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Anti-inflammatory Activities of Ethanolic Extracts of *curcuma* Longa (Turmeric) and *cinnamon (Cinnamomum verum)*

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Abstract Curcuma longa and Cinnamon are used in folkloric medicine and thought to have different pharmacological activities including anti-inflammatory effects. The objective of this work is to evaluate the *in vitro* inhibitory effect of Curcuma longa and Cinnamon ethanolic extracts on Lipopolysacaride (LPS)-induced Interlukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) by polymorphonuclear Cells (PMNCs). Polymorphonuclear cells were isolated from the whole blood using Histopaque (Ficol-1077) method and then cultured in an enriched Roswell Park Memorial Institute (RBMI) medium. The concentrations of TNF-α and IL-6 in the supernatant were measured after 24 h and compared using paired-samples t test. The Curcuma longa and Cinnamon extracts have shown significant reduction in the levels of both Il-6 and TNF-α. HPLC analysis of Curcuma longa extract revealed that it contains curcumin, demethoxycurcumin, and bisdemethoxycurcumin while the major compound in the extract of cinnamon was found to be cinnamic acid. Reduction in the levels of IL-6 and TNF-α upon effect of the plants" extract is an indication of their anti-inflammatory effects. The observed anti-inflammatory effect may be due to the presence of curcuminoids and cinnamic acid from Curcuma longa and Cinnamon, respectively.

Keywords: *anti-inflammatory, Curcuma longa, cinnamon, TNF-alpha, curcuminoids, cinnamic acid*

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1. Introduction

Cinnamon and curcumin are among the herbal plants that have been used in many different cultures to treat patients who suffered from different diseases. They have pharmacological activities such as anticancer, anti-inflammatory, antimicrobial and antioxidant activities [1,2,3].

Curcumin is a pigment with yellow color commonly used as a food coloring agent and found in turmeric roots, mostly planted in India and widely used to cure many diseases such as hepatic disorders, rheumatism, skin diseases, dyspepsia, blood sugar, colic inflammation, and amenorrhoea [4]. Due to its highly effective properties, many companies are currently providing curcumin in the form of capsules, soft drinks, gels, nasal sprays, and tablets [5]. Cinnamon (Cinnamomum zeylanicum) contains a high proportion of eugenol which has antispasmodic, antiparasitic, antibacterial, and antidiarrheal effects. Thus, such herb has been used to treat many disorders. Also, it has a wound healing activity [6,7].

Lipopolysaccharide (LPS) is an endotoxin and a part of the cell wall of the gram negative bacteria. It is important to the integrity of the bacterial structure and helps in protection of the bacteria from attacks by certain chemicals. LPS enhance the immune response of the immune system by binding to Toll Like Receptor 4 (TLR 4) in many immune cells, as Dendritic cells, B cells, Monocytes and Macrophages [8,9,10].

Interlukin-6 and Tumor Necrosis Factor – α are proinflammatory mediators that are released from many immune cells as Monocytes, B cells, and Macrophages upon exposure to an inflammatory signal such as the exposure to LPS [11,12,13]. Anti-inflammatory agents decrease the production of these pro-inflammatory cytokines (IL-6 and TNF-α).

Many investigations have been conducted to clarify the anti-inflammatory effect of curcumin as well as cinnamon [5,14-19]. In this research, the anti-inflammatory effect of Curcuma longa and Cinnamon was evaluated using LPS-induced mononuclear cells isolated from the buffy coat of whole blood. IL-6 and TNF-α were measured before and after the addition of the LPS and the extracts [20,21]. Levels of the cytokines were measured using Enzyme Linked Immune Sorbent Assay (ELISA) method. Analysis of the active compounds of Curcuma longa and Cinnamon extracts by HPLC-UV was also conducted in this study.

2. Materials and Methods

2.1. Plant Material

Grinded cinnamon and curcumin were purchased from Alsirisi market, Jenin, Palestine and classified by Mostafa Amarni (botanical specialist at the Ministry of Agriculture, Jenin, Palestine). Cinnamon identification number is 09061100 and curcumin identification number is 969516.

2.2. Plant Extraction

Fifty gm of powder of cinnamon and 50 gm powder curcumin were mixed separately in 500 ml of 96% ethanol and kept shaking for one week at room temperature. The solution was filtered using a Whatman filter paper and the filtered product was evaporated using rotary evaporator (BUCHI brand) under vacuum at 50°C. The extract was collected and kept at -20°C until further use.

2.3. Cell Culture

Polymorphonuclear cells were collected from a 5 ml whole blood by a method as described by Qabaha et al [22]. The cells were cultured in Roswell Park Memorial Institute medium (RPMI) enriched with 100 Uml^{-1} penicillin, 100-µg ml^{-1} streptomycin, and with 10% heat-inactivated Fetal Bovine Serum (FBS). The mixtures were divided into a 12 well tray in 5% CO_2 incubator at 37°C for 24 hour. Each well contains one ml of the mixture which contains one million cells. Wells were exposed to different concentrations of the plant extracts in the presence and absence of Lipopoly Sacharide (LPS) (1 µg/ well).

2.4. Cytotoxicity Test-trypan Blue Exclusion Test

Cytotoxicity of the curcumin and cinnamon extract was evaluated using Trypan Blue Exclusion Test as described by Avelar-Freitas et al. 2014 [23]. The dead cells will be stained with trypan blue while the viable cells will be colorless.

2.5. Immunoassay for IL-6 And TNF-alpha

Enzyme Linked Immunoassay was used to quantify TNF-alpha and IL-6 according to manufacturer's instructions.

2.6. HPLC Instrumentation Systems

The analytical HPLC is Waters Alliance (e2695 separations module), quipped with 2998 Photo diode Array (PDA). Data acquisition and control were carried out using Empower 3 chromatography data software (Waters, Germany).

2.7. Chromatographic Conditions

The HPLC analytical experiments of the crude water, 80% ethanol and 100% ethanol extracts were run on ODS column of Waters (XBridge, 4.6 ID x 150 mm, 5 µm) with guard column of Xbridge ODS, 20 mm x 4.6mm ID, 5 µm. The mobile phase is a mixture of 0.5% acetic acid solution (A) and acetonitrile (B) ran in a linear gradient mode. The start was a 100% (A) that descended to 70% (A) in 40 minutes. Then to 40% (A) in 20 minutes and finally to 10% (A) in 2 minutes and stayed there for 6 minutes and then back to the initial conditions in 2 minutes. The HPLC system was equilibrated for 5 minutes with the initial acidic water mobile phase (100 % A) before injecting next sample. All the samples were filtered with a 0.45 µm PTFE filter. The PDA wavelengths range was from 210-500. The flow rate was 1 ml/min. Injection volume was 20 µl and the column temperature was set at 25°C. The HPLC system was then equilibrated for 5 minutes with the initial mobile phase composition prior injecting the next sample. All the samples were filtered via 0.45 µm micro porous disposable filter.

3. Results and Discussion

3.1. HPLC Analysis of the Extracts

3.1.1. Curcuma Longa (Turmeric) Ethanolic Extract

Curcuma longa (turmeric) has a significant influence as an anti-inflammatory agent. Turmeric constituents include three major curcuminoids, namely, curcumin which is responsible for its yellow color, demethoxycurcumin, and bisdemethoxycurcumin, see Figure 1.

Upon injection of the ethanolic extract to analytical HPLC chromatograph, it has been noticed that the curcumin extract comprises mainly the three major curcuminoids (Figure 2), while there were only negligible amount of phenolics in the range between 3-45 minutes when compared to standard phenolic mixture which ran under the same chromatographic conditions at the same monitoring wavelength (Figure 3).

Therefore, the anti-inflammatory influence is probably due to these three major curcuminoids compounds.

3.1.2. Cinnamon Ethanolic Extract

The Cinnamon ethanolic extract revealed one major peak eluted at 36.7 minutes along with some other minor ones at 290 nm. Standard cinnamic acid was injected and the retention time and UV-Vis were matched exactly with cinnamic acid from the extract indicating its identity (Figure 4). This major peak with the other minor existing peaks did not match any of the phenolic and flavanoidic standard mixture retention or UV-Vis as shown in Figure 5. It is reported that cinnamic acid derivatives and esters have a tangible anti-inflammatory effect [24,25]. This also strengthens the role cinnamic acid plays as an anti-inflammatory agent.

3.2. Cytotoxicity of Curcumin and Cinnamon Extracts

Curcumin and Cinnamon extracts at concentration 300 µg/ ml and LPS at concentration of 1 µg/ ml have no negative effect on the viability of the Poly Morpho Nuclear cells as shown by Table 1.

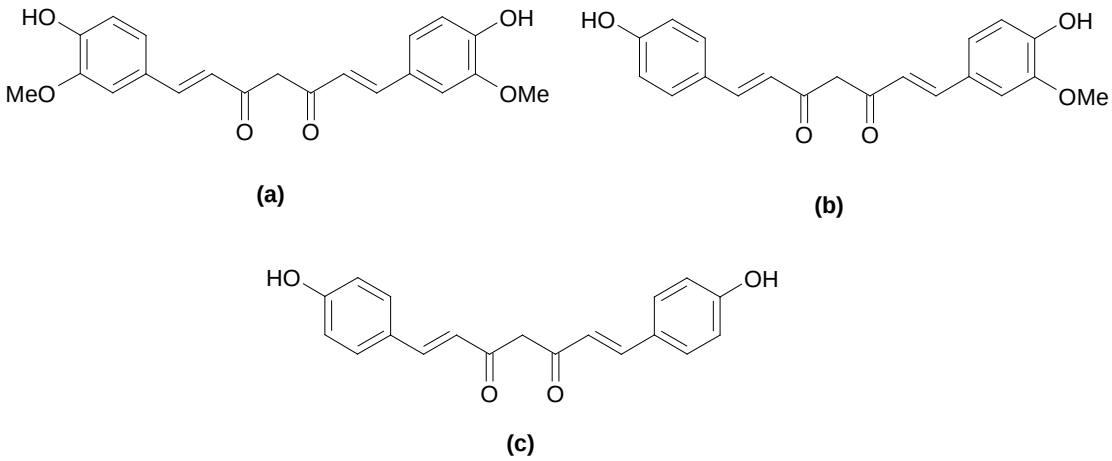

Figure 1. Structure of the main three compounds in curcumin ethanolic extract (curcumin (a), demethoxycurcumin (b) and bisdemethoxycurcumin (c))

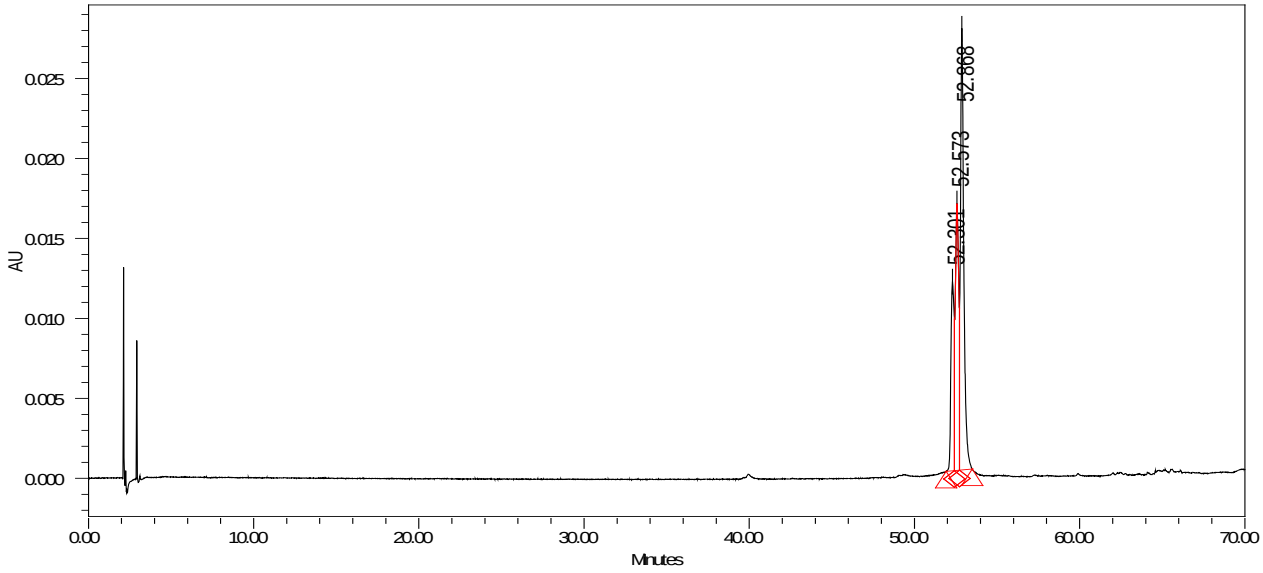

Figure 2. HPLC-PDA chromatogram of crude curcumin 100% ethanol extract at 425 nm. The overlaid UV-Vis spectra of the 3 main curcumin peaks have maximum adsorption at 416.8 nm (retention of 52.302 minutes, compound c), 420.4 nm (retention of 52.752 minutes, compound b) and 425.3 nm (retention of 52.867 minutes, compound a)

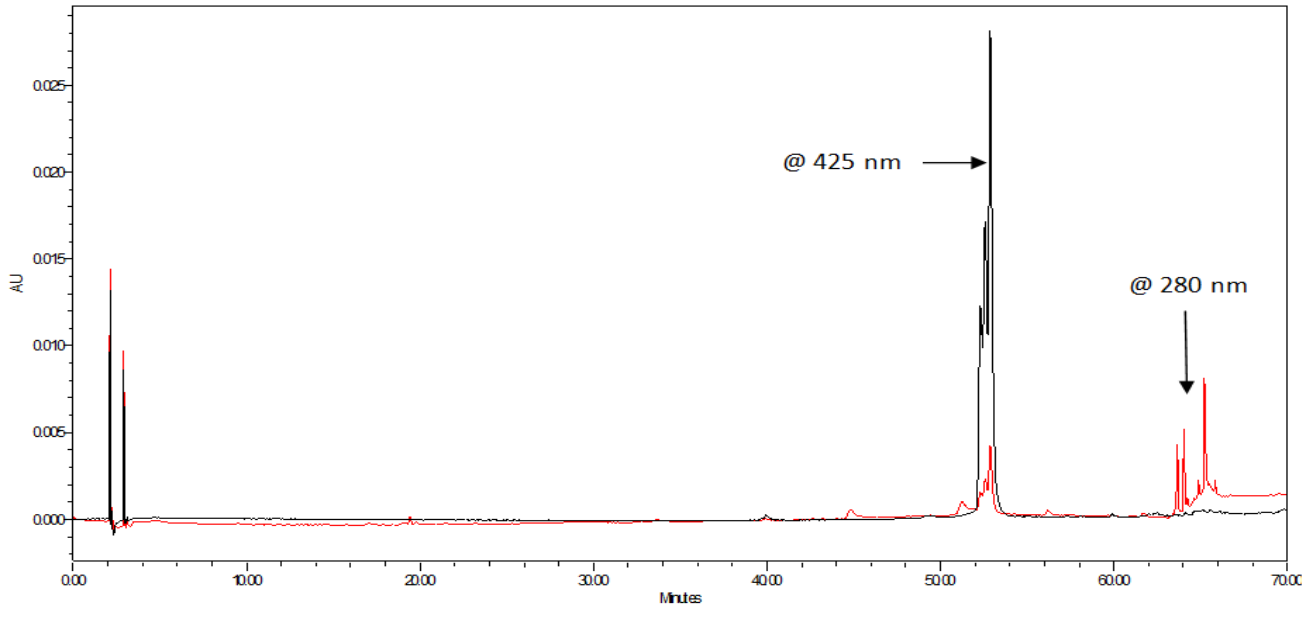

Figure 3. Overlaid HPLC-PDA chromatograms of crude curcumin 100% ethanol extract at 425 nm and at 280 nm where most of the phenolics have a significant absorption

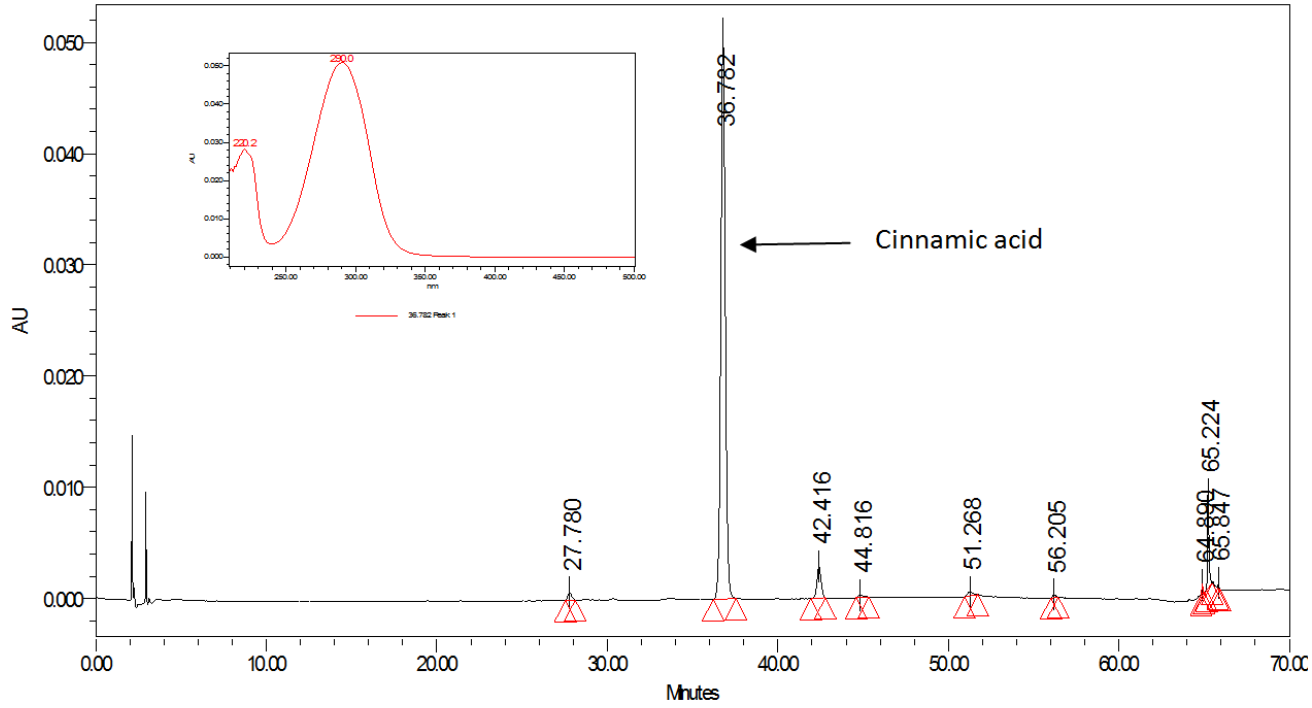

Figure 4. HPLC-PDA chromatogram of crude ethanolic extract of cinnamon at 290 nm. The UV-Vis of the major compound (cinnamic acid of retention 36.7 minutes) has a maximum absorption at 290 nm

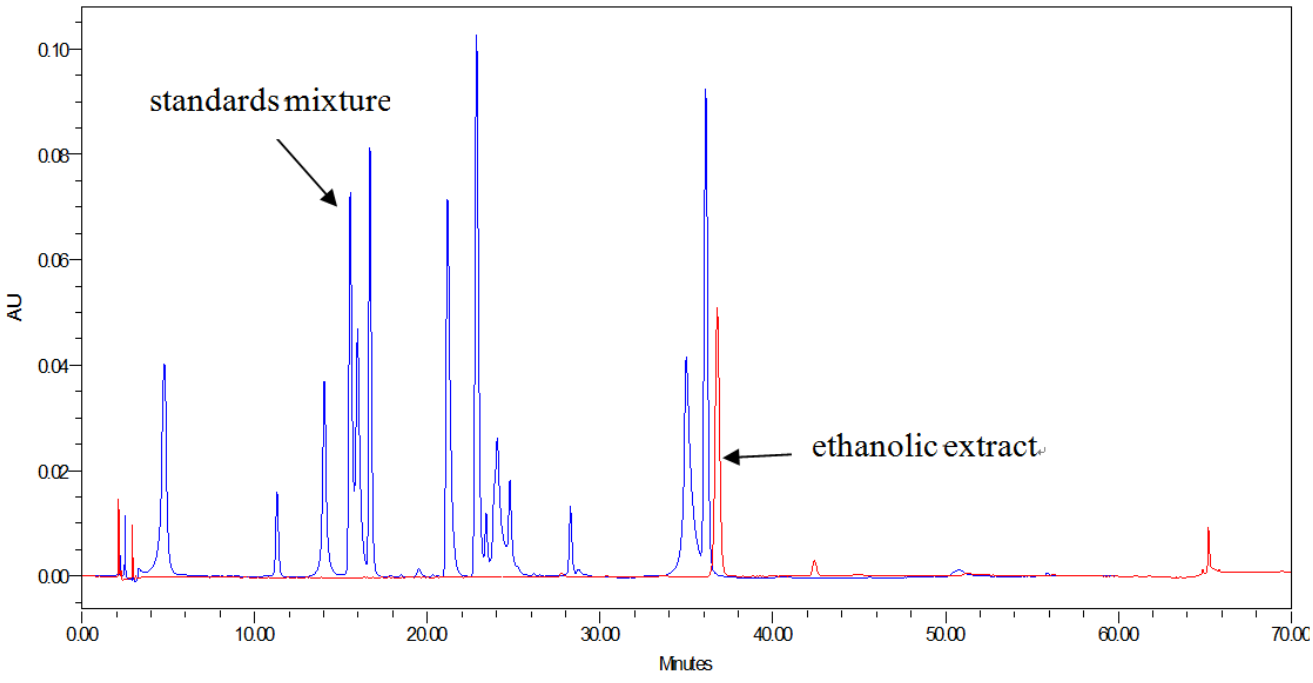

Figure 5. HPLC-PDA overlaid chromatogram of 18 phenolic and flavonoid standards mixture at 290 nm with cinnamon ethanolic extract

Table 1. Effects of Curcumin and Cinnamon extracts on PMNCs viability

Contents	Viability
PMNCs only	96.7±0.8
PMNCs with LPS	94.4±0.7
PMNCs with LPS and 300 µg/ ml CurcuminExtract	92.6±0.4
PMNCs with LPS and 300 µg/ ml Cinnamon Extract	91.2±0.6

3.3. Anti-inflamatory Effect of Curcumin and Cinnamon Extracts

Upon stimulation of PMNCs with 1 µg/ ml LPS, the production of IL-6 and TNF-α has increased significantly after 24 hour. However, after treatment with the plant extracts, the production of both cytokines has decreased as the concentration of the plant extract increased in a dose-dependent manner (Table 2). Furthermore, the differences in the inhibition of the cytokine production upon the effect of each dose were significant (different small letters within column indicate significant difference.

Table 2. Effect of Curcumin and Cinnamon extract on TNf-α and IL-6 production by PMNCs

	TNf-α and IL-6 (pg/ml)			
	Curcumin extract		Cinnamon extract	
	TNf-α*	IL-6	TNf-α	IL-6
1	68±2.4 e	114.7±2.7 e	63.7±2.6 e	109.3±1.2 e
2	1031±4.5 a	629.3±3.2 a	1009±8.3 a	587.4±2.1 a
3	873.3±7.4 b	374±2.9 b	842.7±4.5 b	455.3±4.1 b
4	712±5.7 c	184.3±3.7 c	654±3.7 c	403±2.5 c
5	543±5.9 d	140±2.9 d	475.3±4.1 d	314±2.0 d

1: Cytokine without any treatment
2: Cytokine with LPS only
3: Cytokine with LPS and 75 µg of plant extract
4: Cytokine with LPS and 150 µg of plant extract
5: Cytokine with LPS and 300 µg of plant extract
* Results are expressed as average of three samples. Different small letters within column indicate significant difference ($p < 0.05$, $n = 3$).

Poly morpho nuclear cells play an important role in host defense as well as in inflammation. LPS- stimulated PMNCs secrete many pro-inflammatory mediators such as TNf-α and IL-6. Many disease causative agents secrete LPS that in turn initiate inflammation in the host. In our study, we mimicked the disease by adding LPS to the PMNCs to release pro-inflamatory mediators.

In order to evaluate the antiinflamatory effect of the extract of cinnamon and curcumin, different concentrations of both extracts were added on the LPS-stimulated PMNCs and both TNf-α and IL-6 levels were measured after 24hours of incubation at 37C in 5% CO2 incubator.

It was found that cinnamon extract has stronger effect in the inhibition of the release of TNf-α compared to curcumin, while the reverse was observed for IL-6 where Curcumin showed stronger effect in the inhibition of IL-6 release compared to cinnamon, Table 2.

The anti-inflammatory effects of both Curcuma longa and Cinnamon are consistent with the findings of Jurenka [14], Menon and Sudheer [15], Jacob et al. [26], Mashhadi et al [16], Hong et al. [17], Joshi et al. [18], and Rao et al. [19].

4. Conclusion

Curcuma longa and Cinnamon extracts showed strong reduction in the production of IL-6 and TNF-α from the LPS-induced PMNCs, indicating strong anti-inflammatory effects, which make Curcuma longa and Cinnamon a source of anti-inflammatory candidates to be used in the pharmacological industry. Both extracts at concentration of 300 µg/ml were not toxic to the PMNCs which show that these extracts are able to inhibit IL-6 and TNF-α production while maintaining cell viability. The anti-inflammatory effects of Curcuma longa are attributed to its main three compounds (curcumin, demethoxycurcumin, and bisdemethoxycurcumin), while this activity for Cinnamon is attributed to cinnamic acid present in cinnamon.

Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations and Nomenclature

LPS: Lipopolysacaride
IL-6: Interlukin-6
TNF-α: Tumor Necrosis Factor-α
PMNCs: Polymorphonuclear Cells
HPLC: High Performance Liquid Chromatography
ELISA: Enzyme Linked Immune Sorbent Assay
RPMI: Roswell Park Memorial Institute medium
FBS: Fetal Bovine Serum.

References

[1] Iwata, N., Kainuma, M., Kobayashi, D., Kubota, T., Sugawara, N., Uchida, A., Ozono, S., Yamamuro, Y., Furusyo, N., Ueda, K., Tahara, E., Shimazoe, T., 2016. "The Relation between Hepatotoxicity and the Total Coumarin Intake from Traditional Japanese Medicines Containing Cinnamon Bark." Front. Pharmacol. 7: 174.

[2] Bimonte, S., Barbieri, A., Leongito, M., Piccirillo, M., Giudice, A., Pivonello, C., de Angelis, C., Granata, C., Palaia, R., Izzo, F., 2016. "Curcumin AntiCancer Studies in Pancreatic Cancer." Nutrients 8(7), 433.

[3] Hugar, S. S., Patil, S., Metgud, R., Nanjwade, B., Hugar, S. M. 2016. Influence of application of chlorhexidine gel and curcumin gel as an adjunct to scaling and root planing: A interventional study. Journal of Natural Science, Biology, and Medicine, 7(2), 149-154.

[4] Sarkar, A., De, R., Mukhopadhyay, A. K., 2016. Curcumin as a potential therapeutic candidate for Helicobacter pylori associated diseases. World Journal of Gastroenterology, 22(9), 2736-2748.

[5] He, Y., Yue, Y., Zheng, X., Zhang, K., Chen, S., Du, Z. 2015. Curcumin, Inflammation, and Chronic Diseases: How Are They Linked? *Molecules*. 20(5): 9183-9213.

[6] Akilen, R., Tsiami, A., Devendra, D., Robinson, N., 2012. Cinnamon in glycaemic control: systematic review and meta analysis. Clin Nutr. 31 (5): 609-615.

[7] Zainol, N. A., Ming, T. S., Darwis, Y., 2015. Development and Characterization of Cinnamon Leaf Oil Nanocream for Topical Application. Indian Journal of Pharmaceutical Sciences, 77(4), 422-433.

[8] Rossato, M., Curtale, G., Tamassia, N., Castellucci, M., Mori, L., Gasperini, S., Bazzoni, F. 2012. IL-10-induced microRNA-187 negatively regulates TNF-α, IL-6, and IL-12p40 production in TLR4-stimulated monocytes. Proceedings of the National Academy of Sciences of the United States of America, 109(45), E3101-E3110.

[9] Skelly D.T., Hennessy E., Dansereau M-A., Cunningham C., 2013. Correction: A Systematic Analysis of the Peripheral and CNS Effects of Systemic LPS, IL-1β, TNF-α and IL-6 Challenges in C57BL/6 Mice. PLoS ONE 8(12).

[10] An, X., Lee, S. G., Kang, H., Heo, H.J., Cho,Y.S., Kim, D.O., 2016. Antioxidant and Anti-inflammatory Effects of Various Cultivars of Kiwi Berry (Actinidia arguta) on Lipopolysaccharide-stimulated RAW 264.7 Cells. J Microbiol Biotechnol. 28; 26(8):1367-74.

[11] Jeong, Y.Y., Ryu, J.H., Shin, J.H., Kang, M.J., Kang, J.R., Han, J., Kang, D. 2016. Comparison of Anti-Oxidant and Anti-Inflammatory Effects between Fresh and Aged Black Garlic Extracts. *Molecules (Basel, Switzerland)*. 21, 430.

[12] Jin, S.E., Kim, O.S., Yoo, S., Seo, C., Kim, Y., Shin, H., Jeong, S. 2016. "Anti-inflammatory effect and action mechanisms of traditional herbal formula Gamisoyo-san in RAW 264.7 macrophages." BMC Complement Altern Med, 16: 219.

[13] Chen, C.-C., Lin, M.-W., Liang, C.-J., Wang, S.-H., 2016. The Anti-Inflammatory Effects and Mechanisms of Eupafolin in Lipopolysaccharide-Induced Inflammatory Responses in RAW264.7 Macrophages. PLoS ONE, 11(7), e0158662.

[14] Jurenka J.S., 2009. Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research, Altern Med Rev. 14(2): 141-53.

[15] Menon V.P., Sudheer A.R., 2007. Antioxidant and anti-inflammatory properties of curcumin, Adv Exp Med Biol. 595: 105-125.

[16] Mashhadi, N. S., Ghiasvand, R., Askari, G., Feizi, A., Hariri, M., Darvishi, L., Hajishafiee, M. 2013. Influence of Ginger and Cinnamon Intake on Inflammation and Muscle Soreness Endued by Exercise in Iranian Female Athletes. Int J Prev Med. 4 (Suppl 1), S11-S15.

[17] Hong, J.-W., Yang, G.-E., Kim, Y. B., Eom, S. H., Lew, J.-H., Kang, H., 2012. Anti-inflammatory activity of cinnamon water extract in vivo and in vitroLPS-induced models. BMC Complementary and Alternative Medicine, 12, 237.

[18] Joshi, K., Awte, S., Bhatnagar, P., Walunj, S., Gupta, R., Joshi, S., Sabharwal, S., Bani, S. Padalkar, A.S., 2010. Cinnamomum zeylanicum extract inhibits proinflammatory cytokine TNFμ: in vitro and in vivo studies, Research In Pharmaceutical Biotechnology, 2(2), 14-21.

[19] Rao, P.V., Gan, S.H., 2014. Cinnamon: A Multifaceted Medicinal Plant, Evidence-Based Complementary and Alternative Medicine, Volume 2014, Article ID 642942, 12.

[20] Muhammad, J. S., Zaidi, S. F., Shaharyar, S., Refaat, A., Usmanghani, K., Saiki, I., Sugiyama, T. 2015. "Anti-inflammatory Effect of Cinnamaldehyde in Helicobacter pylori Induced Gastric Inflammation." Biological and Pharmaceutical Bulletin 38(1): 109-115.

[21] Zhu, W., Wu, Y., Meng, Y.-F., Wang, J.-Y., Xu, M., Tao, J.-J., Lu, J., 2015. Effect of curcumin on aging retinal pigment epithelial cells. Drug Design, Development and Therapy, 9, 5337-5344.

[22] Qabaha, K., Abu Ras, S., Abbadi, J., Al-Rimawi, F., 2016. Ant-inflammatory of Eucalyptus spp. and Pistascia lentiscus leaf Extracts, Afr J Tradit Complement Altern Med. 13(5):1-6.

[23] Avelar-Freitas, B. A., Almeida, V. G., Pinto, M. C. X., Mourão, F. A. G., Massensini, A. R., Martins-Filho, O. A., Brito-Melo, G. E. A., 2014. Trypan blue exclusion assay by flow cytometry. Brazilian Journal of Medical and Biological Research, 47(4), 307-3015.

[24] Godoy, M. E. Rotelli, A., Pelzer, L., Tonn, C. E., 2000, Antiinflammatory Activity of Cinnamic Acid Esters, Molecules, 5, 547-548.

[25] Liao, J.-C., Deng, J.-S., Chiu, C.-S., Hou, W.-C., Huang, S.-S., Shie, P.-H., Huang, G.-J., 2012. Anti-Inflammatory Activities of Cinnamomum cassia Constituents In Vitro and In Vivo. Evidence-Based Complementary and Alternative Medicine: eCAM, 2012, 429320.

[26] Jacob, A., Wu, R., Zhou, M., Wang, P., 2007. Mechanism of the Anti-inflammatory Effect of Curcumin: PPAR-γ Activation, PPAR Research, Volume 2007, Article ID 89369, 5.