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Aloe vera Protective Effect on Lipopolysaccharide-Induced RAW 264.7 Inflamed Cells

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***Abstract*—During inflammation, the immune system releases proinflammatory mediators. Therefore, suppression of inflammatory mediators has been one of the most valuable strategies for treating inflammatory diseases. Research has shown that medicinal plants have anti-inflammatory properties. This study aimed to investigate the cytotoxicity and protective effect of *Aloe vera* gel extract (AVGE) on inflamed LPS-stimulated macrophage cells (RAW 264.7 ATCC TIB 71). Based on the cytotoxicity test, *Aloe vera* gel extract (AVGE) concentrations of 12.5 µg/mL and 75.0 µg/mL were chosen in this research as more than 90% cell viability was seen. Results showed that the level of critical inflammatory mediators observed in this study was significantly reduced by *Aloe vera* gel extract (AVGE) 75.0 µg/mL concentration and percentage inhibition over positive control, that is, PGE_2 (627.42 ± 27.77 pg/mL and 29.79 ± 3.11 pg/mL), TN α (265.7 ± 36.57 pg/mL and 54.24 ± 6.30 pg/mL), and IL-1β (191.33 ± 0.66 pg/mL and 56.23 ± 0.15 pg/mL). Microscopically, the cytokine production was reduced significantly post-treated with *Aloe vera* gel extract 75.0 micro g/mL concentration. These findings confirmed the protective effect of *Aloe vera* gel extract (AVGE) towards inflamed cells.**

Keywords—Aloe vera, anti-inflammation, inflammatory mediators, LPS, RAW 264.7

I. Introduction

Inflammation is an immune system response to any pathogens, mechanical damage, infection, toxin compounds, or chemical irritation in the tissue [1]. Inflammatory pathways aid the body's protection and it is a defense mechanism of immune cells against any damage that has occurred in the body [2]. Inflammation consists of an organized chain of responses involving mediators, namely prostaglandin in macrophage, tumor necrosis factor-α, and interleukin [3]. Prostaglandin functions to induce pain in the inflammatory response [4]. Tumor necrosis factor-α, a pro-inflammatory cytokine produced by macrophages and monocytes, aggregate the inflamed area to attract more immune cells and chemokines [5,6]. TNF-α and IL-1β cytokines, which cause fever during inflammation, are blocked in anti-inflammatory interventions [7]. IL-1β plays a role in both the acute and chronic inflammatory processes as a typical pro-inflammatory mediator that produces pleiotropic effects on various cells [8].

Suppression of inflammatory mediators has been one of the most valuable strategies for treating inflammatory diseases [9]. Research shows that medicinal plants have anti-inflammatory properties attributed to their phytochemical compounds [10,11]. Alkaloids, flavonoids, and saponins found in *Berberis vulgaris*, *Prunella vulgaris*, *Rhodiola Rosea*, *Gendarussa vulgaris*, and *Annona muricata* L. have great potential as anti-inflammatory agents [10-15].

Aloe vera is a juicy plant in the *Liliaceae* family and consists of more than 75 ingredients beneficial for health [16]. It has excellent potential in inhibiting inflammation due to its phytochemical compounds [17]. Studies have proven that *Aloe vera* rind and gel extracts contain large numbers of alkaloids and small amounts of saponins, flavonoids, phenols, and steroids [18,19]. Various natural plants, such as colored avocado seed extract, *Nelumbo* leaf extract, and *Glycyrrhiza glabra* L. (licorice) leaf extract, showed alkaloids and flavonoids, saponins and phenols compounds in these extracts were responsible for their anti-inflammatory properties [20-22]. Alkaloids decrease prostaglandin in inflammatory processes [23]. The anti-inflammatory mechanism of saponin works by inhibiting exudate formation and reducing vascular permeability [24]. There are two mechanisms in which flavonoids reduce inflammation that is by inhibiting arachidonic acid and the regulation of lysosomes; or by impeding endothelial enzymes to stop the proliferation and exudation of the inflammatory processes [25,26].

The RAW 264.7 cell line is an appropriate model of macrophage-like cells for analyzing anti-inflammatory compounds from plant extracts [27,28]. This myeloid cell line was derived from BALB/c mice and recommended by ATCC (American Type Culture Collection) suppliers to use anti-inflammation studies [29]. The Gram-negative bacteria consist of a component of the lipopolysaccharide that stimulates macrophages in their cell wall, causing them to release inflammatory mediators and trigger specific organized responses [30]. Lipopolysaccharide (LPS) is commonly used in models studying inflammation as it mimics the real inflammatory effects and signals [31].

This preliminary study aimed to investigates the cytotoxicity and protective effect of *Aloe vera* gel extract (AVGE) on lipopolysaccharide-induced inflamed murine RAW 264.7 (ATCC TIB-71) cells. Firstly, this study assessed the cytotoxicity of various *Aloe vera* gel extract concentrations. Afterward, the degree of primary inflammatory mediators and microscopic characteristics of

the cells were observed to determine their anti-inflammatory activities. We believe this study may provide helpful information on *Aloe vera* gel extract effectiveness towards inflammation as there is no previous research in investigating the cytotoxicity and protective effect of *Aloe vera* gel extract. As the result, the data presented is expected to add to the current body of knowledge about anti-inflammatory properties in medicinal plant and promote their application for therapeutic values.

II. Materials and Methods

A. Aloe Vera Gel Extracts Preparation

Fresh *Aloe vera* (L.), Burm. f. was obtained from Semplak village, Semplak district, Bogor regency, Indonesia. The maceration method was used to obtain *Aloe vera* gel extract (250 g). The 70% ethanol filtrate was filtered every 24 hours until a colorless filtrate was developed and evaporated to form a paste [32].

B. RAW 264.7 Cell Culture

The RAW 264.7 (ATCC TIB-71) macrophage cell was acquired from Aretha Utama's Biomolecular and Biomedical Research Center in Bandung, Indonesia. The RAW 264.7 cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 1% antibiotic, and anti-mycotic, and later incubated at 37ºC humidified atmosphere with 5% CO_2 until confluent. Centrifugation was performed at 1600 rpm for 5 minutes [33].

C. Cytotoxicity Test

The cytotoxicity test was performed to measure the viability of the cells through their metabolic activities. The analysis was made to decide the concentration for the next assessments. MTS (3-(4.5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay was used to determine the RAW 264.7 cell viability. Cells (5 x 103 cells per well) were measured using a hemocytometer after being planted in a 96-well plate and cultured for 24 hours at 37ºC with 5% CO_2. After 24 hours of incubation, the medium was altered with 180 µl new medium and addition of 20 µl (12.5; 25.0; 50.0; 100.0 µg/mL which served as the final concentration) of the sample on each well and then incubated for 24 hours on 37ºC with 5% CO_2. Each well received 20 µl of MTS after 24 hours of treatment. They were incubated for 3 hours at 37ºC with 5% CO_2. Assessment of the absorbance was conducted through a spectrophotometer at 490 nm. The cells which were not given treatment would be considered as the negative control [34].

D. BSA Total Protein

There is three characteristics of Bradford coomassie brilliant blue G-250 protein-binding dye, namely: neutral (green), cationic (red), and anionic (blue). The calculation is based on the binding results of coomassie brilliant blue G-250 dye to the essential amino acid residues at acidic pH and hydrophobic interactions in proteins. The color may change to blue as a result of this. Bovine serum albumin was diluted and then placed on the well-plate until the color change to blue occurred. The absorbance was assessed at 595 nm [35].

E. Pro-Inflammatory Initiation of RAW 264.7 Cell

A modified method was used to perform the pro-inflammatory initiation of the cells. The cells were plated in 6-well plates at a density of 1 x 106 cells per well, then incubated for 24 hours at 37ºC with 5% CO_2. After being incubated for 24 hours, we noticed a changed in the culture medium, and later supplemented it with 1600 µL growth medium and 200 µL of *Aloe vera* gel extract (AVGE) in various concentrations (12.5 µg/mL and 75.0 µg/mL) and followed by 1 to 2 hours of incubation before adding LPS. The cells were then incubated for 24 hours. The conditioned medium was centrifuged at 2000 x g for 10 minutes. The supernatant was stored for measuring the levels of PGE_2, TNF-α, and IL-1β [34,36].

F. Assessment of PGE_2, TNF-α, and IL-1β

The assessment of PGE_2, TNF-α, and IL-1β was conducted using a Mouse ELISA kit. The cells that had been activated with LPS acted as a positive control. Meanwhile, the cells that had not been treated were categorized in a negative control group. Next, the standard solution was inserted into each 100 µL of wells, wrapped, and incubated for 90 minutes at 37°C. After removing the solution, 100 µL/well Biotinylated Detection Ab was applied and incubated at 37°C for 1 hour. Next, the solution was rinsed away. The HRP Conjugate was then applied to each well and incubated for 30 minutes at 37°C. After a second wash, 90 µL of the substrate was supplemented and incubated at 37°C for 15 minutes. Finally, an assessment of the optical density using a spectrophotometer was performed at 450 nm [34,36].

G. Statistical Analysis

The tests were presented as mean ± standard deviations (Mean ± SD). Data collected from the triplicate measurements were analyzed using SPSS (version 20.0). Comparisons between groups were evaluated with one-way ANOVA, followed by the Tukey HSD post-hoc test. The results were considered significantly different at $p \leq 0.05$.

III. Results and Discussions

A. Cell Cytotoxicity Test

Initially, the cytotoxicity of *Aloe vera* gel extracts (AVGE) on RAW 264.7 macrophage cells were being studied. By comparing the mean value of treatments to the control, cell viability was calculated. Sample from control group and the other four AVGE concentrations groups of 12.5 µg/mL, 25.0 µg/mL, 50.0 µg/mL, and 100.0 µg/mL have cell viability's mean of 100.00 ± 2.45%; 130.75 ± 5.82%; 116.20 ± 5.38%; 104.02 ± 3.08% and 81.99 ± 2.06%, respectively "Table. I".

Viable cells were noticeable at *Aloe vera* gel extracts (AVGE) sample groups of 12.5 µg/mL, 25.0 µg/mL, and 50.0 µg/mL concentrations. The above was suitable for further analysis in this study. AVGE concentration of 100.0 µg/mL showed toxicity, in which the value was below 80% cell viability. The safe range of concentration was from 12.5 µg/mL and below 100.0 µg/mL. Thus, based on the BSA linear regression analysis, 75.0 µg/mL concentrations were accounted for the highest safe range of AVGE concentrations, as more than 90% cell viability was observed.

TABLE I. EFFECT OF AVGE AT VARIOUS CONCENTRATIONS ON CELL VIABILITY OF RAW 264.7 CELL LINES

Sample	Control	AVGE 12.5	AVGE 25.0	AVGE 50.0	AVGE 100.0
Sample 1	98.91	128.06	110.53	106.68	81.41
Sample 2	102.81	137.42	116.80	100.65	84.28
Sample 3	98.28	126.76	121.25	104.73	80.28
Mean ± SD Cell Viability (%)	100.00 ± 2.45	130.75 ± 5.82	116.20 ± 5.38	104.02 ± 3.08	81.99 ± 2.06

B. Inflammatory Mediator PGE_2, Cytokines TNF-α, and IL-1β Levels

The results of ELISA tested on PGE_2 level showed that *Aloe vera* gel extracts (AVGE) 75.0 µg/mL had a significant difference ($p \leq 0.05$) (627.42 ± 27.77 pg/mL) compared to the positive control (893.57 ± 49.58 pg/mL) and negative control (404.68 ± 27.50 pg/mL) on Tukey HSD post hoc test. In terms of inhibition percentage, AVGE 75.0 µg/mL (29.79 ± 3.11 pg/mL) was more effective ($p \leq 0.05$) compared to AVGE 12.5 µg/mL (8.48 ± 2.52 pg/mL). The results confirmed that AVGE 75.0 µg/mL was effective in reducing the PGE_2 level. Therefore, in terms of inhibition percentage, it aided in suppressing the inflammation process in LPS-inducted RAW 264.7 up to halve the control negative group "Table. II".

This study revealed that *Aloe vera* gel extracts (AVGE) 75.0 µg/mL significantly ($p \leq 0.05$) lowered the TNF-α level from concentration 580.83 ± 30.21 pg/mL to 265.79 ± 36.57 pg/mL. AVGE 75.0 µg/mL displayed the percentage of inhibition over positive control of 54.24 ± 6.30 pg/mL, approaching the negative control of 61.76 ± 0.47pg/mL. The result indicated that AVGE 75.0 µg/mL was effective in reducing the inflammation process in LPS-induced RAW 264.7. The proportion of AVGE 75.0 µg/mL was over doubled the 12.5 µg/mL class. In current study, IL-1β level substantially decreased ($p \leq 0.05$) with AVGE 75.0 µg/mL at 191.33 ± 0.66 pg/mL concentration, compared to AVGE 12.5 µg/mL (373.71 ± 0.51 pg/mL) and positive control (437.16 ± 26.48 pg/mL).

The percentage of IL-1β inhibition over positive control of AVGE 75.0 µg/mL (56.23 ± 0.15 pg/mL) was significantly ($p \leq 0.05$) increased compared to 12.5 µg/mL concentration (14.51 ± 0.12 pg/mL), approaching the negative control value of (68.24 ± 2.00 pg/mL). The percentage of inhibition of AVGE 75.0 µg/mL was above triplet of those in AVGE 12.5 µg/mL. The outcome suggested that AVGE 75.0 µg/mL was effective in reducing IL-1β levels; hence it can control the inflammation process in LPS-induced RAW 264.7 macrophage cells. In LPS-induced macrophage cell, the AVGE 75.0 µg/mL sample group inhibited the synthesis of PGE_2, TNF-α, and IL-1β in a concentration-dependent manner.

TABLE II. EFFECT OF AVGE AT VARIOUS CONCENTRATION ON PGE_2, TNF-A, AND IL-1B LEVELS IN RAW 264.7 CELL LINE

Mediator		Experimental Groups			
		Control Negative	*AVGE 75 .0 µg/mL*	*AVGE 12.5 µg/mL*	*Control Positive*
PGE_2 (pg/mL)	Sample 1	373.29	595.48	840.84	933.65
	Sample 2	424.52	645.90	795.90	908.94
	Sample 3	416.23	640.87	816.74	838.13
	Mean ± SD (µg/mL)	404.68 ± 27.50^{d}	627.42 ± 27.77^{b}	817.8 ± 22.49^{cd}	893.57 ± 49.58^{a}
TNF-α (pg/mL)	Sample 1	236.75	286.63	436.06	613.75
	Sample 2	245.50	223.56	448.81	554.38
	Sample 3	240.00	287.19	475.50	574.38
	Mean ± SD (µg/mL)	240.75 ± 4.42^{b}	265.7 ± 36.57^{d}	453.46 ± 20.13^{ac}	580.83 ± 30.21^{a}
IL-1β (pg/mL)	Sample 1	132.88	192.08	373.59	451.86
	Sample 2	134.76	190.82	373.27	406.59
	Sample 3	148.88	191.09	374.27	453.02
	Mean ± SD (µg/mL)	138.84 ± 8.75^{b}	191.33 ± 0.66^{d}	373.71 ± 0.51^{c}	437.16 ± 26.48^{a}

$^{a,b,c,d.}$ The different superscript showed significant difference ($p \leq 0.05$) on Tukey HSD post hoc test. AVGE: Aloe vera gel extract. Data presented in the form of Mean ± SD.

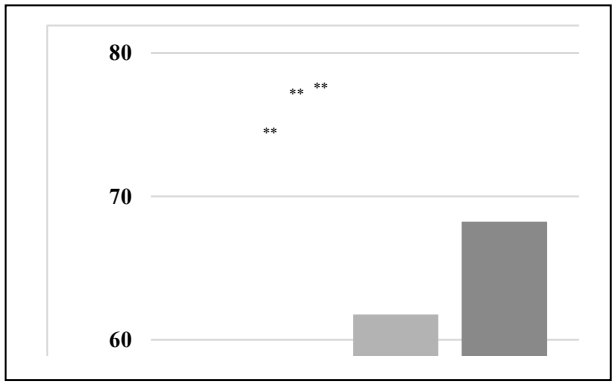

Fig. 1. Inhibition percentage (Mean ± SD) of inflammatory mediators over positive control in four experimental groups. The AVGE 75.0 µg/mL group (**) were significantly different ($p \leq 0.05$) compared to control negative.

The protective impact of *Aloe vera* gel extracts on PGE_2, TNF-α, and IL-1β levels in LPS-induced RAW 264.7 cells were investigated using an ELISA test kit. AVGE 75.0 µg/mL sample group demonstrated a strong protective power on pro-inflammatory cytokines compared to positive control. In addition, the inhibition percentage of the *Aloe vera* gel extracts 75.0 µg/mL sample group on TNF-α and IL-1β cytokines were comparable to the negative control "Fig. 1".

A current study found that the protective effects of *Aloe vera* gel extracts were obvious in 75.0 µg/mL concentration, in which it significantly leveled down the numbers of chemokines receptors (PGE_2) and pro-inflammatory cytokines (TNF-α, and IL-1β). LPS-induced inflammation is mimicking the microbial products, the primary inflammatory stimuli. Inflammatory stimuli activated intracellular signaling pathways by increasing the fabrication of inflammatory mediators [37,38]. Prostaglandin in macrophages, one of the chemokines receptors, is a derivative of arachidonic acid that plays a key role as an immunomodulatory lipid mediator. PGE_2 was formed by stimulating two cyclooxygenases (COX) isomorphic enzymes, that is, the main expression COX-1 and generated COX-2 [39]. PGE_2 reduces inflammation and immune activities by preventing the initiation of macrophage, leukocyte, ROS, cytokines, and chemokines alteration [40].

Aloe vera contains constituents that are possibly acted as anti-inflammatory, antiseptic, and analgesic [41]. Phytochemical screening of *Aloe vera* gel extracts (AVGE) revealed that alkaloids, flavonoids, phenols, tannins, saponins, steroids and terpenoids, compounds were detected. Alkaloids phytocompounds extracted from various plants have been used as medicinal substances [42-44]. Alkaloids can be categorized based on biosynthetic constituents and chemical structures, such as indole, quinazoline, isoquinoline, and other alkaloid types [45-48]. Alkaloids and flavonoids have been reported to attribute to many functional characteristic properties, including anti-inflammatory, antifungal, antimicrobial, anti-allergic, anti-aging, antioxidant, vascular, and cytotoxic antitumor activities [49,50]. Saponins have been considered a bioactive antibacterial agent [51,52]. *Phenylalanine* and *tryptophane,* amino acid components in *Aloe vera*, were reported for their anti-inflammatory activity [53]. In addition, salicylic acid properties inside *Aloe vera* were proven to prevent the biosynthesis of prostaglandins from arachidonic acid. Thus, AVGE can reduce inflammation and reversed the reactions of immune systems by blocking prostaglandins synthesis and modulate the production of lymphocyte and macrophage derivatives mediators (lymphokines), including interleukins and interferons. In addition, their vital components can act as a wound healing stimulator by promoting antibody production [54,55]. This study explains how AVGE used in this study can reduce vasodilation and the vascular effect of histamine, serotonin, bradykinin, and other mediators' inflammation. These findings indicated the association between phytochemical components in AVGE and its protective effect on RAW 264.7 macrophage cells. The present study may provide a basis for further studies on the efficacy of AVGE in treating oral mucosa lesions that provoke an inflammatory response, namely recurrent aphthous stomatitis, oral submucous fibrosis, oral lichen planus, radiation-induced oral mucositis, gingivitis, and periodontitis. Nonetheless, the anti-inflammatory effect of AVGE requires advanced laboratory and clinical experiments to confirm their immune regulatory effect, molecular mechanisms, and signaling pathways by adding additional parameters.

C. Morphologic features of RAW 264.7 Cells

The morphologic characteristics of pre-and post-treated RAW 264.7 cells were examined using an Inverted Phase Contrast Microscope with 40x magnification. Pre-treated cells had a normal ultrastructure that was smooth and clean on the cell surface "Fig. 2A". However, LPS-induced inflamed cells presented with cytokine production on the outer part of the cell "Fig. 2B". Post-treated cells with *Aloe vera* gel extracts 12.5 µg/mL showed a slight difference in LPS-induced inflamed cells "Fig. 2C". Microscopically, cytokine production was reduced significantly post-treatment with AVGE 75.0 µg/mL concentration "Fig. 2D". These histologic features confirmed *Aloe vera* gel extracts' ability to reduce the inflammation triggered by LPS cellularly.

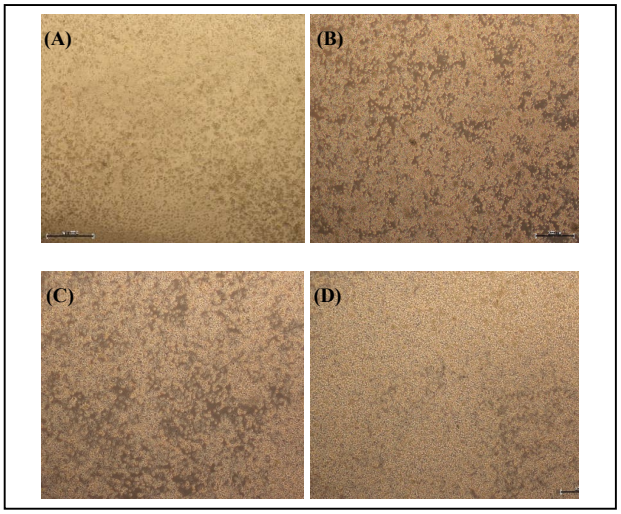

Fig. 2. Study of Cell morphology using inverted phase contrast microscope (40X) (A). Normal cells; (B). LPS-induced RAW 264.7 cells; (C). LPS-induced RAW 264.7 cells + AVGE 12.5 µg/mL; (D). LPS-induced RAW 264.7 cells + AVGE 75.0 µg/mL.

IV. Conclusion

Cytotoxicity test indicated the safest range of AVGE concentration from 12.5 µg/mL to 75.0 µg/mL as more than 90% cell viability was observed. Reduction in the vital inflammatory mediators, PGE_2, TNF-α, and IL-1β, confirmed the protective value of AVGE in the current study. The effectiveness of AVGE in inhibiting the inflammation tripled in percentage, as the amounts of concentrations increased from 12.5 µg/mL to 75.0 µg/mL. Compared to the positive control group, the AVGE 75.0 µg/mL concentration group significantly decreased cytokine values ($p \leq 0.05$). In addition, the microscopic morphology of pre-and post-treated RAW 264.7 cells suggested that cytokine production declined after treatment with concentration and percentage inhibition over positive control of AVGE 75.0 µg/mL on PGE_2 (627.42 ± 27.77 pg/mL and 29.79 ± 3.11 pg/mL), TNF-α (265.7 ± 36.57 pg/mL and 54.24 ± 6.30 pg/mL), and IL-1β (191.33 ± 0.66 pg/mL and 56.23 ± 0.15 pg/mL). These findings provide evidence that AVGE is a potent source of anti-inflammation, and it justifies its use as a medicinal plant.

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References

[1] N. Yahfoufi, N. Alsadi, M. Jambi, and C. Matar, "The Immunomodulatory and Anti-Inflammatory Role of Polyphenols," Nutrients. J, Sept 2018, vol. 10.

[2] S. Paul, D. Modak, S. Chattaraj, D. Nandi, A. Sarkar, J. Roy, T. K. Chaudhuri, and S. Bhattacharjee, "*Aloe vera* Gel Homogenate Shows Anti-Inflammatory Activity Through Lysosomal Membrane Stabilization and Downregulation of TNF-α and Cox-2 Gene Expressions in Inflammatory Arthritic Animals," Future. J. Pharm. Sci, 2021, vol. 7, no. 12.

[3] C. C. Yong, Y. Yoon, H. S. Yoo, and S. Oh, "Effect of *Lactobacillus* Fermentation on the Anti-Inflammatory of Turmeric," J. Microbiol. Biotechnol, 2019, vol. 29, no. 10, pp. 1561-1569.

[4] A. Kawabata, "Prostaglandin E_2 and Pain- An update," Biol. Pharm. Bull, 2011, vol. 34, no. 8, pp. 1170-1173.

[5] S. Kany, J. T. Vollrath, and B. Relja, "Cytokines in Inflammatory Disease", Int. J. Mol. Sci, 2019, vol. 20, no.6008.

[6] A. Saha, A. Biswas, S. Srivastav, M. Mukherjee, P. K. Das, and A. Ukil, "Prostaglandin E2 Negatively Regulates the Production of Inflammatory Cytokines/Chemokines and IL-17 in Visceral Leishmaniasis," J. Immunol, 2021, vol. 193, pp. 2330-2339.

[7] T. Poongodi, T. H. Nazeema, and B. Ranjini, "Enhanced Anti Inflammatory Effect of Polyherbal Formulation (MKA) Comprising of Three Selected Plants in Lipopolysaccharide (LPS)–Induced RAW 264.7 Macrophage Cell Line," Indian J Pharm Sci 2020, vol. 82, no. 4, pp. 692-697.

[8] K. Ren and R. Torres, "Role of Interleukin-1β During Pain and Inflammation," Brain. Res. Rev, April 2009, vol. 60, no. 1, pp. 57-64.

[9] N. M. D. Sandhiutami, M. Moordiani, D. R. Laksmitawati, N. Fauziah, M. Maesaroh, and W. Widowati, "*In Vitro* Assessment of Anti-Inflammatory Activities of *Coumarin* and *Indonesian Cassia* in Extract in RAW264.7 Murine Macrophage Cell Line," Iran. J. Basic. Med. Sci, Jan 2017, vol. 20, no. 1.

[10] R. N. Kalmarzi, S. N. Naleini, D. A. Larky, I. Peluso, L. Jouybari, A. Rafi, F. Ghorat, N. Heidari, F. Sharifian, J. Mardaneh, P. Aiello, S. Helbi, and W. Kooti, "Anti-Inflammatory and Immunomodulatory Effects of Barberry (*Berberis vulgaris*) and its Main Compounds," Oxidative. Med. & Cellular. Longevity, 2019.

[11] F. Shahzad, D. Anderson, and M. Najafzadeh, "The Antiviral, Anti-Inflammatory Effects of Natural Medicinal Herbs and Mushrooms and SARS-CoV-2 Infection," Nutrients, 2020, vol. 12, no. 2573.

[12] W. L. Pu, M. Y. Zhang, R. Y. Bai, L. K. Sun, W. H. Li, Y. L. Yu, Y. Zhang, L. Song, Z. X. Wang, Y. Fei. Peng, H. Shi, K. Zhou, and T. X. Li, "Anti-Inflammatory Effects of *Rhodiola Rosea* L.: A Review," Biomedicine. & Pharmacotherapy. J, 2020.

[13] M. I. Alkhalaf, W. S. Alansari, E. A. Ibrahim, and M. E. A. Elhalwagy "Anti-Oxidant, Anti-Inflammatory and Anti-Cancer activities of avocado (*Persea americana*) Fruit and Seed Extract," J. King. Saud. University. Sci. 31, 2019, pp.1358-1362.

[14] D. R. Laksmitawati, A. J. Prasanti, N. Larasinta, G. A. Syauta, R. Hilda, H. U. Ramadaniati, A. Widyastuti, N. Karami, M. Afni, D. D. Rihibiha, H. S. W. Kusuma, and W.Widowati, "Anti-Inflammatory Potential of Gandarusa (*Gendarussa vulgaris* Nees) and Soursop (*Annona muricata L*) Extracts in LPS Stimulated-Macrophage Cell (RAW 264.7)," J. Natural. Remedies, Apr 2016, vol. 16, no. 2.

[15] R. Yang, B. C. Yuan, Y. S. Ma, S. Zhou, and Y. Liu, "The Anti-Inflammatory Activity of *Licorice*, a widely used Chinese herb," Pharmaceutical. Bio, 2017, vol. 55, no. 1, pp. 5-18.

[16] N. Y. A. E. Irma, S. Phillippe, A. Abdoukarim, Y. A. K. Alassane, A. C. Pascal, M. Daouda, and S. K. C. Dominique, "Evaluation of *Aloe vera* Leaf Gel as a Natural Flocculant: Phytochemical Screening and Turbidity Removal Trials of Water by Coagulation Flocculation," Res. J. Recent. Sci, Jan. 2016, vol. 5, no. 1, pp. 9-15.

[17] M. Sanchez, E. G. Burgos, I. Iglesias, and M. P. G. Serranillos, "Pharmacological Update Properties of *Aloe vera* and its Major Active Constituents," Molecules, 2020, vol. 25, no. 1324.

[18] P. Muthukumaran, R. Divya, E. Indhumathi, and C. Keerthika, "Total phenolic and flavonoid content of membrane processed *Aloe vera* extract: a comparative study," Int. Food. Research. J, 2018, vol. 25, no. 4, pp. 1450-1456.

[19] D. Dharajiya, N. Pagi, H. Jasani, and P. Patel, "Antimicrobial Activity and Phytochemical Screening of *Aloe vera* (*Aloe* barbadensis Miller)" Int. J. Curr. Microbiol. App. Sci, 2017, vol. 6, no. 3, pp. 2152-2162.

[20] E. Park, G. D. Kim, M. S. Go, D. Kwon, I. K. Jung, J. H. Auh, and J. H. Lim, "Anti-Inflammatory Effect of *Nulembo* Leaf Extracts and Identification of Their Metabolites," Nut. Research. Practice, 2017, vol. 11, no. 4, pp. 265-274.

[21] L. Frattaruolo, G. Carullo, M. Brindisi, S. Mazzotta, L. Bellissimo, V. Rago, R. Curcio, V. Dolce, F. Aiello, and A. R. Cappello. "Antioxidant and Anti-Inflammatory Activities of Flavanones from *Glycyrrhiza glabra* L. (licorice) Leaf Phytocomplexes: Identification of Licoflavanone as a Modulator of NF-KB/ MAPK Pathway," Antioxidants, 2019, vol. 8, no. 186.

[22] E. G. Martinez, I. Andujar, A. Y. Carmen, J. Prohens, and N. M. Navarrete, "Antioxidant and Anti-Inflammatory Activities of Freeze-Dried Grapefruit Phenolics as Affected by Gum Arabic and Bamboo Fibre Addition and Microwave Pretreatment," J. Sci. Food. & Agriculture, 2017.

[23] A. L. Souto, J. F. Tavares, M. S. Silva, M. F. F. M. Diniz, P. F. A. Filho, and J. M. B. Filho, "Anti-Inflammatory Activity of Alkaloids: An Update from 2000-2010," Molecules, 2011, vol. 16, pp. 8515-8534.

[24] P. Dey, S. Dutta, A. Chowdhury, A. P. Das, and T. K. Chaudhuri, "Variation in Phytochemical Composition Reveals Distinct Divergence of *Aloe vera* (L.) Burm. F. From Other *Aloe* Species: Rationale Behind Selective Preference of *Aloe vera* in Nutritional and Therapeutic Use," J. Evidence. Based. Complementary. & Alternative. Med, 2017, vol. 22, no. 4, pp. 624-631.

[25] M. M. Namadina, U. Sunusi, M. H. Abdulrazak, U. I. Hamza, F. M. Musa, F. I. Ismail, A. U. Bashir, F. M. Yau, H. A. Barde, M. Kaila, and T. Y. Makin, "Phytochemical Screening, Antifungal and Antioxidant Activities of *Aloe* Barbadensis Miller (*Aloe vera*) Leaves," DUJOPAS, December 2020, vol. 6, no. 4, pp. 15-17.

[26] S. P Azarfam, H. Nadian, A. Moezzi, and A. Gholami, "Effect of Silicon on Phytochemical and Medicinal Properties of *Aloe vera* Under Cold Stress," Ecology. Environmental. Research, 2020, vol. 18, no. 1, pp. 561-575.

[27] J. Xu, Y. Zhao, and H. A. Aisa, "Anti-Inflammatory Effect of Pomegranate Flower in Lipopolysaccharide (LPS)-Stimulated RAW264.7 Macrophages," Pharmaceutical. Biology, 2017, vol. 55, no. 1, pp. 2095-2101.

[28] D. Rusmana, M. Elisabeth, W. Widowati, N. Fauziah, and M. Maesaroh, "Inhibition of Inflammatory Agent Production by Ethanol Extract and Eugenol of *Syzygium aromaticum* (L.) Flower Bud (Clove) in LPS-Stimulated RAW 264.7 Cells," Res. J. Med. Plant, 2015, vol. 9, no. 6, pp. 264-274.

[29] B. Taciak, M. Bialasek, A. Braniewska, Z. Sas, P. Sawicka, L. Kiraga, T. Rygiel, and M. Krol, "Evaluation of Phenotypic and Functional Stability of RAW 264.7 Cell Line Through Serial Passages," PLoS ONE, 2018, vol. 13, no. 6.

[30] L. A. Abdulkhaleq, M. A. Assi, R. Abdullah, M. Z. Saad, Y. H. Taufiq-Yap, and M. N. M. Hezmee, "The Crucial roles of Inflammatory mediators in inflammation: A Review," Veterinary World, 2018, vol. 11, no. 5, pp. 627-635.

[31] J. Y. Lee, H. Song, O. Dash, M. Park, N. E. Shin, M. W. Mclane, J. Lei, J. Y. Hwang, and I. Burd, "Administration of Melatonin for Prevention of Preterm Birth and Fetal Brain Injury Associated with Premature Birth in a Mouse Model," AJRI, 2019, vol. 82, no. 3.

[32] S. B. Mahadi, A. S. Handayani, W. Widowati, Wilsen, Y.Dewani, E. Fachrial, and I. N. E. Lister, "Antioxidant and Anti-Tyrosinase Activities of *Aloe vera* Rind and Gel Extracts," Global. Medical. Health. Communication, December 2019, vol. 7, no. 3.

[33] A. Novilla, D. S. Djamhuri, B. Nurhayati, D.D. Rihibiha, E. Afifah, and W. Widowati, "Anti-Inflammatory Properties of Oolong Tea (*Camellia sinensis*) Ethanol Extract and Epigallocatechin Gallate in LPS-Induced RAW 264.7 cells," Asian. Pac. J. Trop. Biomed, 2017, vol. 7, no. 11, pp. 1005-1009.

[34] D. R. Laksmitawati, A. Widyastuti, N. Karami, E. Afifah, D.D. Rihibiha, H. Nufus, and W. Widowati, "Anti-Inflammatory Effects of *Anredera Cordifolia* and *Piper Crocatum* Extracts on Lipopolysaccharide-Stimulated Macrophage Cell Line," Bangladesh. J. Pharmacol, 2017, vol. 12, pp. 35-40.

[35] J. M. Walker, The Protein Protocols Handbook-second edition, Humana. Press. Totowa, New. Jersey, 2002 pp. 15.

[36] W. Widowati, L. Darsono, J. Suherman, N. Fauziah, M. Maesaroh, and P. P. Erawijantari, "Anti-inflammatory Effect of Mangosteen (*Garcinia mangostana L.*) Peel Extract and its Compounds in LPS-induced RAW 264.7 Cells," Natural. Product. Sci, 2016, vol. 22, no. 3.

[37] I. M. Jou, C. F. Lin, K. J. Tsai, and S. J. Wei, "Macrophage-Mediated Inflammatory Disorders," Hindawi, 2013.

[38] E. Ricciotti and G. A. Fitzgerald, "Prostaglandins and Inflammation," Arterioscler Thromb Vasc Biol, 2011, vol. 31, no. 5, pp. 986-1000.

[39] J. L. Wallace, A. Bak, W. Mcknight, S. Asfaha, K. A. Sharkey, and W. K. Macnaughton, "Cyclooxygenase 1 Contributes to Inflammatory Responses in Rats and Mice: Implications for Gastrointestinal Toxicity," Gastroenterology, 1998, vol. 115, pp. 101-109.

[40] P. Ruytinx, P. Proost, J. V. Damme, and S. Struyf, "Chemokines-Induced Macrophage Polarization in Inflammatory Conditions," Front. Immunol, 2018, vol. 9, no. 1930.

[41] A. Kumar, A. Mahajan, and Z. Begum, "Phytochemical Screening and *In vitro* Study of Free Radical Scavenging Activity of Flavonoids of *Aloe vera*," Research. J. Pharm. And. Tech, 2020, vol. 13, no.2.

[42] A. Shah and S. A. Nik, "The Role of Phytochemicals in the Inflammatory Phase of Wound Healing," Int. J. Mol. Sci, 2017, vol. 18, no.1068.

[43] L. Zhang, C. Virgous, and H.Si, "Synergistic Anti-Inflammatory Effects and Mechanisms of Combined Phytochemicals," J. Nut. Bio, 2019 vol. 69, pp. 19-30.

[44] A. Roy, "A Review on the Alkaloids an Important Therapeutic Compound from Plants," Int. J. Pub. Bio, 2017, vol. 3, no. 2, pp. 1-9.

[45] N. Bribi, "Pharmacological activity of Alkaloids: A Review," Asian. J. Botany, 2018. vol. 1.

[46] A. L. Souto, J. F. Tavarez, M. S. Silva, M. F. F. M. Diniz, P. F. A. Filho, and J. M. B. Filho, "Anti-inflammatory Activity of Alkaloids: An Update from 2000 to 2010," Molecules, 2011, vol. 16, pp. 8515-8534.

[47] J. M. B. Filho, M. R. Piuvezam, M. D. Moura, M. S. Silva, K. V. B. Lima, E. V. L. Cunha, I. M. Fechine, and O. S. Takemura, "Anti-inflammatory Activity of Alkaloids: A Twenty-Century Review," Brazilian J. Pharm, 2006, vol. 16, no. 1, pp. 109-139.

[48] N. Bribi, F. Algieri, A. R. Nogales, J. G. Mesa, T. Vezza, F. Maiza, M. P. Utrilla, M. E. R. Cabezas, and J. Galvez, "Antinociceptive and Anti-Inflammatory Effects of Total Alkaloid Extract from *Fumaria capreolata*," Hindawi, 2015.

[49] M. Sonam, R. P. Singh, and S. Pooja, "Phytochemical Screening and TLC Profiling of Various Extracts of *Reinwardtia indica*," IJPPR, 2017, vol. 9, no. 4.

[50] F. F. Neto, C. R. F. Volobuff, M. M. C. Pederiva, Z. V. Pereira, M. H. Sarragiotto, C. A. L. Cardoso, C. A. L. Kasuya, and A. S. N. Formagio, "Anti-Inflammatory action of an alkaloid, fraction and extract from *Alchornea glandulosa* in mice," J. Ethnopharmacology, 2019, vol. 231, pp. 66-72.

[51] N. Z. Malik, M. Riaz, Q. Q. Noshad, N. Rashid, Q. U. Ain, and A. Hussain, "Morphological, Phytochemical and Antifungal Analysis of *Aloe vera* L. Leaf Extracts," Asian. J. Agri. & Biol, 2017, vol. 5, no. 4, pp. 177-187.

[52] D. Tungmunnithum, A. Thongboonyou, A. Pholboon, and A. Yangsabai, "Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview," Medicines. J, 2018, vol. 5, no. 93.

[53] S. Sadaf, M. S. Jamali, M. H. Jan, K. Mehmood, Z. Mehmood, M. Anwar, M. Israr, and A. Akbar, "Comprehension Study on Phytochemicals and Antibacterial Activity of *Aloe* vera Barbadensis Found in Different Regions of Balochistan," J. Sci. Techl, 2017, vol. 6, pp. 186-189.

[54] Y. Kartika, E. L. Nyoman, F. Edy, and N.G. Chrismis (2021), "Potential Extract Ethanol of *Aloe vera* Gel as a Rejuvenation Agent,' Asian. J. Pharmaceutical Research & Development, 2021, vol. 9, no. 1, pp. 46-50.

[55] S. A. Hashemi, S. A. Madani, and S. Abediankenari, "The Review on Properties of *Aloe vera* in Healing of Cutaneous Wounds," Biomed. Research. International, 2015.