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**Vitamin C and E contents, antioxidant, antibacterial and antiproliferative activities of
Citrus suhuiensis peel**

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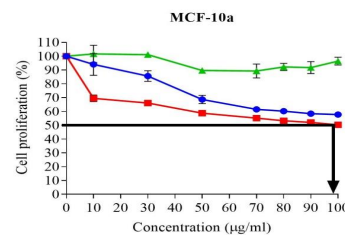
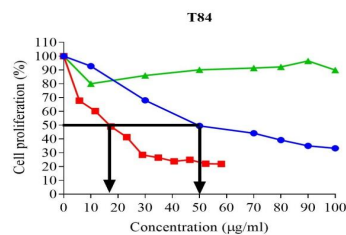
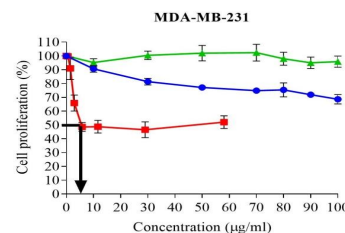
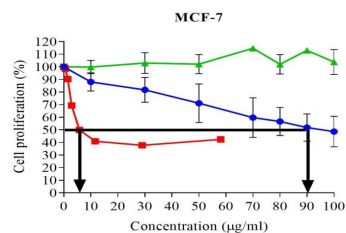


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Vitamin C and E contents, antioxidant, antibacterial and antiproliferative activities of *Citrus suhuiensis* peel

Graphical Abstract



● Orange peel ■ Lapatinib +ve control ▲ DMSO -ve control



Abstract

Citrus suhuiensis is a citrus fruit with greenish yellow peel. It is consumed as fresh produce but the peels are often discarded as waste which might also contain biological potentials. This study aimed to determine the vitamin C and E contents, antioxidant, antibacterial and antiproliferative properties of *Citrus suhuiensis* peel crude extract. Vitamin C and E contents were measured following the AOAC method. The total phenolic content was evaluated using Folin-Ciocalteu assay. The capability of the extract to scavenge free radical was elucidated using DPPH assay. Agar disk diffusion method was used to evaluate the antibacterial effect. The antiproliferative effects of *Citrus suhuiensis* crude extract on MCF-7 (estrogen-dependent breast cancer), MDA-MB-231 (non-estrogen-dependent breast cancer), T84 (colon cancer) and MCF10a (normal fibroblast) cell lines were assessed via MTS assay. Vitamin C (19.18 ± 0.14 mg/100 g sample) in *C. suhuiensis* peel was significantly higher than vitamin E (0.018 ± 0.00 mg/100 g sample) ($p < 0.001$). *Citrus suhuiensis* crude extract was unable to scavenge 50% of DPPH free radicals. The extract showed no antimicrobial properties against all bacterial strains tested. *Citrus suhuiensis* crude extract inhibits 50% MCF-7 estrogen-dependent breast cancer and T84 colon cancer cell growth at 90.03 ± 0.18 and 50.11 ± 1.46 $\mu\text{g/ml}$, respectively. No inhibition was observed on MDA-MB-231 non-estrogen-dependent breast cancer and MCF10a normal fibroblast cell growth. Although *C. Suhuiensis* peel has very limited antibacterial and DPPH radical scavenging activities but it shows antiproliferative effect against estrogen-dependent breast cancer and colon cancer cell growth.

Keywords: *Citrus suhuiensis* peel crude extract, vitamin C and E, antioxidant, antibacterial, antiproliferative



Introduction

- Citrus suhuiensis (local name; limau langkat or madu) is grown mainly in Terengganu state of Malaysia.
- Health benefits of citrus fruits are attributed to the presence of high antioxidants such as phenolics (e.g., flavanone glycosides, hydroxycinnamic acids), vitamin C and carotenoids.
- A very large amount of citrus fruit residues such as peels are derived every year (Manthey & Grohmann, 2001). To minimize the burden of environmental waste, we should utilize the orange peels in an effective and beneficial way.
- However, very few studies have investigated the antioxidant, antibacterial and anticancer activity of citrus fruit peel and no studies are available which investigated all these properties of the local species of *C. suhuiensis* peel.
- This study was conducted to investigate the presence of antioxidant vitamins C and E, antioxidant, antibacterial and anticancer activities of suhuiensis peel extract.



Results and discussion

Vitamin C and E content

HPLC analysis of *Citrus suhuiensis* orange peel showed that it contains both vitamin C and vitamin E. *Citrus suhuiensis* peels contained significantly higher vitamin C (19.72 ± 0.14 mg/100 g sample) than vitamin E (0.02 ± 0.005 mg/100 g sample) ($p < 0.01$) (**Figure 1**). This finding tallied with our expectation as *Citrus suhuiensis* fruit pulp is well-known for its vitamin C content (de Moraes Barros et al., 2012).

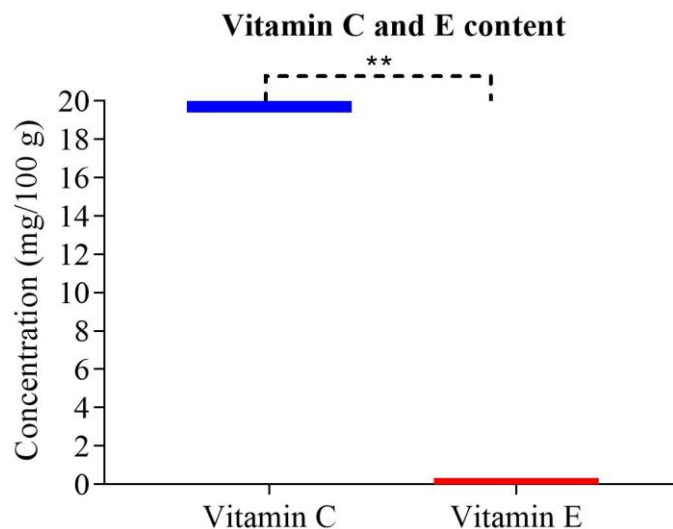


Figure 1. Vitamin C and vitamin E content in *Citrus suhuiensis* orange peel as analysed using HPLC following AOAC method. Vitamin C (19.72 ± 0.14 mg/100 g sample) was significantly higher than vitamin E (0.02 ± 0.005 mg/100 g sample) ($p < 0.01$).



Results and discussion

Antioxidant activities

The total phenolic content in *C. suhuiensis* orange peel crude extract was evaluated using Folin-Ciocalteu assay. BHT and vitamin E were used as standards. BHT contained the highest phenolic content (235.66 ± 1.41 mg GAEs/g sample) which was significantly higher than *C. suhuiensis* orange peel crude extract (108.00 ± 1.0 mg GAEs/g sample) and vitamin E (104.25 ± 0.35 mg GAEs/g sample) ($p < 0.0001$) (**Figure 2**). No significant difference was observed between total phenolic content in *C. suhuiensis* orange peel crude extract and vitamin E (**Figure 2**).

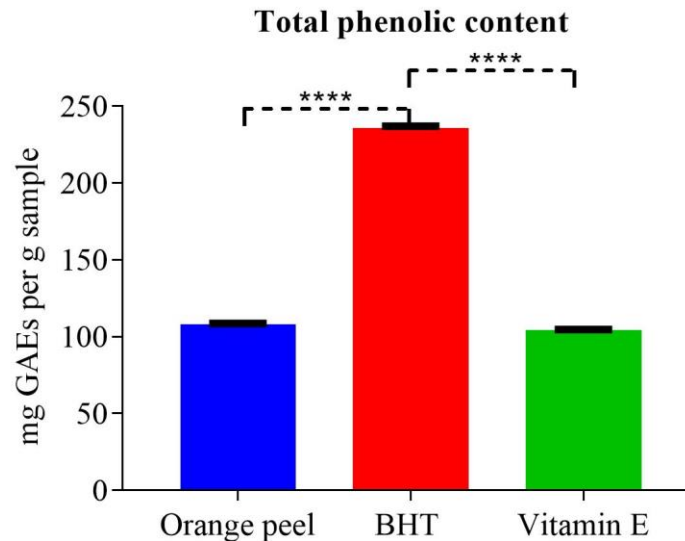


Figure 2. Total phenolic content in *Citrus suhuiensis* orange peel crude extract, BHT and vitamin E as evaluated using Folin-Ciocalteu assay. Results are expressed as mg gallic acid equivalents (GAEs) per g sample. BHT (235.66 ± 1.41 mg GAEs/g sample) showed significantly higher total phenolic content compared to orange peel crude extract (108.00 ± 1.0 mg GAEs/g sample) and vitamin E (104.25 ± 0.35 mg GAEs/g sample) ($p < 0.0001$). Data shown as mean \pm S.E.M; $n=4$.

Results and discussion

The ability of *C. suhuiensis* orange peel crude extract to scavenge DPPH free radical was measured using DPPH free radical scavenging assay. BHT and vitamin E were used as standards. The DPPH free radical can easily receive an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule (Aksoy et al., 2013). The dose-response curve for the free radical scavenging activity of *C. suhuiensis* peel crude extract and standards at different concentrations are presented in **Figure 3**. Only BHT was able to scavenge 50% DPPH free radical at $150 \pm 0.57 \mu\text{g/ml}$. Both *C. suhuiensis* orange peel crude extract and vitamin E were not able to scavenge 50% of DPPH free radical.

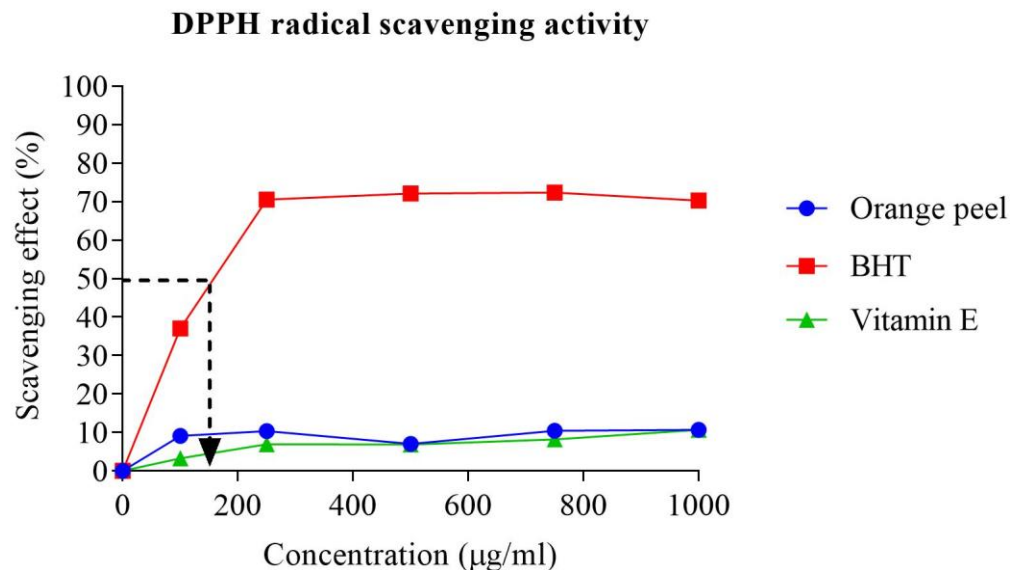


Figure 3. DPPH free radical scavenging effect of *Citrus suhuiensis* orange peel crude extract, BHT and vitamin E. Only BHT was able to scavenge 50% DPPH free radical at $150 \pm 0.57 \mu\text{g/ml}$. Both *Citrus suhuiensis* orange peel crude extract and vitamin E were not able to scavenge 50% of DPPH free radical. Data shown as mean \pm S.E.M; n=4.

Results and discussion

C. suhuiensis orange peel crude extract contained high amount of phenolic compound but did not scavenge free radicals which contradicts with previous studies that suggest low free radical scavenging activities contribute to low antioxidant activities which are due to low phenolic content (Ramkissoon et al., 2013). Nevertheless, use of several antioxidant methods are required to confirm the findings as different antioxidant methods have different principles which evaluate different forms of antioxidant activity (Moharram and Youseff, 2014).

Results and discussion

Antibacterial activities

In this study, we investigated the antibacterial activity of *C. suhuiensis* orange peel extract against *S. aureus*, *E. coli* and *P.aeruginosa*. Gentamycin was used as positive control. Among the three microorganisms tested, *C. suhuiensis* orange peel extract showed a significantly higher antibacterial effect against gram-positive bacteria *S. aureus* (1.23 ± 0.03 mm inhibition zone) compared to gram-negative bacteria *E. coli* (0.90 ± 0.00 mm inhibition zone) and *P.aeruginosa* (0.83 ± 0.03 mm inhibition zone) ($p < 0.001$) (**Figure 4**). However, the strength is not adequate as an antibacterial agent against all tested microorganisms. Gentamycin possessed the largest zone of inhibition against all three types of bacteria tested (*S. aureus*: 2.50 ± 0.00 mm inhibition zone, *E. coli*: 2.63 ± 0.07 mm inhibition zone, *P. aeruginosa*: 2.40 ± 0.00 mm inhibition zone). The results were significantly higher than *C. suhuiensis* orange peel extract ($p < 0.0001$) (**Figure 4**).

Results and discussion

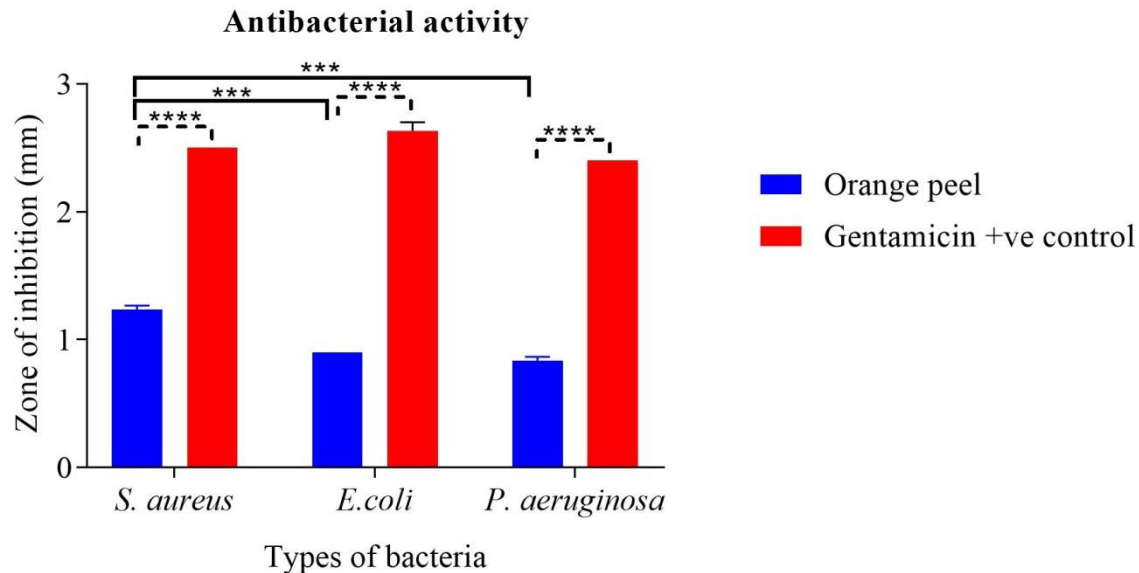


Figure 4. Antibacterial activity of *C. suhuiensis* orange peel crude extract against *S. aureus*, *E. coli* and *P. aeruginosa* as assessed using disc diffusion method. Results were compared with gentamicin (positive control) and between all microorganisms tested. Gentamycin possessed the largest zone of inhibition against all three types of bacteria tested (*S. aureus*: 2.50 ± 0.00 mm inhibition zone, *E. coli*: 2.63 ± 0.07 mm inhibition zone, *P. aeruginosa*: 2.40 ± 0.00 mm inhibition zone). The results were significantly higher than *C. suhuiensis* orange peel extract ($p < 0.0001$). *C. suhuiensis* orange peel crude extract showed significantly higher antibacterial effect against *S. aureus* (1.23 ± 0.03 mm inhibition zone) as compared to *E. coli* (0.90 ± 0.00 mm inhibition zone) and *P. aeruginosa* (0.83 ± 0.03 mm inhibition zone) ($p < 0.001$). Data shown as mean ± S.E.M; n=4.

Results and discussion

Antiproliferative activities

MCF-7, MDA-MB-231, T84 and MCF-10a cell lines were treated with *C. suhuiensis* orange peel crude extract at a series of concentrations (10-100 $\mu\text{g/ml}$) to determine the concentration of the extract that could inhibit 50% cell growth (**Figure 5**). Results were also compared with lapatinib, an anticancer drug, as a positive control treatment in which lapatinib inhibits 50% of all the cell lines tested (MCF-7: $6.01 \pm 1.11 \mu\text{g/ml}$; MDA-MB-231: $5.81 \pm 0.98 \mu\text{g/ml}$; T84: $17.43 \pm 0.87 \mu\text{g/ml}$; MCF-10a: $98.05 \pm 0.54 \mu\text{g/ml}$). However, *C. suhuiensis* orange peel crude extract inhibits 50% of MCF-7 and T84 cell growth at $90.03 \pm 0.18 \mu\text{g/ml}$ and $50.11 \pm 1.46 \mu\text{g/ml}$, respectively. DMSO did not cause 50% cell inhibition at any of the concentrations, which signifies that the vehicle did not influence the cytotoxic effect of the extract and lapatinib on the cell line (**Figure 5**).

Results and discussion

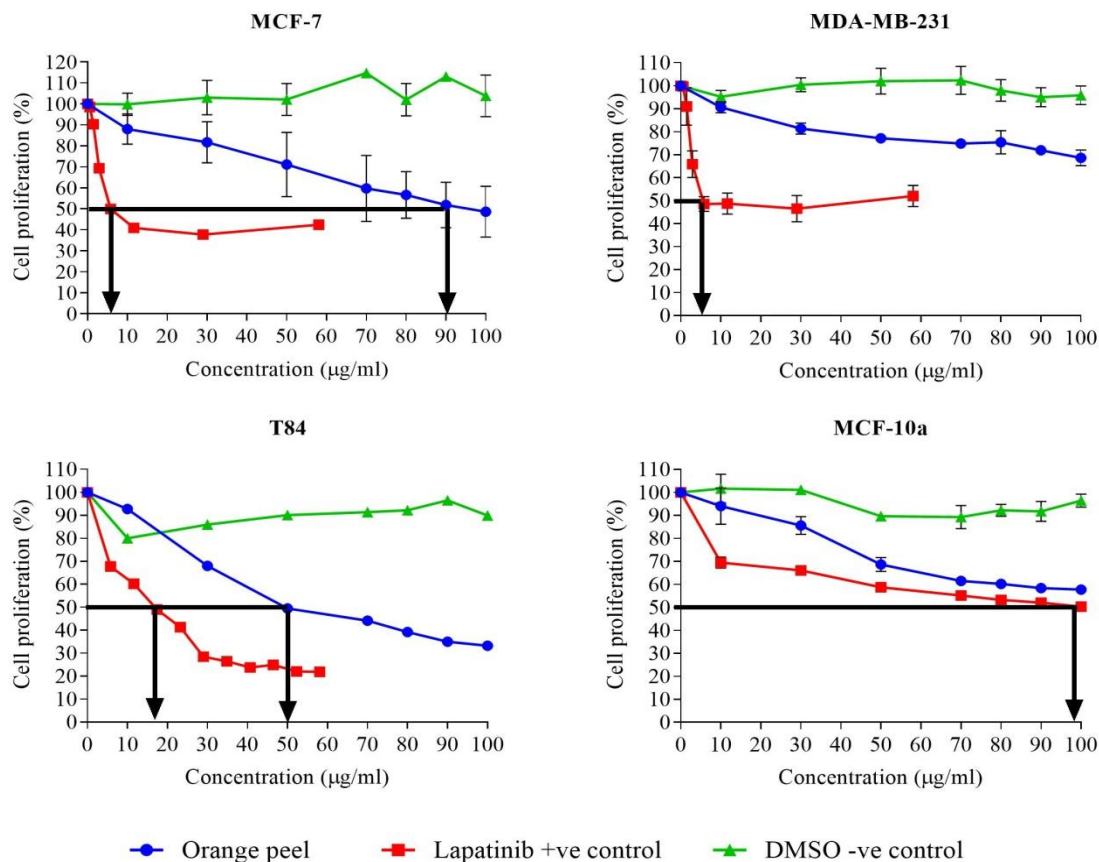


Figure 5. Antiproliferative activities of *C. suhuiensis* orange peel crude extract, lapatinib (positive control) and DMSO (vehicle/negative control) on cancer and normal cell lines as assessed using MTS assay. *C. suhuiensis* orange peel crude extract inhibits 50% cell growth of MCF-7 (IC₅₀=90.03 ± 0.18 µg/ml) and T84 (IC₅₀= 50.11 ± 1.46 µg/ml) cell lines while lapatinib inhibits 50% cell growth of all cell lines tested (MCF-7: 6.01 ± 1.11 µg/ml; MDA-MB-231: 5.81 ± 0.98 µg/ml; T84: 17.43 ± 0.87 µg/ml; MCF-10a: 98.05 ± 0.54 µg/ml) DMSO does not inhibit 50% cell growth of all cell lines which signifies that the vehicle does not influence the IC₅₀ values. Graph shown for each cell line is¹² representative of experiments conducted. Data shown as mean ± S.E.M; n=3.

Results and discussion

C. suhuiensis orange peel crude extract showed antiproliferative activities on the MCF-7 human estrogen-dependent breast cancer and T84 human colon cancer cell lines. The peel crude extract does not inhibit 50% MCF-10a normal fibroblast cell growth. The phenolic content in the crude extract might contribute to *C. suhuiensis* orange peel crude extract growth inhibitory effect. It is believed that inhibition of cell growth is closely associated with antioxidant compound of the extract (Rodriguez 2016).

Conclusions

C. suhuiensis orange peel has very limited antibacterial and free radical scavenging activities but it shows antiproliferative effect against estrogen-dependent breast cancer and colon cancer cell growth which could be due to its vitamin C and E and total phenolic contents. Further investigations are required to increase understanding on the underlying mechanisms.



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