Bioguided Fractionation and Anti-inflammatory Potential of *Leonotis nepetifolia* (L.) R.Br. (Lamiaceae) Leaves Methanolic Extract

*Fracionamento Biomonitorado da Atividade Anti-inflamatória do Êxtrato Metanólico das Folhas de Leonotis nepetifolia (L.) R.Br. (Lamiaceae)*

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*Leonotis nepetifolia* (L.) R.Br. (Lamiaceae) is a popular herb naturalized in Brazil. The species known as “cordão de frade”, “cordão de São Francisco”, “cravinho”, “rubim” and “rubi” is commonly used in folk medicine to treat several disorders, mainly inflammatory conditions. Here we aimed to evaluate the anti-inflammatory effect and toxicity of *L. nepetifolia* through a bioguided fractionation. The leaves methanolic extract was fractionated by means of liquid-liquid extraction and further separated by High-Performance Countercurrent Chromatography (HPCCC). Anti-inflammatory activity has been achieved by measuring NO and TNF-α inhibition in RAW 264.7 murine macrophages. Also, cytotoxicity was evaluated through the MTT method. The hexane fraction (LNFMH) inhibited the production of NO and TNF-α not displaying toxicity. Further fractionation of LNFMH revealed two fractions (2.4 and 2.11) with inhibitory capacity on NO and TNF-α. Gas Chromatography coupled to Mass Spectrometry (GC-MS) analysis of 2.4 and 2.11 showed the presence of major compounds such as n-hexadecanoic acid, n-hexadecanoic acid methyl ester and geranylgeraniol that might be responsible for the anti-inflammatory effect.

**Keywords:** *Leonotis nepetifolia*; anti-inflammatory activity; bioguided fractionation; countercurrent chromatography.

1. Introduction

*Leonotis* genus (Lamiaceae) comprises nine species of African origin readily recognized due to its flowering which exhibits dense whorls with predominantly orange flowers. *Leonotis nepetifolia* (L.) R.Br. is the unique species of the genus distributed worldwide in tropical regions.  

In addition, accounts of its medicinal utility in Brazil goes back to the 19th century in the book “Formulary and Medical Guide” (Chernoviz, 1864), describing the plant as “exciting” and “for children’s baths”. Also, the use as a syrup was described in the first edition of the “Brazilian Pharmacopeia” (1929), but was excluded from later editions due to the lack of evidence of its medicinal use. Also, ethnopharmacological studies point out the utilization of *L. nepetifolia* leaves in Brazilian folk medicine, especially due to its anti-inflammatory, anti-rheumatic and wound healing activities.

Its phytochemistry is partially known consisting of several metabolic classes, such as labdanes and bis-spirolabdanes diterpenes, glycosylated phenylethanoids, glycosylated and acylated iridoids, flavones, flavonols, coumarins and steroids. Labdane diterpenes are reported as potentially anti-inflammatory substances. The effect is mainly attributed to the inhibition of NF-κB activity, a transcription factor that regulates immunity and inflammation, but also by modulating arachidonic acid metabolism and reducing nitric oxide (NO) production.

Given the utilization of *L. nepetifolia* in Brazilian folk medicine for the treatment of several inflammatory conditions, this work aimed to evaluate the anti-inflammatory potential and toxicity through a bioguided fractionation of *L. nepetifolia* leaves methanolic extract.
2. Experimental

2.1. Plant material

Leaves of *Leonotis nepetifolia* (L.) R.Br. (Lamiaceae) were collected in Santo Antônio de Pádua countryside (Rio de Janeiro State, Brazil), in April 2018 (autumn season). A voucher specimen (HUENF 11009) was deposited in the State University of Norte Fluminense Darcy Ribeiro herbarium.

2.2. Extraction and fractionation

Air-dried and powdered leaves of *L. nepetifolia* (600 g) were extracted with methanol (4.2 L) in Soxhlet apparatus for 24 h. The crude methanolic extract was concentrated under reduced pressure on a rotatory evaporator and then lyophilized yielding 127.5 g of dried methanolic extract (LNFM). Then, LNFM (40 g) was suspended in H₂O – MeOH (20%) solution and successively partitioned by liquid-liquid extraction using solvents with increasing polarity degree. Four fractions were obtained: hexane fraction (LNFMH, 6.97 g); dichloromethane fraction (LNFM, 7.92 g); ethyl acetate fraction (LNFMFA, 3.88 g); aqueous fraction (LNFMFAq, 21.42 g).

2.3. HPCCC separation

The hexane fraction (LNFMH) was subjected to High-Performance Countercurrent Chromatography (HPCCC). Separation was performed in a Dynamic Extractions Spectrum HPCCC equipped with a 142 mL multilayer coil columns (1.6 mm i.d.). The β-value ranges from 0.52 to 0.86 and revolution speed can be set up to 1600 rpm. The system comprises two Knauer Smartline 100 V5010 pumps, a Knauer Smartline 2500 V7604 UV absorbance detector, a Büchi C-660 fraction collector, a LabTech Smart H-150–240 mL of sample was injected. The eluate was collected in 3 mL fractions. Obtained fractions were combined in 24 main fractions after comparison of chemical profile by TLC: 2.1 (19.1 mg); 2.2 (50.7 mg); 2.3 (29.5 mg); 2.4 (7.1 mg); 2.5 (7.6 mg); 2.6 (30.4 mg); 2.7 (5.6 mg); 2.8 (7.8 mg); 2.9 (2.6 mg); 2.10 (2.2 mg); 2.11 (9.3 mg); 2.12 (7.9 mg); 2.13 (4.1 mg); 2.14 (1.1 mg); 2.15 (1.3 mg); 2.16 (2.6 mg); 2.17 (5.7 mg); 2.18 (3.0 mg); 2.19 (5.9 mg); 2.20 (16.3 mg); 2.21 (5.7 mg); 2.22 (12.2 mg); 2.23 (6.5 mg); 2.24 (7.4 mg).

2.4. Gas chromatography-mass spectrometry (GC-MS)

The bioactive fractions 2.4 and 2.11 were analyzed by GC-MS on an Agilent GC 7890A equipped with a HP-5ms column (30 m, 0.25 mm, 0.25 µm) coupled to an Agilent MS 5975C with Triple-Axis Detector. Helium was used as carrier gas at a 1.0 mL/min flow rate. The injection volume was 1 µL with a split ratio of 1:10 at 280 °C. Oven initial temperature was 80 °C then ramped 10 °C/min until reaching 280 °C. Mass spectrometer was set up at 280 °C.

2.5. In vitro anti-inflammatory activity

RAW 264.7 macrophages were treated with LPS (1 µg/mL) in the presence of test samples at the concentration of 0.8, 4, 20 and 100 µg/mL, for 24 h. The supernatant were collected and nitrite concentration was measured by Griess. Non-treated cells were used as negative control and LPS-treated cells were used as positive control of macrophages stimulation. L-NMMA-treated cells were used as positive control of nitric oxide (NO) inhibition.

Tumor necrosis factor-α (TNF-α) inhibitory effect was evaluated by indirect bio-assay using L929 murine fibroblasts. Non-treated and non-LPS-stimulated macrophages were used as negative control. Non-treated and LPS-stimulated macrophages were used as positive control. TNF-α concentration was calculated by obtaining a standard curve with recombinant murine TNF-α.

2.6. Cell viability assay

Cytotoxicity was evaluated by measuring cell viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The toxicity percentage was obtained in relation to the negative control (non-treated macrophages) and to the positive control (macrophages culture stimulated and treated with Triton X-100 1% (v/v)).

2.7. Statistical analyses

IC₅₀ and CC₅₀ were calculated by non-linear regression. Data are expressed as the mean ± the standard error of the mean and analyzed using one way analysis of variance (ANOVA) followed by Tukey’s post-hoc test, using GraphPad Prism software version 4.0.
3. Results and Discussion

3.1. *L. nepetifolia* methanolic extract and fractions

*L. nepetifolia* leaves methanolic extract (LNFM) and its hexane (LNFMH), dichloromethane (LNFMD), ethyl acetate (LNFMA), and aqueous (LNFMAq) fractions were evaluated *in vitro* for anti-inflammatory activity. The five samples were tested at the concentration of 0.8, 4, 20 and 100 µg/mL through the capacity to inhibit the production of inflammatory mediators (NO, TNF-α) and cytotoxicity on RAW 264.7 macrophages.

Four samples presented inhibitory effect on NO production: LNFM (IC$_{50}$ = 11.95 ± 1.2 µg/mL), LNFMH (IC$_{50}$ = 14.70 ± 1.3 µg/mL), LNFMD (IC$_{50}$ = 16.31 ± 1.2 µg/mL) and LNFM (IC$_{50}$ = 22.38 ± 1.3 µg/mL), as detailed in Figure 1 and Table 1. However, only LNFMH showed inhibitory effect on TNF-α production (IC$_{50}$ = 21.81 ± 1.4 µg/mL).

![Figure 1](image-url)

*Figure 1.* Effect of *L. nepetifolia* leaves methanolic extract (LNFM) and fractions – hexane (LNFMH), dichloromethane (LNFMD), ethyl acetate (LNFMA), and aqueous (LNFMAq) – in NO and TNF-α production on LPS-stimulated RAW 264.7 macrophages and cytotoxic evaluation. Tested concentrations from the left to right were: 0.8, 4, 20, and 100 µg/mL.
In general, samples demonstrated low cytotoxicity in the MTT assay, but toxicity increased in the higher concentration tested (100 μg/mL). CC50 determination showed cytotoxicity only by LNFMD (CC50 = 42.27 ± 1.6 μg/mL), due to the effect in the higher concentration tested (100 μg/mL).

These results suggest the anti-inflammatory effect of L. nepetifolia might be related with the inhibition of NO production by macrophages, since only LNFMH presented inhibition on TNF-α production.

The effectiveness of L. nepetifolia leaves in anti-inflammatory activity had been reported by Parra-Delgado and coworkers (2004) by in vivo TPA-induced edema model in mice. The hexane extract of the leaves reduced the edema by 55.06%. In this study, the authors determined the anti-inflammatory activity of leonotinin, a labdane diterpenoid isolated from the hexane extract.14

3.1.2. Hexane sub-fractions

The hexane fraction (LNFMH) of L. nepetifolia leaves methanolic extract inhibited the production of both NO (IC50 = 14.70 ± 1.3 μg/mL) and TNF-α (IC50 = 21.81 ± 1.4 μg/mL) not displaying toxicity (CC50 = 98.70 ± 1.2 μg/mL). Thus, this fraction was selected for fractionation and further evaluation of anti-inflammatory activity of its sub-fractions.

LNFMH fractionation was achieved by means of High-Performance Countercurrent Chromatography (HPCCC). Different biphasic solvent systems have been tested by the shake-flask method19 including the Arizona20 (hexane-ethyl acetate-methanol-water) and also non-aqueous solvent systems. The one constituted by hexane-acetonitrile-ethyl acetate (5:5:1, v,v,v)31 were tested with the hexane fraction but emulsion formation has been observed. Two more tests were performed using ethanol and isopropanol in substitution of ethyl acetate. The system comprising hexane-acetonitrile-isopropanol (5:5:1, v,v,v) showed a better sample distribution in both upper and lower phases.

HPCCC fractionation of the hexane fraction resulted in 144 fractions that were analyzed by Thin-Layer Chromatography (TLC) and further combined in 24 sub-fractions due to similar TLC profile. Due to the low mass obtained in some sub-fractions, only eleven could be used and have been selected for biological tests: 2.1 (19.1 mg); 2.2 (50.7 mg); 2.3 (29.5 mg); 2.4 (7.1 mg); 2.5 (7.6 mg); 2.6 (30.4 mg); 2.8 (7.8 mg); 2.11 (9.3 mg); 2.20 (16.3 mg); 2.24 (7.4 mg). The effects of the eleven tested samples in NO and TNF-α production, and cytotoxicity evaluation are shown in Figure 2 and Table 2.

| Samples | NO | TNF-α | Cytotoxicity |
|---------|----|-------|-------------|
|         | IC50 (µg/mL) | sd (±) | IC50 (µg/mL) | sd (±) | CC50 (µg/mL) | sd (±) |
| LNFMH   | 14.70a | 1.3 | 21.81 | 1.4 | 98.70 | 1.2 |
| LNFMD   | 16.31a | 1.2 | >100 | - | 42.27 | 1.6 |
| LNFMAq  | >100 | - | >100 | - | - | - |

*aLNFM (methanolic extract); LNFMH (hexane fraction); LNFMD (dichloromethane fraction); LNFMA (ethyl acetate fraction); LNFMAq (aqueous fraction)
**sd – standard deviation; SI – Selectivity Index (CC50/IC50)

| Samples | NO | TNF-α | Cytotoxicity |
|---------|----|-------|-------------|
|         | IC50 (µg/mL) | sd (±) | SI | IC50 (µg/mL) | sd (±) | SI | CC50 (µg/mL) | sd (±) |
| 2.1     | 12.27 | 1.3 | 3.7 | 8.88a | 1.1 | 5.1 | 45.07 | 1.7 |
| 2.2     | 3.28 | 1.3 | 8.0 | 33.11 | 1.4 | 0.8 | 26.10 | 1.2 |
| 2.3     | 11.54 | 1.5 | 3.5 | 36.65 | 1.2 | 1.1 | 40.38 | 1.5 |
| 2.4     | 8.23 | 1.5 | 6.9 | 12.35 | 1.2 | 4.6 | 57.08 | 1.5 |
| 2.5     | 9.10 | 1.2 | 5.2 | >100 | - | - | 47.30 | 1.6 |
| 2.6     | 3.64 | 1.4 | 12.1 | 29.48 | 1.3 | 1.5 | 43.88 | 1.7 |
| 2.8     | 17.34 | 1.2 | 2.8 | >100 | - | - | 48.56 | 1.6 |
| 2.11    | 12.95 | 1.4 | 4.9 | 8.16a | 1.3 | 7.8 | 63.59 | 1.5 |
| 2.12    | 11.92 | 1.2 | 3.3 | >100 | - | - | 39.43 | 1.2 |
| 2.20    | 7.02 | 1.3 | 2.9 | >100 | - | - | 20.35 | 1.3 |
| 2.24    | 10.09 | 1.3 | 3.8 | >100 | - | - | 38.80 | 1.5 |

*a sd – standard deviation; SI – Selectivity Index (CC50/IC50)
All eleven selected hexane sub-fractions inhibited NO production (IC_{50} = 3.28 – 17.34 µg/mL). The strongest NO inhibition was demonstrated by fractions 2.2 (IC_{50} = 3.28 µg/mL) and 2.6 (IC_{50} = 3.64 µg/mL). Moreover, fraction 2.6 were the most selective sample to inhibit NO production (SI = 12.1).

However, only six fractions (2.1, 2.2, 2.3, 2.4, 2.6 and 2.11) inhibited TNF-α production with an IC_{50} ranging from 8.16 to 36.65 µg/mL. Remarkable results were displayed by fractions 2.1 (IC_{50} = 8.88 µg/mL), 2.4 (IC_{50} = 12.35 µg/mL) and 2.11 (IC_{50} = 8.16 µg/mL). Although similar results obtained by fractions 2.1 and 2.11 regarding inhibition of TNF-α production, 2.11 presented a higher selectivity index (SI = 7.8) in comparison to 2.1 (SI = 5.1).

The cytotoxicity evaluation of samples demonstrated a CC_{50} ranging from 20.35 to 63.59 µg/mL, with the lowest toxicity observed by fractions 2.4 and 2.11 (CC_{50} = 57.08 and 63.59 µg/mL). Toxicity has been observed in all samples at the tested concentration of 100 µg/mL. The fraction 2.20 was the most toxic one (CC_{50} = 20.35 µg/mL).

The Selectivity Index (SI = CC_{50}/IC_{50}) was calculated for the inhibitory potential of NO and TNF-α demonstrating fractions 2.4 and 2.11 were at least four times more selective to promote an immunomodulatory action (SI for NO: 2.4 = 6.9 and 2.11 = 4.9; SI for TNF-α: 2.4 = 4.6 e 2.11 = 7.8). At 20 µg/mL concentration, fractions 2.4 and 2.11 displayed activity and low toxicity, although at 100 µg/mL toxicity has been observed.

An increase in toxicity was noted in the sub-fractions (CC_{50} = 20.35 – 63.59 µg/mL) when compared to LNFMH (CC_{50} = 99.2 ± 0.2 µg/mL). However, LNFMH and its sub-fraction 2.4 presented similar SI, as for NO (6.6 and 6.9, respectively), as for TNF-α (both = 4.6).

The bioactive sub-fractions 2.4 and 2.11 have been analyzed by GC-MS in order to achieve their chemical profile. Fatty acids were the main compounds observed in both samples as shown in Table 3. The presence of n-hexadecanoic acid has been observed in both sub-fractions (2.4 and 2.11). Also, n-hexadecenoic acid methyl ester and the isoprenoid geranylgeraniol have been identified as one of the major compounds in the sub-fraction 2.4.
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Table 3. Identified compounds in the bioactive fractions 2.4 and 2.11 by Gas Chromatography-mass spectrometry (GC-MS)

| Fraction | Compound                  | Retention Time (min) | m/z (relative intensity)                  | Library* similarity (%) |
|----------|---------------------------|----------------------|------------------------------------------|-------------------------|
| 2.4      | n-hexadecanoic acid       | 14.26                | 55(83.3); 57(74.3); 60(90.3); 69(49.3); 73(100); 129(49.1); 213(33.4); 256(39.9) | 93                      |
|          | n-hexadecanoic acid methyl ester | 18.99              | 55(80.3); 57(86.3); 74(66.6); 98(100); 239(79) | 91                      |
|          | geranylgeraniol           | 21.35                | 55(24.4); 57(42.9); 70(31.3); 83(15); 112(24.1); 185(100) | 95                      |
| 2.11     | n-hexadecanoic acid       | 14.44                | 55(67.9); 57(65.2); 60(78.8); 69(41.7); 73(100); 129(52.5); 213(34.5); 256(46.7) | 94                      |

*NIST library

The anti-inflammatory activity of the identified compounds is documented in the literature. The n-hexadecanoic acid inhibits the phospholipase A₂, demonstrating anti-inflammatory effect by inhibiting the activation of nuclear factor-kappa B (NF-κB) and also reduced NO production.

4. Conclusions

The hexane fraction (LNFMH) and its sub-fractions 2.4 and 2.11 obtained from the leaves methanolic extract of L. nepetifolia showed in vitro anti-inflammatory potential by inhibiting both NO and TNF-α production. Furthermore, GC-MS analysis revealed the presence of the major compounds n-hexadecanoic, n-hexadecanoic methyl ester and geranylgeraniol that might be responsible for the anti-inflammatory effect.

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References

1. Iwarsson, M.; Harvey, Y.; Monograph of the Genus Leonotis (Pers.) R. Br. (Lamiaceae). Kew Bulletin 2003, 58, 597. [CrossRef]
2. Antar, G. M.; Leonotis in Flora do Brasil 2020 em construção. Jardim Botânico do Rio de Janeiro: Rio de Janeiro, 2021. [Link]
3. Cruz, V. B.; Tresvenzol, L. M. F.; Ferreira, H. D.; Paula, J. R.; Paulino, N.; Leonotis nepetifolia (L.) R. Br. (Cordão-de-frade): Biologia e uso tradicional. Revista de Pesquisa e Inovação Biológica e uso tradicional. Revista de Pesquisa e Inovação Biológica 2011, 3, 15. [Link]
4. Di Stasi, L. C.; Oliveira, G. P.; Carvalhaes, M. A.; Queiroz-Junior, M.; Tien, O. S.; Kakinami, S. H.; Reis, M. S.; Medicinal plants popularly used in the Brazilian Tropical Atlantic Forest. Fitoterapia 2002, 73, 69. [CrossRef] [PubMed]
5. Agra, M. F.; Freitas, P. F.; Barbosa-Filho, J. M.; Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Brazilian Journal of Pharmacognosy 2007, 17, 114. [CrossRef]
6. Albuquerque, U. P.; Medeiros, P. M.; Almeida, A. L. S.; Monteiro, J. M.; Freitas, E. M. L. N.; Melo, J. G.; Santos, J. P.; Medicinal plants of the caatinga (semi-arid) vegetation of NE Brazil: A quantitative approach. Journal of Ethnopharmacology 2007, 114, 325. [CrossRef] [PubMed]
7. Costa, V.; Mayworm, M. A.; Plants medicinals utilizadas pela comunidade do bairro dos Tenentes - município de Extrema, MG, Brasil. Revista Brasileira de Plantas Medicinais 2011, 13, 282. [CrossRef]
8. Neves, F. S.; Trabalho de Conclusão de Curso, Universidade Estadual do Norte Fluminense Darcy Ribeiro, 2012. [Link]
9. Silva, A. F.; Sousa, R. L.; Silva, S. G.; Costa, J. M.; Albuquerque, L. C. S.; Pereira, M. G. S.; Mesquita, S. S.; Silva, E. C.; Condeiro, Y. E. M.; Etnobotânica de plantas medicinais aromáticas: preparações e usos da flora local em cinco comunidades rurais localizadas na região do Baixo Tocantins, Pará, Brasil. Research, Society and Development 2021, 10, e9510111284. [CrossRef]
10. Ricardo, L. M.; Paula-Souza, J.; Andrade, A.; Brandão, M. G. L.; Plants from the Brazilian Traditional Medicine: species from the books of the Polish physician Piotr Czerniewicz (Pedro Luiz Napoleão Chernoviz, 1812–1881). Revista Brasileira de Farmacognosia 2017, 27, 388. [CrossRef]
11. Brandão, M. G. L.; Cosenza, G. P.; Moreira, R. A.; Monte-Mor, R. L. M.; Medicinal plants and other botanical products from the Brazilian Official Pharmacopoeia. Revista Brasileira de Farmacognosia 2006, 16, 408. [CrossRef]
12. Brandão, M. G. L.; Zanetti, N. N. S.; Oliveira, G. R. R.; Goulart, L. O.; Monte-Mor, R. L. M.; Other medicinal plants and botanical products from the first edition of the Brazilian Official Pharmacopoeia. Revista Brasileira de Farmacognosia 2008, 18, 127. [CrossRef]
13. Takeda, T.; Narukawa, Y.; Hada, N.; Studies on the Constituents of Leonotis nepetaefolia. Chemical & Pharmaceutical Bulletin 1999, 47, 284. [CrossRef]
14. Parra-Delgado, H.; García Ruiz, G.; Nieto Camacho, A.; Martínez-Vázquez, M.; Anti-inflammatory Activity of Some Extracts and Isolates from Leonotis nepetaefolia on TPA-induced Edema Model. Revista de la Sociedad Química de México 2004, 48, 293. [Link]
15. Li, J.; Fronczek, F. R.; Ferreira, D.; Burandt, C. L.; Setola, V.; Roth, B. L.; Zjawiony, J. K.; Bis-spirolabdan diterpenoids from Leonotis nepetefolia. Journal of Natural Products 2012, 75, 728. [CrossRef] [PubMed]

16. Ueda, F.; Iizuka, K.; Tago, K.; Narukawa, Y.; Kiuchi, F.; Kasahara, T.; Tamura, H.; Funakoshi-Tago, M.; Nepetaefuran and leonotinin isolated from Leonotis nepetefolia R. Br. potently inhibit the LPS signaling pathway by suppressing the transactivation of NF-κB. International Immunopharmacology 2015, 28, 967. [CrossRef] [PubMed]

17. Oliveira, A. P.; Guimarães, A. L.; Pacheco, A. G. M.; Aratijo, C. S.; Oliveira Júnior, R. G.; Lavor, E. M.; Silva, M. G.; Araújo, E. C. C.; Mendes, R. L.; Rolim, L. A.; Costa, M. P.; Farias, H. C. L.; Pessoa, C. D. O.; Lopes, N. P.; Marques, L. M. M.; Almeida, J. R. G. S.; Phytochemical study on antimicrobial and cytotoxic activity of specimens of Leonotis nepetifolia L. (Br). Química Nova 2015, 39, 32. [CrossRef]

18. Tran, Q. T. N.; Wong, W. S. F.; Chai, C. L. L.; Labdane diterpenoids as potential anti-inflammatory agents. Pharmacological Research 2017, 124, 43. [CrossRef] [PubMed]

19. Berthod, A.; Carda-Broch, S.; Determination of liquid–liquid partition coefficients by separation methods. Journal of Chromatography A 2004, 1037, 3. [CrossRef] [PubMed]

20. Berthod, A.; Hassoun, M.; Ruiz-Angel, M. J.; Alkane effect in the Arizona liquid systems used in countercurrent chromatography. Analytical and Bioanalytical Chemistry 2005, 383. [CrossRef] [PubMed]

21. Ramos, A. C.; Oliveira, R. R.; One-step separation of terpenoids from leaves extracts of Solanum cernuum by high-performance countercurrent chromatography. Journal of Liquid Chromatography and Related Technologies 2016, 39, 8. [CrossRef]

22. Aparna, V.; Dileep, K. V.; Mandal, P. K.; Karthe, P.; Sadasivan, C.; Haridas, M.; Anti-Inflammatory Property of n-Hexadecanoic Acid: Structural Evidence and Kinetic Assessment. Chemical Biology & Drug Design 2012, 80, 434. [CrossRef] [PubMed]

23. Giriwono, P. E.; Shirakawa, H.; Ohsaki, Y.; Hata, S.; Kuriyama, H.; Sato, S.; Goto, T.; Komai, M.; Dietary supplementation with geranylgeraniol suppresses lipopolysaccharide-induced inflammation via inhibition of nuclear factor-κB activation in rats. European Journal of Nutrition 2013, 52, 1191. [CrossRef] [PubMed]

24. Giriwono, P. E.; Shirakawa, H.; Ohsaki, Y.; Sato, S.; Aoyama, Y.; Ho, H.-J.; Goto, T.; Komai, M.; Geranylgeraniol Suppresses the Expression of IRAK1 and TRAF6 to Inhibit NFκB Activation in Lipopolysaccharide-Induced Inflammatory Responses in Human Macrophage-Like Cells. International Journal of Molecular Sciences 2019, 20, 2320. [CrossRef] [PubMed]

25. Marcuzzi, A.; Tommasini, A.; Crovella, S.; Pontillo, A.; Natural isoprenoids inhibit LPS-induced-production of cytokines and nitric oxide in aminobisphosphonate-treated monocytes. International Immunopharmacology 2010, 10, 639. [CrossRef]