PUBCHEM. And then compounds were virtually evaluated for ADME and toxicity using an online tool pkCSM and pharmacokinetics and druglike properties were analysed by SwissADME.

Results and discussion

Yield of the essential oils

By using Clevenger apparatus essential oils from the collected peels sample of the citrus varieties were extracted by hydrodistillation method. It was observed that there was variability in the percentage of yield of oils from the samples as shown in the Table 1.

Table 1. Yield variability of EOs from peels of four different varieties of citrus species

Name of the sample	Sample Code	Yield of EOs (% V/W)
Citrus jambhiri	PL1	7.5
Citrus medica	PL2	3.5
Citrus limon (elongated)	PL3	5.3
C. aurantifolia (rough lemon)	PL4	6.2

The percentage yield of essential oil found to be highest for *Citrus jambhiri* followed by *Citrus aurantifolia* (rough lemon), *Citrus limon* (elongated) and *Citrus medica* that is 7.5%, 6.2%, 5.3% and 3.5% respectively. The findings of the present study are comparable with previous reports of Bibi Sharmeen Jugreet *et al.*, 2020 and Fancello *et al.*, 2016, in which they reported the yield of essential oils from rhizome of *Curcuma longa* and ripe fruits of *Morinda citrifolia* were 1.21% and 0.35% respectively. The findings are also in accordance with finding of Francesco Fancello *et al.*, 2016, in which they showed yield of essential oil from *Citrus limon var. pompia* leaf between 0.43 percent to 0.52%.

Effects of essential oils on growth pattern of the bacterial strains

The effects of essential oils on growth pattern of *Bacillus cereus* MTCC430 and *Yersinia enterocolitica* MTCC859 were investigated for 20 hours duration in different concentration like MIC, 1/2MIC and 1/4MIC are shown in the Fig. 1 and 2. The optical density of the bacterial samples without EOs treatment (Control) showed rapid increase compared to the EOs treated samples. When *Bacillus cereus* MTCC430 treated with MIC, ½ MIC and 1/4MIC concentration of PL1, the bacterial growth delayed and optical density increased from 0.041 to 0.492 (at MIC), 0.072 to 1.646 (at 1/2MIC) and 0.079 to 1.799 (at 1/4MIC). Similarly, when treated PL2 the optical density decreases compared to the control i.e. the optical density changed from 0.080 to 0.395 (at MIC), 0.074 to 1.646 (at 1/2MIC) and 009 to 1.76 (at 1/4MIC). Moreover, the similar delay in growth was observed in PL3 and PL4 treated samples. In PL3 treated samples the change in optical density observed from 0.058 to 0.594 at MIC level, 0.061 to 1.687 at 1/2MIC level and 0.073 to 1.730 at 1/4 MIC level. However, in PL4 treated samples the difference in optical density was observed compared the control. The change of optical density was from 0.086 to 0.524 at MIC level, 0.082 to 1.729 at 1/2 MIC level and 0.085 to 1.730 at 1/4 MIC level respectively. Similarly, the effects of these EOs on the growth of *Yersinia enterocolitica* MTCC859 was analysed and inhibited growth pattern was observed in the treated samples compared to the control sample. In PL1 treated sample extended log phase was observed and there was rapid rise in optical density of the control from 0.234 to 1.924 whereas there was delay in growth pattern from optical density 0.234 to 0.382 at MIC, 0.092 to 1.846 at 1/2MIC and 0.159 to 1.769 at 1/4MIC of the oil for duration of 20 hours. Further assessment with PL2 treatment, the change in optical density from 0.234 to 0.379 at MIC level, 0.234 to 1.646 at 1/2MIC and 0.259 to 1.969 at 1/4MIC were observed in given time duration. The extended lag phase was observed in the PL3 treated sample compared to the control (without oil treatment), the change optical density from 0.246 to 0.304 at MIC, 0.241 to 1.929 at 1/2MIC and 0.285 to 1.930 at 1/4MIC were observed.

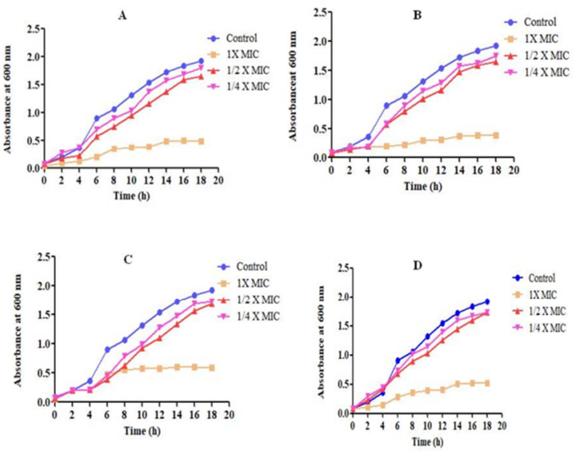

Fig. 1. Effects of essential oils on growth curve of the *Bacillus cereus* MTCC430. The effect of PL 1(A), The effect of the PL2 (B), The effect of PL 3 (C) and The effect of the PL 4(D).

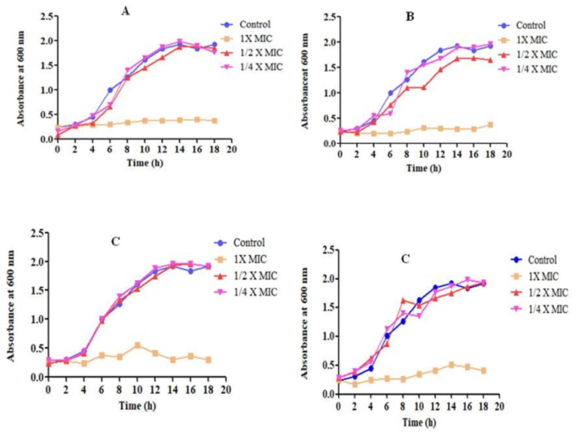

Fig. 2. Effects of essential oils on growth curve of the *Yersinia enterocolitica* MTCC859. The effect of PL1 (A), The effect of PL2 (B), The effect of PL3 (C) and The effect of PL4 (D).

However, treatment with the PL4 also resulted change in growth pattern of the bacteria, the change in optical density was from 0.246 to 0.404 at MIC, 0.282 to 1.929 at 1/2MIC and 0.285 to 1.930 at 1/4MIC. The finding of the present study is in conformity with findings of Abdelhakim Bouyahya *et al.*, 2019, in which they reported essential oil of *O. compactum* showed the alternation of the growth kinetics of bacteria which affected the different phases of the bacteria at 2MIC, 1/2MIC and MIC concentration. The essential oil of *Oregano* at MIC the growth *V. vulnificus* completely inhibited and at 1/2MIC the lag phase was prolonged and below 1/4MIC there was no effects on the growth pattern (Fancello *et al.*, 2016). Another report showed that antimicrobials of essential oils prolonged lag phase and reduced the exponential growth rate (Valero *et al.*, 2006).

ADMET profiling of selected compounds

The pharmacokinetics and drug like properties of the selected compounds are shown in the Table 2. The compounds analysed were found to meet the Lipinski Rule of five and values for TPSA (Topological polar surface area) were found to be less than 30 Å2 which indicates good brain penetration and lipophilicity behaviour as consensus Log Po/w values of the compounds were found to be 2.31 to 4.96. The bioavailability score of all the compounds were 0.55 which indicates more drugs like properties and none of the compounds were substrate of P-glycoprotein (P-gp) which indicates good intestinal absorption.

Table 2. The pharmacokinetic and drug like property of the selected compounds

Compound	P-gp substrate	Lipinski	TPSA (Å2)	Consensus LogPo/w	Bioavailability score
1	No	Yes	0.00	3.44	0.55
2	No	Yes	0.00	3.37	0.55
3	No	Yes	0.00	4.24	0.55
4	No	Yes	0.00	4.38	0.55
5	No	Yes	20.23	2.92	0.55
6	No	Yes	17.07	2.71	0.55
7	No	Yes	0.00	4.96	0.55
8	No	Yes	0.00	4.50	0.55
9	No	Yes	0.00	3.42	0.55
10	No	Yes	20.23	2.31	0.55

***.** 1: Alpha-Pinene, 2: Limonene, 3: Caryophyllene, 4: Beta-Bisabolene, 5: Citronellol, 6: Citral, 7: Alpha-Farnesene, 8: Longifolene, 9: Beta-Pinene, 10: Trans-Verbenol.

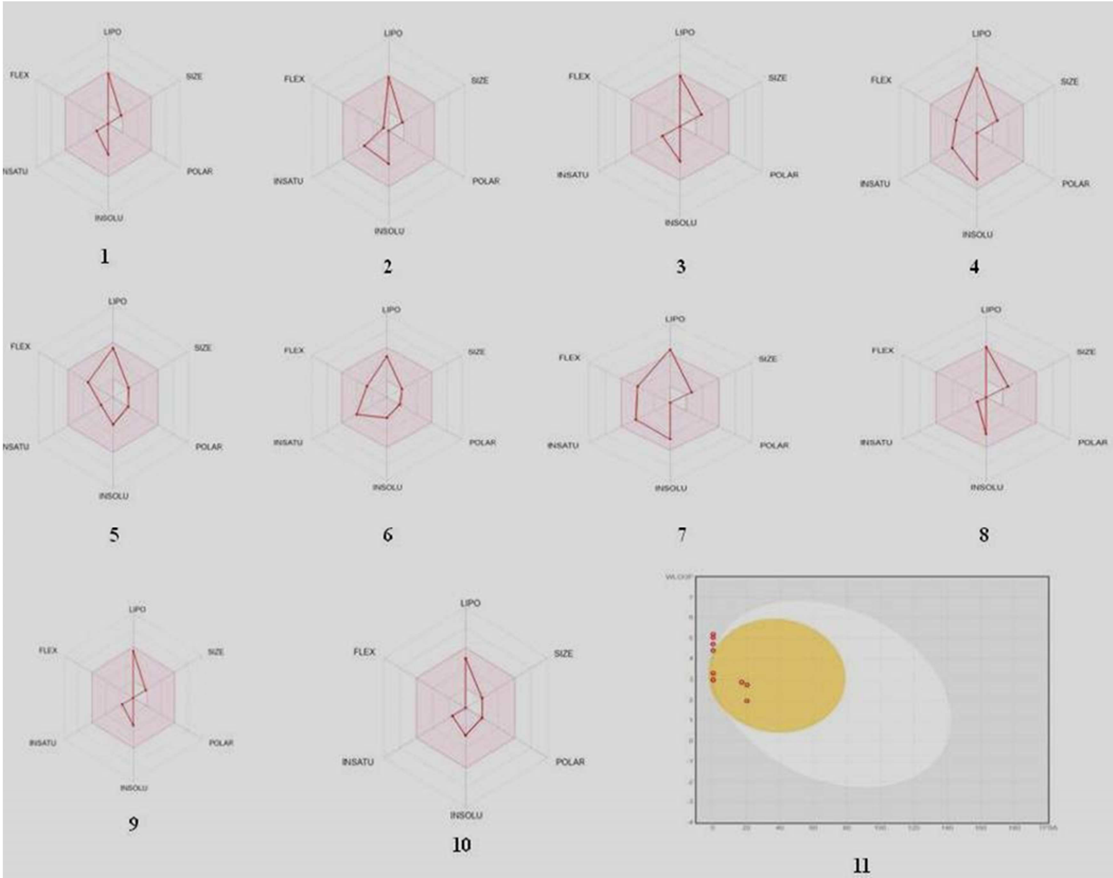

Fig. 3. Radar plot and boiled egg graph of the selected compounds. 1: Alpha-Pinene, 2: Limonene, 3: Caryophyllene, 4: Beta-Bisabolene, 5: Citronellol, 6: Citral, 7: Alpha-Farnesene, 8: Longifolene, 9: Beta-Pinene, 10: Trans-Verbenol, 11: Boiled egg plot of selected compounds

Moreover, the radar plots (Fig. 3) of all the compounds were inside the pink areas are indicative of good bioavailability score along with more drugs like properties. Further the compounds were evaluated by the boiled egg model and most of the compounds were found in the yellow region that confirms the probability of the brain penetration is high. The findings of the present study are in accordance with Alminderej *et al.*, 2020; Karakoti *et al.*, 2022, in which they reported that compounds of *Piper cubeba* L. fruit and *Vitex* species essential oil met the Lipinski Rule of five and TPSA values less than 30 Å2 with good brain penetration, more drug likeness property and good bioavailability profile.

Conclusion

The current study mainly focused on the growth inhibition properties of Citrus peel's essential oils against foodborne pathogens**.** The EOs of the citrus peels has the potential of inhibiting the foodborne pathogens by affecting the growth pattern of the pathogens. The ADMET profiling of the revealed that most of the compounds of the essential oils have safety profile better brain penetration, lipophilicity behaviour, good intestinal absorption and good bioavailability score. So essential oils from Citrus varieties showing safety profile could be a potential natural agent that affects growth patterns of foodborne pathogens having safety profiles. However, it is necessary to investigate safety profiles of the essential oils in vivo model system for better efficacy.

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