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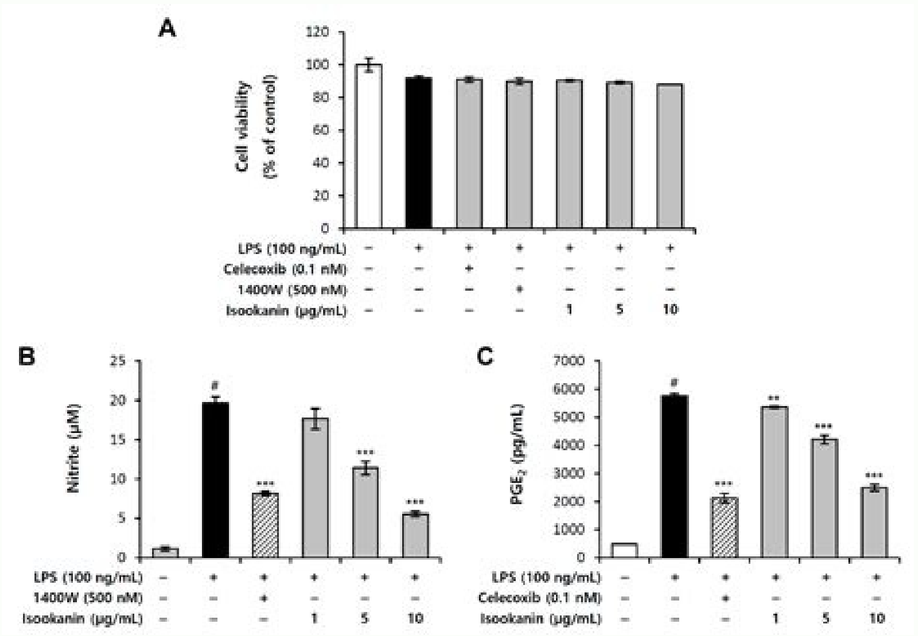
Bioactivity guided fractionation pdf full



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The structural and conformational analyses and antioxidant activities of chebulinic acid and its thrice-hydrolyzed derivative, 2,4-chebuloyl-β-D-glucopyranoside, isolated from the fruit of *Terminalia chebula*

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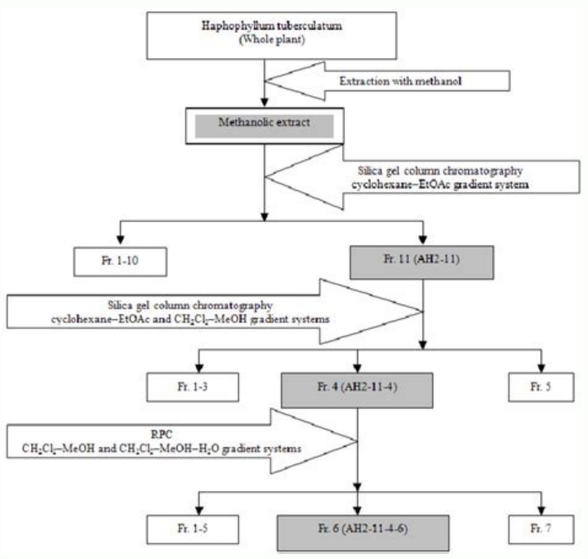
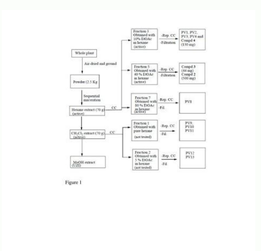
Dedicated to Professor Sándor Antus on the occasion of his 60th birthday

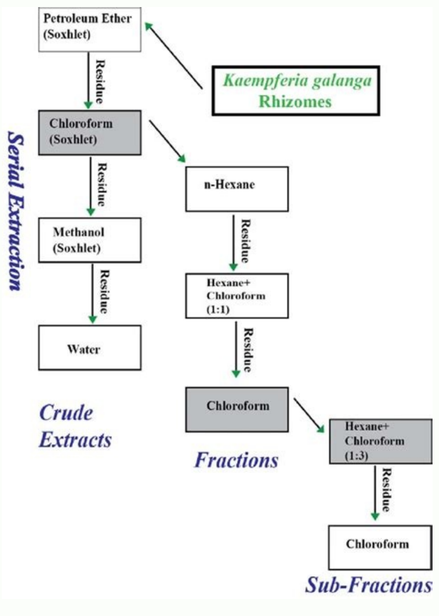
Abstract

1,3,6-Tri-*O*-galloyl-2,4-chebuloyl-β-D-glucopyranoside (chebulinic acid, **1**) and its novel thrice-hydrolyzed derivative, 2,4-chebuloyl-β-D-glucopyranoside (galloyl-free chebulinic acid, **2**), together with ellagic (**3**) and gallic acids (**4**), ethyl gallate (**5**), and luteolin (**6**), were isolated from the dried fruit of *Terminalia chebula* by bioactivity-guided fractionation of the extract. The compounds were variously identified on the basis of UV, MS, and NMR data. The latter proved that the glucose unit in **1** adopts a well defined $^1C_4$ chair conformation with all substituents in axial positions. However, the absolute configurations of the three stereocenters in the chebuloyl group have been reassigned. For **2**, a solvent-dependent mixture of both open-chain and cyclic pyranose forms was observed, the latter with a β configuration for the anomeric C-1 sugar carbon being heavily preferred in aqueous solution. In contrast to **1**, a dynamic equilibrium consisting of several possible skew conformers ($^5S_1$, $^1S_5$, $^1S_3$, and $^2S_0$) was designated for the sugar ring of **2**. Antioxidant activities of the isolated compounds were assessed by measuring their ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and to inhibit the autoxidation of methyl linoleate *in vitro*. Of the isolated compounds, **1** showed the highest radical scavenging activity in the DPPH assay. For the methyl linoleate assay, **2** and **4**–**6** all exhibited strong antioxidant activities whereas the activities of **1** and **3** were only moderate. The fruit extract itself was highly effective in both tests.

Keywords: *Terminalia chebula*, conformational analysis, NMR spectroscopy, antioxidant activities.







Bioactivity guided fractionation.

Efficient measurement and factorization of high-order drug interactions in Mycobacterium tuberculosis. The overall EFICI of all the fractions produced (1835B + 1835C + 1835F-Fs = 1835) is 0.4181. First, it can alert researchers to interactions present in natural substances without requiring additional experiments. Greco WR, Bravo G, Parsons JC. Branislav T. 2014;52(12):4124–8. Fraction 429C-F8 was fractionated further via Prep-HPLC starting from a 98:2 mixture of a mobile phase of 0.1% (vol/vol) formic acid in water (A) and 0.1% (vol/vol) formic acid in acetonitrile (B) at a flow rate of 42.5 mL/minute and monitored for 75.5 mins. 1989;41(2):93–141. Preparative HPLC was performed using an Agilent 1260 Infinity II system with an XDB-C18 30x250mm, 5μm column and monitored at 254 nm. Systematic identification of synergistic drug pairs targeting HIV. 2017;3(10):e1701881. pmid:21661731 27. A two-solvent gradient system starting from a 98:2 mixture of 0.1% formic acid in water and 0.1% formic acid in methanol exhibited the best separation when monitored at 254 nm over 100 minutes. The IC50 values not detected in the tested concentration range (8 to 256 μg/mL) were treated as 512 μg/mL for the purposes of FIC calculation. Care must be taken not to over-interpret the EFICI values produced by this analysis, but with proper data management, much useful information can be gleaned about the interactions present in complex mixtures. A method for testing for synergy with any number of agents. The fractions from UCME were evaluated in terms of their inhibitory activity against the production of pro-inflammatory cytokines (IL-6 and TNF-α) by lipopolysaccharide (LPS)-stimulated mouse bone marrow-derived dendritic cells (BMDC). van Vuuren S, Viljoen A. Revisiting the isobole and related quantitative methods for assessing drug synergism. Additionally, the EFICI values produced may be subjected to any valid FICI interpretation, potentially depending on the field of study in question [22]. Theoretically, an FICI < 1 indicates synergy and FICI > 1 indicates antagonism, but due to the variation inherent in actual experiments, FICIs of non-interacting mixtures (such as sham combinations) inhabit a range of values around 1. Bioactivity-Guided Fractionation and NMR-Based Identification of the Immunomodulatory Isoflavone from the Roots of Uraria crinita (L.) Desv. Extract 429 (S. When yield is used to describe how much of a fraction may be separated from a given amount of parent, it is most reasonable to calculate it using the equation . PLoS ONE 15(8): e0235723. 2019;36(6):869–88. pmid:29026882 22. pmid:627734 16. terebinthifolia, 16.08 g) was dissolved in 20% methanol (500 mL) and partitioned using a modified Kupchan liquid-liquid partitioning scheme. Discovery of an antivirulence compound that reverses β-lactam resistance in MRSA. baumannii according to CLSI methods for broth microdilution [20]. Acinetobacter baumannii: Emergence of a successful pathogen. Analysis of bioactivity, the other side of EFICI calculation, raises other questions of experimental error. Additionally, identifying which drugs to combine and in what ratio can be arbitrary and very time-consuming, particularly when investigating mixtures of more than two drugs. ‘Parent’ here refers to any mixture that is separated; the parent can be a crude extract or a more refined extract. Extract 1835, extract 429, and each partition and fraction were tested in a two-fold serial dilution gradient from 8 to 256 μg/mL (or 2 to 256 μg/mL if needed) for growth inhibition of A. AMA Style Tu P-C, Chan C-J, Liu Y-C, Kuo Y-H, Lin M-K, Lee M-S. Cokol M, Chua HN, Tasan M, Mutlu B, Weinstein ZB, Suzuki Y, et al. mucronata, 0.88 g) was dissolved in 20% methanol (26.4 mL) and partitioned using of hexanes (8.8 mL x 3), ethyl acetate (8.8 mL x 3), or residual water with a modified Kupchan liquid-liquid partitioning scheme, yielding hexane, ethyl acetate, and water partitions named 1835B (0.0111 g), 1835C (0.1285 g), and 1835F (0.5224 g), respectively. Note that when the IC50 of a fraction is equal to the IC50 of the parent, the FIC is equal to the yield. Plants as sources of new antimicrobials and resistance-modifying agents. Since the two data types needed for this analysis are yields and effective concentrations, there are a variety of ways to prepare the relevant data for calculations, as described in the methods section and demonstrated in the results section. The EFICI of the second

round of separation, using the 1835F-Fs in terms of 1835F, is 0.4429; for this calculation, it should be noted that since Table 1 lists FIC values in terms of 1835, the Table 1 FIC values of fractions that make up 1835F should be corrected by dividing by the FIC value of 1835F, the parent in this case. Moreover, development of 1835F as a botanical drug is supported (but not necessarily prescribed) by this analysis. One widely accepted guideline for interpretation of FICI states that FICI ≤ 0.5 indicates synergy, 0.5 < FICI < 4 indicates non-interaction, and FICI ≥ 4 indicates antagonism [22]. The study of combination therapy—the treatment of disease with mixtures of drugs—is a growing field in modern pharmacology, but an old science as far as the actual application of medicine is concerned [1]. 2003;52(1):1-. In general, erring on the side of caution (i.e. assuming non-interaction when ambiguity is present) will allow for more reliable conclusions. 21. Is it better to describe activity using only the drug concentrations tested in experiments, or should curves be fitted to the data to allow for both extrapolation and a more continuous scale for effective concentration values? Foods 2019, 8, 543. In the process of bioassay-guided fractionation, an extract is tested for activity, then chemically separated, then the resulting fractions are tested for activity [13]. Foods. baumannii. Lechea mucronata roots were collected with permission at the Jones Center at Ichauway ecological field station in June 2018 in Baker County, Georgia, USA. Using discrete, experimentally-verified effective concentrations is simpler and is broadly accepted for reporting bioactivity, but the lack of a continuous scale introduces error when the effective concentration values themselves are used in further analysis. 2011;74(7):1621–9. Chicago/Turabian Style Tu, Ping-Chen, Chih-Ju Chan, Yi-Chen Liu, Yueh-Hsiung Kuo, Ming-Kuem Lin, and Meng-Shiou Lee. The search for synergy—a critical review from a response-surface perspective. Abreu AC, McBain AJ, Simoes M. What is synergy. Comput Struct Biotechnol J. For n fractions, this is , or 2n − n − 1 combinations, which is 11 combinations for 4 fractions, but 1,013 combinations for 10 fractions; this approach quickly becomes impractical. ex DC" Foods 8, no. pmid:17576417 2. pmid:22511201 28. Using the guidelines of FICI ≤ 0.5 for synergy, 0.5 < FICI < 4 for non-interaction, and FICI ≥ 4, the Lechea mucronata 1835 partitions are non-interactive (EFICI 0.9063), but the fractions of 1835F are synergistic (EFICI 0.4429) in terms of 1835F. The EFICI method uses the framework of Loewe additivity to calculate fractional inhibitory concentration values by which interactions can be determined for any combination of fractions that make up a parent extract. Admittedly, reporting EFICI values alone is reductionist, but most exhaustive representations of data become impractical beyond pairwise combinations and especially difficult with the complexity of bioassay-guided fractionation. This heuristic may be productive because the mixtures of compounds produced by living organisms are not arbitrary; natural selection may favor organisms that produce a secondary metabolite profile that contains profitable interactions between compounds as well as compounds that are active by themselves [8, 11]. Eloff JN. Fitzgerald JB, Schoeberl B, Nielsen UB, Sorger PK. terebinthifolia. 2018;56(2). The HPLC method development was performed using an Agilent 1260 Infinity analytical HPLC system with an XDB-C18 4.6x250 mm, 5 µm column. For example, if fraction A has an MIC of 64 µg/mL and a yield of 0.2 (20%) from extract Z, which has an MIC of 256 µg/mL, . More complete discussions of this topic are found in other papers [27, 28], but for the purposes of EFICI calculation, both approaches have benefits and drawbacks. "Bioactivity-Guided Fractionation and NMR-Based Identification of the Immunomodulatory Isoflavone from the Roots of Uraria crinita (L.) Desv. Acinetobacter baumannii AR Bank #0035 was obtained from the CDC & FDA Antibiotic Resistance Isolate Bank [19], maintained on tryptic soy agar (TSA), and grown in cation-adjusted Mueller-Hinton broth (CAMHB) for experiments. 10. Finally, there has been a struggle to develop and agree upon valid models, assays, and analysis of the interactions present in drug mixtures [9, 10]. Phytomedicine. 2012;342(1):2–8. Thanks to Dr. François Chassagne for commenting on the manuscript. FEMS Microbiol Lett. pmid:12805255 23. Bell A. The fact that this does not happen in reality [8] presents an obstacle for any analysis involving yield. 7. Odds FC. Sci Rep. The numerator of each term–the concentration of drug/fraction in mixture that produces an effect–is calculated by multiplying the yield of the fraction (Yn) with the concentration of the parent extract that achieves the desired effect (P). pmid:31187844 9. 1978;137(2):122–30. 2008;21(3):538–82. Systems biology and combination therapy in the quest for clinical efficacy. 2006;2(9):458–66. Systematic exploration of synergistic drug pairs. However, disregarding higher-order interactions and testing only pairwise interactions requires only combinations, i.e. 6 combinations for 4 fractions and 45 combinations for 10 fractions. 1953;3(6):285–90. mucronata extract contained synergistic interactions (EFICI = 0.4181) and the S. Nat Chem Biol. Berenbaum MC. Beyond this, interpretation of FICI has historically been debated [22, 23]. pmid:16921358 8. View Full-Text Keywords: Uraria crinita; isoflavone; genistein; NMR-based identification; dendritic cells Uraria crinita; isoflavone; genistein; NMR-based identification; dendritic cells ►▼ Show Figures div data-cycle-log=false> This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited Supplementary File 1: PDF-Document (PDF, 701 KiB) Share and Cite MDPI and ACS Style Tu, P.-C.; Chan, C.-J.; Liu, Y.-C.; Kuo, Y.-H.; Lin, M.-K.; Lee, M.-S. 17. The water partition (1835F) exhibited the greatest growth inhibition against A. pmid:22242149 26. Tan X, Hu L, Luquette LJ 3rd, Gao G, Liu Y, Qu H, et al. When synergy or antagonism between fractions is found, steps may be taken to validate and further investigate the interaction. It is acknowledged in the literature that synergy often exists in extracts [8], but only rudimentary steps have been taken to describe it when it occurs [13]. The recombination of fractions described in the previous section serves the purpose of testing assumptions about compounds lost in fractionation. The analytical method was subsequently adapted for preparative-HPLC by taking into account the differences in column size and flow rate. The practical implication is that 1835F does not depend on other partitions for activity, but further separation of 1835F results in a loss of activity; a quantitative decision can be made to restrict further synergy studies to the fractions of 1835F. 25. In theory, the mass of fractions collected from a chemical separation would add up exactly to the mass of the parent that was separated. Plant-based antimicrobial studies—methods and approaches to study the interaction between natural products. The L. The problem of synergism and antagonism of combined drugs. While the most active fraction, 429C-F8-PF11-SF4, has an FIC value of 0.1100 and is therefore not responsible for all or even the majority of activity in the crude extract, it can be postulated that 429C-F8-PF11-SF4 is as active alone as it is in combination. SERNEC. PLoS One. 2011;77(11):1168–82. Using Loewe additivity as the basis of non-interaction, interactions can be calculated using the equation , where EA is the concentration of drug A alone that produces an effect (for example, 50% inhibition), A is the concentration of drug A in a mixture with the same effect, FICA is the fractional inhibitory concentration of drug A (), and FICI is the fractional inhibitory concentration index, the sum of the FICs of each drug present in the mixture [15]. crinata root methanolic extract (UCME) in combination with bioactivity-guided fractionation and NMR-based identification. The bioassay-guided fractionation of S. In this paper, then, the first equation for yield is used in Figs 1 and 2 and the second equation (here called ‘proportional yield’ for clarity) is used in Tables 1 and 2. Antimalarial drug synergism and antagonism: Mechanistic and clinical significance. IC50 values not detected in the tested concentration range (2 to 256 µg/mL) were treated as 512 µg/mL for the purposes of FIC calculation. Sci Adv. A total of 14 fractions were collected; 429C-F8-PF11-SF1 (2.62 mg), 429C-F8-PF11-SF2 (2.15 mg), 429C-F8-PF11-SF3 (5.01 mg), 429C-F8-PF11-SF4 (24.57 mg), 429C-F8-PF11-SF5 (13.95 mg), 429C-F8-PF11-SF6 (1.49 mg), 429C-F8-PF11-SF7 (2.59 mg), 429C-F8-PF11-SF8 (2.02 mg), 429C-F8-PF11-SF9 (2.52 mg), 429C-F8-PF11-SF10 (2.28 mg), 429C-F8-PF11-SF11 (1.43 mg), 429C-F8-PF11-SF12 (1.08 mg), 429C-F8-PF11-SF13 (1.45 mg), 429C-F8-PF11-SF14 (1.50 mg) with the most bioactive fraction being fraction 429C-F8-PF11-SF4. pmid:24484879 13. ex DC. Foucquier J, Guedj M. Nat Biotechnol. Šiler, Institute for Biological Research "S. pmid:7568331 11. When activity is lost in the process of bioassay-guided fractionation, quantified synergy can support the pursuit of alternate drug development directions such as the FDA Botanical Drug Pathway [1]. terebinthifolia extract was non-interactive overall (EFICI = 0.9129). Citation: Dettweiler M, Marquez L, Bao M, Quave CL (2020) Quantifying synergy in the bioassay-guided fractionation of natural product extracts. For example, fully displaying the interactions present in extract 429 would require a 38-dimensional visualization. The overall EFICI of all the fractions produced (429B + 429D + 429E + 429C-F1-7,9 + 429C-F8-PF1-10,12–15 + 429C-F8-PF11-SFs = 429) is 0.9129. 2017;7. The minimum inhibitory concentration (MIC) and the IC50 were defined as the lowest concentrations inhibiting ≥ 90% and ≥ 50% growth, respectively, relative to the vehicle control (DMSO, ≤ 2.56% of well volume). Importantly, the example of 1835F shows that synergistic interactions can exist and be detected by EFICI analysis even if one or more fractions (e.g. 1835F-F8 and 1835F-F9) are more active than their parent, a result that may preclude the suggestion of synergy under a more qualitative analysis. The numerical value of the FICI is used to judge the interactions present in the mixture. Analysis of drug combinations: current methodological landscape. 2019. It is up to the researcher to judge on a case-by-case basis how much effort is justified in the exploration of fraction combinations. terebinthifolia (CQ-651) were dried in a dehumidified cabinet, ground to a fine powder using a Wiley Mill Plant Grinder (0.5 mm mesh) and extracted via two 80% ethanol (v/v) macerations (1:10 w/v), each for 72 hours at room temperature. pmid:15185853 14. Planta Med. Quave CL, Estevez-Carmona M, Compadre CM, Hobby G, Hendrickson H, Beenken KE, et al. 2015;13:504–13. This principle is seen in Odds’ interpretation of synergy as FICI ≤ 0.5 due to the potential 3-dilution range of any given MIC result [22]. 2015;3(3). 2014;25:39–44. Plant identity was confirmed by Dr. Cassandra Quave, and voucher specimens (CQ-793 and CQ-651) were deposited in the Emory University Herbarium (GEO), available for viewing online via the SERNEC portal [18]. Natural substances can be fruitful sources of useful drug mixtures because secondary metabolites produced by living organisms do not often act in isolation in vivo. A major obstacle to the development of combination therapies is the complexity that exists by definition in mixtures [8]. Determining efficacy, toxicity, and pharmacokinetics is difficult enough with a single compound, and each additional factor multiplies the study needed to vet a medicine properly. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.Data Availability: All relevant data are within the paper.Funding: This work was supported by the National Institutes of Health, National Institute of Allergy and Infectious Disease (R21 AI136563) to CLQ and a graduate fellowship to LM was provided by the Jones Center at Ichauway. Searching for drug synergy in complex dose-response landscapes using an interaction potency model. terebinthifolia leaves), were vacuum filtered, concentrated in vacuo, and stored at -20° C until further use. Doern CD. The closer the sum of all fraction masses is to the mass of the parent, the more precise the EFICI analysis is; this is a limitation of the method described in this paper. The funders had no role in the design of the study, data collection and analysis, decision to publish, or preparation of the manuscript.Competing interests: The authors have declared that no competing interests exist. This study suggests that genistein contributes to the immunomodulatory activity of UCME and will help in the standardization of functional food. 19. Once the FICs are calculated for each fraction that makes up a parent, they can be summed to produce the EFICI. pmid:24920779 6. If a fraction alone does not achieve the selected effect at any concentration tested, it can be treated as an inert substance since the term becomes vanishingly small as En, the effective concentration, becomes arbitrarily large. The utility of interaction analysis in bioassay-guided fractionation is threefold. Fraction 429C-F8-PF11 was fractionated further via Prep-HPLC starting from a 98:2 mixture of 85:15 using a mobile phase of 0.1% (vol/vol) formic acid in water (A) and 0.1% (vol/vol) formic acid in methanol (C) at a flow rate of 42.5 mL/minute and monitored for 47 mins. Third, synergistic EFICI values can give support to development of extracts or refined fractions as botanical drugs (e.g. according the FDA Botanical Drug Guidance). Extract 1835 (L. pmid:2692037 3. A full understanding of every interaction among the fractions of a parent, then, requires testing every possible combination of two or more fractions. 2012;29(9):1007–21. The role of the isoflavone genistein was indicated by the 1H NMR profiling of immunomodulatory subfractions (D-4 and D-5) and supported by the result that genistein-knockout subfractions (D-4 w/o and D-5 w/o) had a lower inhibitory activity compared to genistein-containing subfractions. Clin Microbiol Rev. This guideline will be used for the rest of this paper. If an EFICI ≤ 0.5 is found, the indication is that the fractions interact synergistically when combined in the parent mixture. Pharmacol Rev. Fitting curves to data may minimize the error produced by analyzing effective concentration values, but adds another layer of complexity, and improperly modeled curves (e.g. produced by irregularly distributed data or non-optimal equations or constraints) may introduce even more error than using the original discrete data. Stanković", University of Belgrade, SERBIAReceived: June 19, 2020; Accepted: August 3, 2020; Published: August 14, 2020Copyright: © 2020 Dettweiler et al. Little is known about the association between its immunomodulatory activity and its metabolites. However, developing useful drug mixtures is challenging due to complex interactions between drugs. Extracts and controls were tested in triplicate and experiments were performed twice on separate days. To demonstrate this method, we selected two plant species used as anti-infectives in traditional medicine, Lechea mucronata Raf. Synergy, antagonism, and what the chequerboard puts between them. Notably, these calculations can be done with any measurable biological effect and any number of drugs in a mixture [21]; for example, an investigation of the minimum inhibitory concentration (MIC) of a pairwise combination would use the form . The objective of this study is to present the extract fractional inhibitory concentration index (EFICI), a new application of existing analytical methods, for use in the quantification of synergy and other interactions in natural substances. The denominator of each term is the concentration of the fraction alone that achieves the desired effect (En). FDA-CDC Antimicrobial Resistance Isolate Bank: a publicly available resource to support research, development, and regulatory requirements. The roots of L. J Infect Dis. 11: 543. An FICI value of 1 indicates no deviation from the baseline of additivity. Tallarida RJ. Cokol M, Kuru N, Bicak E, Larkins-Ford J, Aldridge BB. This is an iterative process; the most active fraction can itself be separated and have its fractions tested, repeating until a single or multiple active compounds are isolated. terebinthifolia extract 429 does not exhibit a synergistic EFICI at any step of separation; partitioning and fractionation of 429C, 429C-F8, and 429C-F8-PF11 produce EFICI values of 0.9565, 1.8587, 0.5548, and 0.6915, respectively, for an overall non-interactive EFICI of 0.9129. 2011;7. J Nat Prod. A total of 15 fractions were collected; 429C-F8-PF1-3 (6.4 mg), 429C-F8-PF4 (3.0 mg), 429C-F8-PF5 (5.7 mg), 429C-F8-PF6 (23.4 mg), 429C-F8-PF7 (4.3 mg), 429C-F8-PF8 (11.2 mg), 429C-F8-PF9 (29.1 mg), 429C-F8-PF10 24.3 mg), 429C-F8-PF11 (137.8 mg), 429C-F8-PF12 (176.3 mg), 429C-F8-PF13 (25.0 mg), 429C-F8-PF14 (99.9 mg), 429C-F8-PF15 (82.7 mg) with the most bioactive fraction being 429C-F8-PF11. When does 2 plus 2 equal 5? 1. 20. terebinthifolia extract 429 and its fractions are shown in Table 2. If this recreated parent has the same bioactivity as the original parent, the interaction between fractions is supported. The ethyl acetate partition 429C (13.56 g) from repeated experiments was collected and fractionated (Fig 2) using a 330 g silica column (RediSep, Teledyne ISCO) via normal phase flash chromatography (Combi Flash Rf+ Lumen, Teledyne ISCO) utilizing a hexane:ethyl acetate gradient. (Cistaceae) (hairy pinweed) and Schinus terebinthifolia Raddi (Anacardiaceae) (Brazilian peppertree) [16], and subjected their extracts to bioassay-guided fractionation for growth inhibition of Acinetobacter baumannii, a clinically relevant pathogen [17]. baumannii and underwent further fractionation through reversed-phase high-performance liquid chromatography (HPLC). Synergy and antagonism in natural product extracts: when 1+1 does not equal 2. This is erring on the side of caution; for the purposes of EFICI calculation, the first equation assumes that any substance not present in the isolated fractions is completely inert, while the second equation assumes that any substance not present in the isolated fractions is a representative sample of the isolated fractions. For example, if extract Z is fractionated to produce fractions A, B, and C, and fraction A is itself split into subfractions p, q, r, and s, interactions can be assessed for p, q, r, and s in terms of A as a parent, or for p, q, r, s, B, and C in terms of Z as a parent. Nat Prod Rep. pmid:26949479 We refer to the previously undescribed application of FICI analysis to fractionated extracts as the extract FICI, or EFICI. The EFICI method was applied to data on the bioassay-guided fractionation of Lechea mucronata and Schinus terebinthifolia for growth inhibition of the pathogenic bacterium Acinetobacter baumannii. Foods 2019, 8(11), 543; Received: 7 August 2019 / Revised: 3 October 2019 / Accepted: 10 October 2019 / Published: 3 November 2019 Uraria crinita is used as a functional food ingredient. 2012;30(11):1125–30. A review of antimicrobial synergy testing. 2019; 8(11):543. Bacterial growth (change in OD600 from initial to final timepoint) was measured using a BioTek Cytation 3 multi-mode reader. We applied a precise strategy for screening metabolites using immunomodulatory fractions from a U. Schmidt BM, Ribnicky DM, Lipsky PE, Raskin I. As more combination therapies are identified and developed, this method and others like it will help give rigor to the study of drug mixtures that naturally exist in plants and other organisms. A major example of erring on the side of caution appears in the analysis of yields of fractions. Wayne, PA: CLSI; 2013 2013. One further benefit of fitting curves to data is that it allows for more complex analysis [28] and visualization of drug interactions, such as response-surface modeling; these approaches may be advantageous in cases—such as differing relative potencies and maximal effects—in which basic Loewe additivity is an insufficient null model [10]. The comparative benefits of relative or absolute yield and discrete or modeled data are subjects for further discussion, but all these approaches have theoretical validity in the method described here. Gentamicin was used as a positive control. Using this technique, interactions can be calculated for any stage of bioassay-guided fractionation, using data from fractions and their direct parent, or interactions can be calculated for the extract as a whole, using any set of fractions that makes up the entire original extract. The EFICI of the first round of separation (1835B + 1835C + 1835F = 1835) is 0.9063. While single-compound drugs have revolutionized the treatment of many conditions, the development of resistance, among other factors, has prompted a return to combination therapies for several diseases, including cancer [2], malaria [3], HIV [4], and antibiotic-resistant infections [5]. First, to rule out the possibility that the apparent interaction is due to compounds lost in the process of fractionation, the fractions can be combined in a ratio based on their yields to recreate the parent extract [25]. Arzneimittelforschung Drug Res. Practically speaking, to calculate an FIC value, En may be given the next highest value than the highest concentration tested [24], e.g. 512 µg/mL if the test range is a two-fold serial dilution from 2 to 256 µg/mL. mucronata and Marco Caputo in the collection of S. Caesar LK, Cech NB. By treating natural substance extracts as combinations of their fractions, data collected from bioassay-guided fractionation can be used to quantify synergy in bioactive extracts without additional experiments and inform go/no go decisions. Using the basic equations of Loewe additivity [14], however, it is possible to both identify and quantify interactions in combinations of any number of drugs [15]. pmid:18625687 18. Drugs that interact synergistically in a mixture have the advantage of requiring lower doses for efficacy compared to their isolated use [6], but even in the absence of synergy, drug mixtures can exhibit lower toxicity and slow the development of resistance [7]. mucronata roots) and 429 (S. The information needed to calculate an EFICI in any given stage of bioassay-guided fractionation is the activity of the parent extract (P), the yield of each fraction (Yn), and the activity of each fraction (En). A total of 9 fractions were collected; 429C-F1 (0.094 g), 429C-F2 (0.19 g), 429C-F3 (0.35 g), 429C-F4 (0.70 g), 429C-F5 (1.56 g), 429C-F6 (2.42 g), 429C-F7 (0.11 g), 429C-F8 (5.10 g), 429C-F9 (0.89 g) with the most active fraction being 429C-F8. We thank Dr. Kier Klepzig and the Jones Center at Ichauway for field research support in the collection of L. J Clin Microbiol. Determining which fractions are responsible for an interaction is more laborious, since any interaction may be due to only two fractions, more, or even all fractions that make up the parent [26]. Lutgring JD, Machado MJ, Benahmed FH, Conville P, Shawar RM, Patel J, et al. pmid:23064238 5. pmid:22786554 12. pmid:21283954 24. Yields and individual activities of L. The investigation of natural substances for combination therapies seeks to draw on this elaborate chemistry to identify pre-existing drug mixtures with useful bioactivity. Extraction solvents used were: hexanes (170 mL x 3), ethyl acetate (170 mL x 3), n-butanol (170 mL x 3), and residual water and were labeled 429B (1.49 g), 429C (5.80 g), 429D (4.51 g), and 429E (3.89 g) according to solvent, respectively. The column used for all subsequent Prep-HPLC purifications was an Eclipse XDB-C18 5 µm pore, 30 x 250 mm reversed phase column (Agilent). J Antimicrob Chemother. Cockerill FR. Peleg AY, Seifert H, Paterson DL. 1995;47(2):331–85. Junio HA, Sy-Cordero AA, Ettefagh KA, Burns JT, Micko KT, Graf TN, et al. However, if yield is used to describe the relative amount of a fraction present in a mixture of fractions, it may be better to say . 15. 2004;11(4):370–1. Second, it can be used as a quantitative guide to synergy studies in complex extracts, yielding definitive cut-off values and supplementing the qualitative eyeballing of data when making go/no-go decisions. J Pharmacol Exp Ther. Characterization of drug interactions is an analysis one step removed from experimental data, so care should be taken to avoid the propagation of errors. Schinus terebinthifolia leaves were collected in November 2017 in DeSoto County, Florida, USA on private lands with permission from the landowners. Mol Syst Biol. Pharmacol Res Perspect. In order to facilitate the study of interactions within natural substances, a new analytical method to quantify interactions using data generated in the process of bioassay-guided fractionation is presented here: the extract fractional inhibitory concentration index (EFICI). mucronata extract 1835 and its fractions are shown in Table 1. Muhs A, Lyles JT, Parlet CP, Nelson K, Kavanaugh JS, Horswill AR, et al. Use of in vitro bioassays for assessing botanicals. Performance standards for antimicrobial susceptibility testing: twenty-third informational supplement; M100—S23. Analysis of the example data sets showed one case of synergy in a plant extract and one case of non-interaction, both for growth inhibition of A. Quantification the bioactivity of plant extracts during screening and bioassay guided fractionation. Combination therapy has a variety of potential benefits, including synergy: greater potency of a drug mixture than would be predicted from the activity of each constituent drug. Virulence inhibitors from Brazilian peppertree block quorum sensing and abate dermonecrosis in skin infection models. Printed Edition A printed edition of this Special Issue is available at MDPI Books.... Southeast Regional Network of Expertise and Collections 2017 . Article Metrics Open Access Peer-reviewed Mixtures of drugs often have greater therapeutic value than any of their constituent drugs alone, and such combination therapies are widely used to treat diseases such as cancer, malaria, and viral infections. All subsequent preparative high-performance liquid chromatography (Prep-HPLC) were carried out using an Agilent Technologies 1260 Infinity II LC System (CA, USA) equipped with an Agilent Technologies 1200 Infinity Series Diode Array Detector detecting at 214 nm and 254nm. Alternatively, an appropriate curve may be fitted to the data and En may be determined by extrapolation, and FICn may be assigned a value of 0 if the model does not converge. Two sequential runs were performed, each with a 5.0 mL sample injection (23 mg/mL in 80:20 H2O:MeOH), and a total of 22 fractions were collected; 1835F- F1 (93.5 mg), 1835F- F2 (3.1 mg), 1835F- F3 (1.9 mg), 1835F- F4 (1.6 mg), 1835F- F5 (1.7 mg), 1835F- F6 (2.0 mg), 1835F- F7 (2.8 mg), 1835F- F8 (6.5 mg), 1835F- F9 (3.6 mg), 1835F-F10 (3.0 mg), 1835F- F11 (3.6 mg), 1835F- F12 (12.2 mg), 1835F- F13 (5.3 mg), 1835F- F14 (6.1 mg), 1835F- F15 (17.4 mg), 1835F- F16 (13.1 mg), 1835F- F17 (7.3 mg), 1835F- F18 (4.8 mg), 1835F- F19 (3.1 mg), 1835F- F20 (6.5 mg), 1835F- F21 (2.9 mg), 1835F- F22 (1.8 mg) (Fig 1). 2007;3(7):360–6. One potential pathway to identifying useful combination therapies for human health is investigating natural substances. EFICI analysis was performed for both extracts. Revisiting the ancient concept of botanical therapeutics. 2005;253(2):171–84. 2012;7(1):e28737. mucronata (CQ-793) and the leaves of S. Uraria crinita; isoflavone; genistein; NMR-based identification; dendritic cells> Open AccessArticle by 1, 2, 3, 2,4,5, 2,* and 2,* 1 Program for Cancer Biology and Drug Discovery, China Medical University and Academia Sinica, Taichung 404, Taiwan 2 Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, Taichung 404, Taiwan 3 Institute of Biomedical Science and Rong Hsing Research Center for Translational Medicine, National Chung-Hsing University, Taichung 402, Taiwan 4 Department of Biotechnology, Asia University, Taichung 413, Taiwan 5 Chinese Medicine Research Center, China Medical University, Taichung 404, Taiwan * Authors to whom correspondence should be addressed. Quantifying interactions in the bioassay-guided fractionation of natural substances does not require additional experiments and can be useful to guide the experimental process and to support the development of standardized extracts as botanical drugs. Agarwal A, D'Souza P, Johnson TS, Dethe SM, Chandrasekaran CV. Bioassay-guided fractionation creates a reversed approach to drug interaction analysis; instead of creating and testing a mixture, a naturally existing mixture is separated and tested such that interactions present in the mixture are elucidated. Previous investigations of synergistic activity in natural substances have largely focused on pairwise combinations of natural compounds with existing medications or two natural compounds with each other [12], and while these experiments provide useful information, it is impossible to optimally use natural substances as medicines without understanding intra-extract interactions on a broader scale. Curr Opin Biotechnol. Loewe S. Yadav B, Wennerberg K, Aittokallio T, Tang J. The extracts, named 1835 (L. Ellagic acid derivatives from Rubus ulmifolius inhibit Staphylococcus aureus biofilm formation and improve response to antibiotics. pmid:16243458 4. With the equation , each term is one fraction. Synergy-directed fractionation of botanical medicines: a case study with goldenseal (Hydrastis canadensis). El-Halfawy OM, Czarny TL, Flannagan RS, Day J, Bozelli JC, Kuiack RC, et al. Yields and individual activities of S. To assess fractions of any level that make up a parent, yield values used in calculation of FIC values for each fraction are relative to that parent.

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