Anti-inflammatory, antibacterial activity and chemical composition of essential oil extracted from *Citrus aurantium* L. leaves using solvent-free microwave extraction technique

D. Jagoda[1,2], G. D. Liyanaarachchi[1], N. Fernando[2], S. M. Handunnetti[2], H. D. Weeratunge[1], W. Divisekera[1], J. K. R. R. Samarasekera[1]

[1]*Industrial Technology Institute, 363, Bauddhaloka Mawatha, Colombo 07, Sri Lanka*
[2]*Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka*

Citrus aurantium is known for its therapeutic properties in managing inflammation associated with disease conditions. Its essential oil (EO) is valued for antiseptic, antimicrobial, astringent properties and is used in aromatherapy, to alleviate anxiety and pain. This study presents the first systematic evaluation of the chemical composition, anti-inflammatory and antibacterial activity of EO extracted from the leaves of *C. aurantium,* using solvent-free microwave extraction (SFME) technique. Fresh leaves (200 g) were subjected to SFME using the NEOS GR microwave extractor to obtain the EO. Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze the chemical composition. Anti-inflammatory activity was evaluated by the inhibitions of enzymes arachidonate 5-lipoxygenase (A5-LOX), arachidonate 15-lipoxygenase (A15-LOX) activities, and inhibition of nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages. Antibacterial activity of EO was evaluated by agar well diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhimurium,* and *Candida albicans.* GC-MS analysis identified D-limonene (25.35%) and α-Citral (17.00%) as major compounds in *C. aurantium* EO. Dose-dependent inhibitions of A5-LOX and A15-LOX activities were recorded with IC_{50} values of 6.97±0.85 and 411.24±2.23 µg/mL respectively ($r=0.995$ & 0.979; $p<0.05$) which were significantly higher compared to the reference standard baicalein (IC_{50}: *2*.09±0.19 µg/mL; $p<0.05$). The *C. aurantium* EO dose-dependently inhibited NO production ($r=0.987$; $p<0.05$), with 74.44±0.58% inhibition at a non-toxic concentration of 1000 µg/mL in LPS-stimulated RAW 264.7 cells, with IC_{50} value of 391.06±31.43 µg/mL. The EO exhibited moderate antimicrobial activity with an inhibition zone of 10 mm for both *E. coli*, and *B. subtilis* at 1000 µg/mL compared to the standard antibiotic, ciprofloxacin (5 mcg/disc). These findings on significant inhibition of A5-LOX, and NO production may support the therapeutic potential of *C. aurantium* leaf EO and emphasize the importance of sustainable and efficient extraction methods for its industrial applications in pharmaceuticals and cosmetics.

Keywords: *Solvent-free microwave extraction, Gas Chromatography-Mass Spectrometry, Essential oil, Anti-inflammatory activity*

Acknowledgements: Financial assistance by the Treasury grant No TG 21/203 received to Industrial Technology Institute is acknowledged.