

Phytochemical Constituents of *Citrus hystrix* DC. Leaves Attenuate Inflammation via Nf- κ B Signaling and Nlrp3 Inflammasome Activity in Macrophages

Watunyoo Buakaew ¹, Rungnapa Pankla Sranujit ², Chanai Noysang ², Yordhathai Thongsri¹, Pachuen Potup ¹, Nitra Nuengchamnon³, Nungruthai Suphrom ⁴ and Kanchana Usuwanthim^{1,*}

¹ Cellular and Molecular Immunology Research Unit, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok 65000, Thailand

² Thai Traditional Medicine College, Rajamangala University of Technology Thanyaburi, Pathum Thani 12130, Thailand

³ Department of Chemistry, Faculty of Science and Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok 65000, Thailand

⁴ Science Laboratory Centre, Faculty of Science, Naresuan University, Phitsanulok 65000, Thailand; Nitran@nu.ac.th

* Correspondence: Kanchanau@nu.ac.th; Tel.: +66-55-966-411

Citation: Buakaew, W.; Sranujit, R.P.; Noysang, C.; Thongsri, Y.; Potup, P.; Nuengchamnon, N.; Suphrom, N.; Usuwanthim, K. Phytochemical Constituents of *Citrus hystrix* DC. Leaves Attenuate Inflammation via Nf- κ B Signaling and Nlrp3 Inflammasome Activity in Macrophages. **2021**, *11*, 105. <https://doi.org/10.3390/biom11010105>

Received: 16 December 2020

Accepted: 12 January 2021

Published: 14 January 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

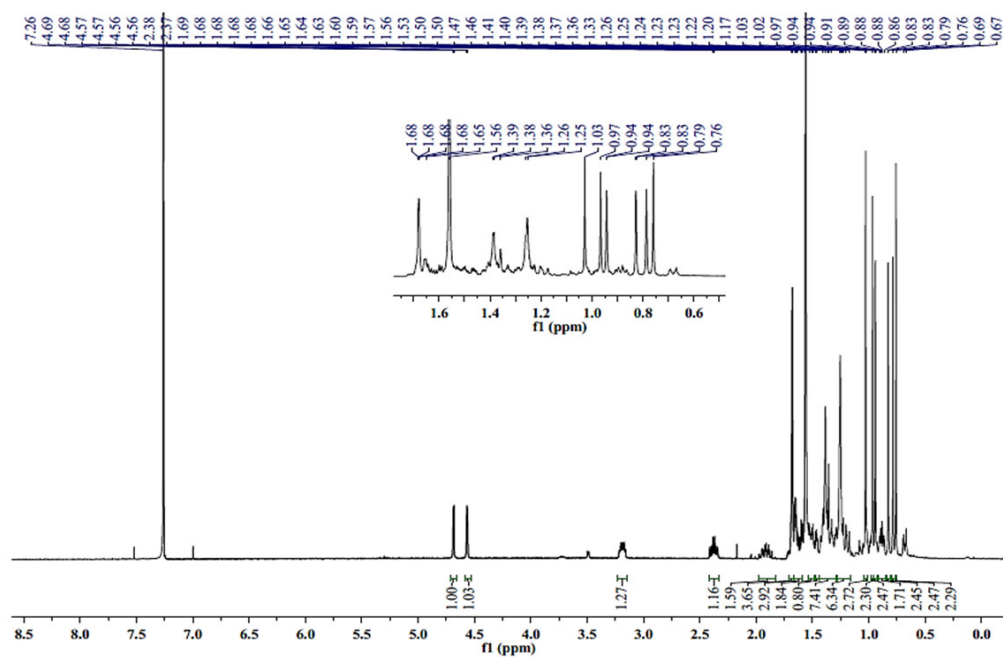


Figure S 1 ^1H NMR spectrum of lupeol (at 400 MHz in CDCl_3)

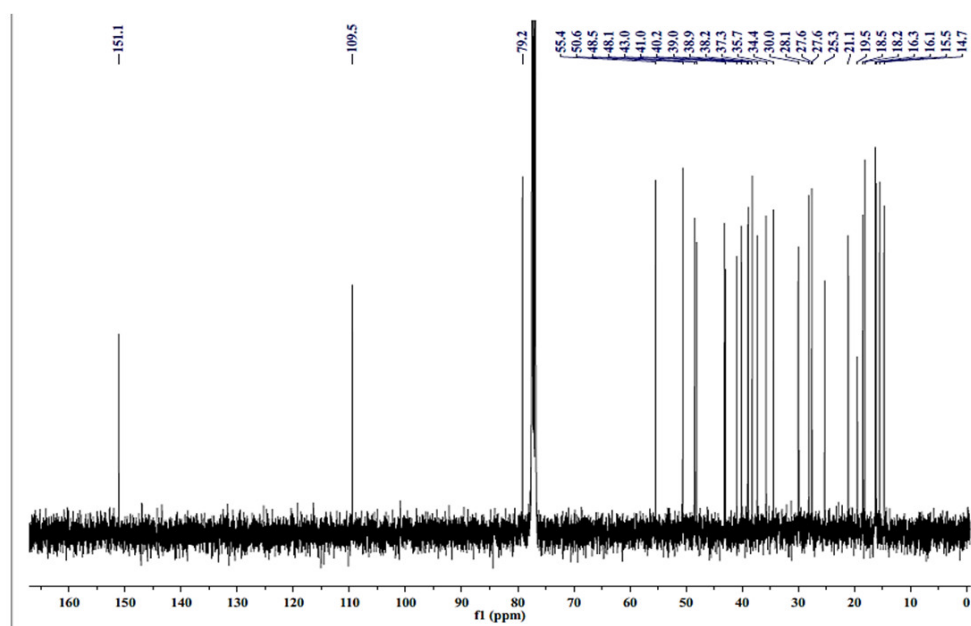


Figure S 2 NMR spectrum of lupeol (at 100 MHz in CDCl₃)

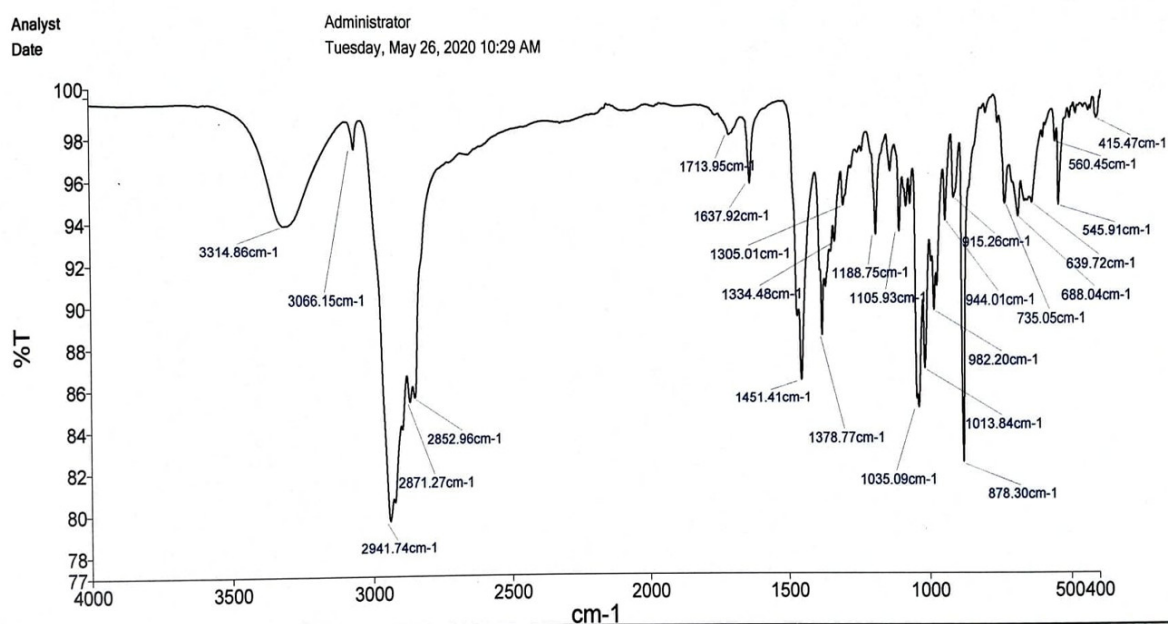


Figure S 2 FT-IR spectrum of lupeol

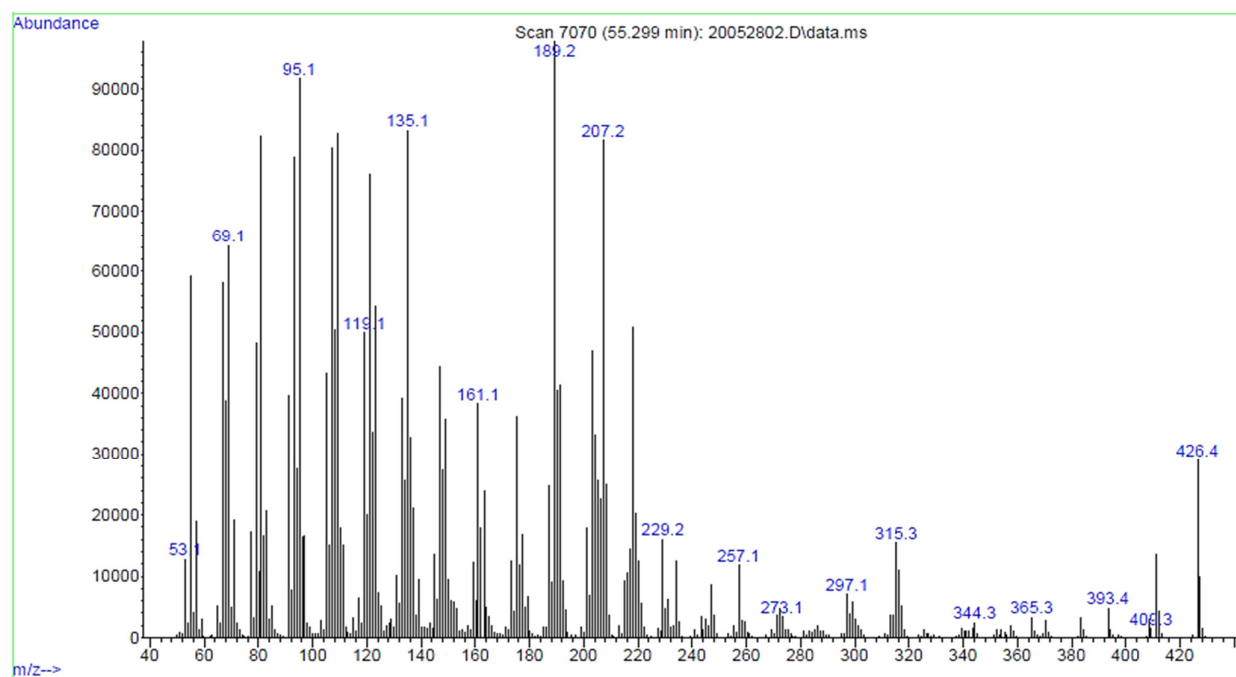


Figure S 3 EI-MS spectrum of lupeol

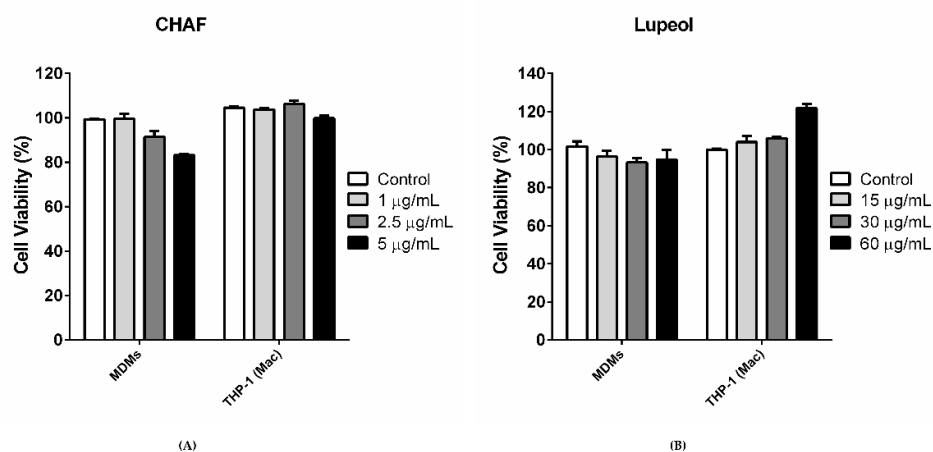
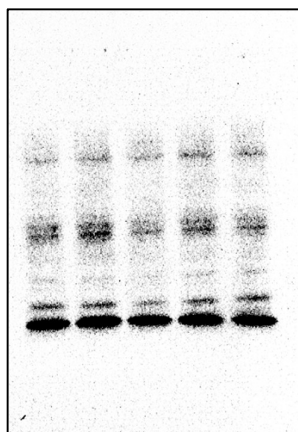
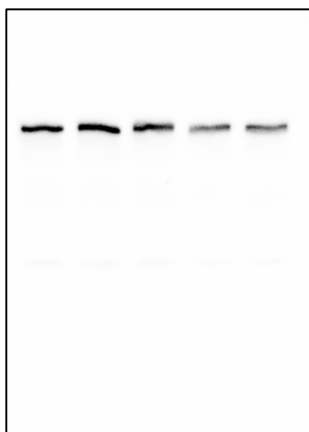


Figure S 4 Cellular cytotoxicity of CHAF and Lupeol. Cellular cytotoxicity of CHAF and Lupeol. (A) The effect of CHAF and (B) Lupeol on cell viability percentage in human MDMs and THP-1-derived macrophages. Cells were seeded in 96-well plate at density 5×10^4 cell/well, then treated with indicated concentration of CHAF and incubated for 24 h. The cell viability was determined using MTT assay. The final concentration of DMSO used in this study was $< 0.1\%$. The data are presented as mean \pm SD.

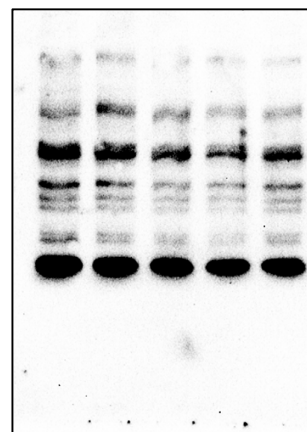
3. Western blotting pictures



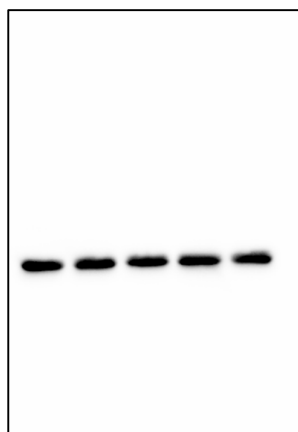
phospho IκB-α



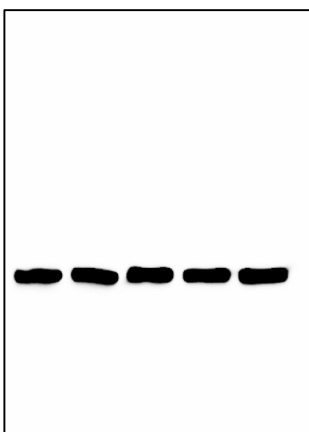
NF-κB p65



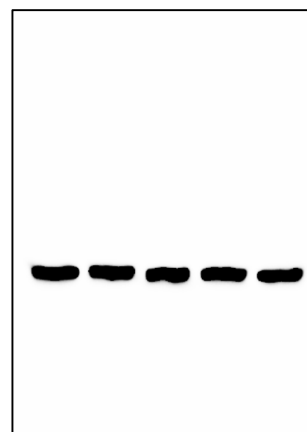
Cyclooxygenase-2



Beta actin



Beta actin



Beta actin