Extracted *Passiflora edulis* Pulp to Reduce Inflammation in LPS-activated Macrophage Cell Line: RAW 264.7

Lily Widjaja
Master of Dental Sciences Program
Faculty of Dentistry
Universitas Prima Indonesia
Medan, Indonesia
ORCID ID. 0000-0002-1017-0789

Cindy Denhara Wijaya
Dept. of Prosthodontics
Faculty of Dentistry
Universitas Prima Indonesia
Medan, Indonesia
ORCID ID. 0000-0001-5208-067X

Mellisa Sim
Dept. of Dental Sciences
Faculty of Dentistry
Universitas Prima Indonesia
Medan, Indonesia
ORCID ID. 0000-0002-1265-4330

Wahyu Widowati
Dept. of Biomolecular
Faculty of Medicine
Universitas Kristen Maranatha
Bandung, Indonesia
ORCID ID. 0000-0002-5401-7794

Lina Hadi
Dept. of Orthodontics
Faculty of Dentistry
Universitas Prima Indonesia
Medan, Indonesia
ORCID ID. 0000-0002-3432-798X

Florenly
Dept. of Dental Sciences
Faculty of Dentistry
Universitas Prima Indonesia
ORCID ID. 0000-0002-6326-3751
ly@unprimdn.ac.id

Abstract—NSAID is known as the gold standard in treating inflammation, despite its reported side effects. Natural products are being explored to find an alternative to treat inflammation. This preliminary study can provide new insight into using *P. edulis* pulp to treat inflammation in the oral mucosa. Plants from which *P. edulis* pulp is extracted are economical and easily found in Indonesia. For this reason, this study was designed to analyze the anti-inflammatory performances of *P. edulis* pulp extract (PEPE) in a pro-inflammatory murine macrophage cell line - RAW 264.7. First, a cell viability test was performed to evaluate the safest range of PEPE concentration, using MTS Cell Proliferation Assay Kit. Next, the RAW 264.7 macrophage cell line was activated with lipopolysaccharide (LPS) to mimic the inflammatory situation in this study. The level of Interleukin, Tumor Necrosis Factor and Prostaglandin were then examined to get the data of inflammatory mediator cytokines. Data obtained from the experiments indicated a significant difference (p<0.05) between negative control, intervention (12,5 and 75 µg/mL) groups, and positive control groups. The inhibition capacity of PEPE 75 µg/mL over positive control was almost similar for both Interleukin and Tumor Necrosis Factor (more than 50%) and around 40% for PGE-2. The histological properties of the four groups showed a meaningful reduction in macrophage level in the groups treated with *P. edulis* pulp extracts. This study concluded that *Passiflora edulis* inhibits inflammation by decreasing Interleukin, Tumor Necrosis Factor, and Prostaglandin levels. In addition, a higher concentration of *Passiflora edulis* enhanced the potential to inhibit inflammation activities.

Keywords—Passiflora edulis, Anti-inflammatory, IL-1β, TNF-α, PGE-2, Lipopolysaccharide, RAW-264.7 cells

I. INTRODUCTION

Body response to various injuries through inflammation, a physiologic reaction that causes direct or indirect tissue damage [1]. Cytokines and chemokines are initiated and transported to the acute inflammation site, marked by the presence of macrophages and neutrophils [2]. These macrophages induce the release of (IL)-1β Interleukin, (TNF)-α Tumor Necrosis Factor cytokines, and Prostaglandin (PGE-2) during the inflammatory process [3]. IL-1β is an immunomodulator, which acted as a mediator in the immune-inflammatory response to T cell and B cell [4]. In addition, macrophages produced Tumor Necrosis Factor through ligand binding to TNF receptor (TNFR1), in which NF-κB or MAPK are triggered and started inflammatory process [5]. Meanwhile, the physiological activity of PGE-2 is facilitated by EP-receptors [6].

NSAID is used as the gold standard medication to treat inflammation. However, it can cause several side effects: allergy, gastrointestinal bleeding, and heart failure exacerbation [7]. Herbal therapy is known as an alternative anti-inflammation agent. Ghalib (2020), in his study, stated that Curcumin decreases the production of inflammatory cytokines [8]. Andriyono et al. (2019) discovered Kaempferia galanga L. and reports that it slows down inflammatory mediators [9]. Wilvia (2020) found that Pachyrizus erosus effectively reduced the amount of IL-1β, TNF-α, and PGE-2 in RAW 264.7 cells induced by LPS [10]. LPS, bacterial endotoxin, induces an initial immune response by regulating inflammatory mediators such as interleukin and tumor necrosis factor [11].

Passion fruit (*Passiflora edulis*) is a native Brazilian plant that is widely found in Indonesia. The fruit can be safely consumed and functions as an anti-bacterial, anti-inflammatory, anti-hypertensive, and sedative [12]. Anabel (2020) found that the diameter of inhibition of purple passion fruit peel (30% - 100%) against Staphylococcus aureus ranges from 12.8±0.27 to 16.7±1.30 mm [13]. In his study, Fachril et al. (2018) revealed that Yellow Passion fruit was effective as an anti-inflammatory, as it helps lower monocyte levels [14]. In addition, Ashly (2018) explained that *Passiflora Edulis* leaves decreases the pro-inflammatory TNF-α and IL-1β cytokines in a rat's colon [15].

The study by Zeraik et al. (2011) revealed that *P. edulis* consists of phenols and flavonoid compounds and can inhibit the inflammatory process [16]. Phenolic acid is known for its ability to exhibit numerous pharmacological properties, including anti-inflammatory, anti-oxidative, and anti-tumor capacities [17,18]. Flavonoids are secondary metabolites found in plants that have an essential role in plant physiology and hold physical benefits through being an anti-oxidant, anti-inflammatory, anti-cancer, antibacterial, and antiviral [19].

The aim of this study was to analyze the anti-inflammatory performance of *P. edulis* pulp extract in LPS-generated pro-inflammatory cell lines (RAW 264.7). *P. edulis* was chosen as it contains lots of vitamins and

2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce) | 978-1-6654-4181-0/21/$31.00 ©2021 IEEE | DOI: 10.1109/InHeNce52833.2021.9537266

Authorized licensed use limited to: UNIVERSITY OF NOTTINGHAM. Downloaded on September 20,2021 at 09:28:27 UTC from IEEE Xplore. Restrictions apply.

beneficial components, is economical, and is easily accessible in Indonesia. We hope the result of this study can provide preliminary data to support further research in reinforcing the usage of *P. edulis* as one of the alternatives for NSAID, especially in treating inflammation cases related to dentistry.

II. Material & Methods

A. Location

P. edulis used in this research were obtained from Percut Sei Tuan Sub-district, Sampali Village, Deli Serdang, Medan. The research was carried out in Bandung, Aretha Medika Utama Biomolecular and Biomedical Research Center, Indonesia.

B. Materials

The consumable materials in this study were *P. edulis* pulp, ethanol 70%, RAW 264.7 Cell Line, LPS, Dulbecco's Modified Eagle Medium supplemented with 10 % fetal bovine serum and 1% ABAM, PBS, MTS Cell Proliferation Assay Kit, Bovine Serum Albumin (BSA) standard, Quick Start Dye Reagent 1X, Mouse IL-1β ELISA kit, Mouse TNF-α ELISA kit, Mouse PGE-2 ELISA kit.

C. Methods

- *P. edulis* Pulp Extraction

P. edulis pulp was extracted with the maceration method using ethanol 70%. The filtrate was collected every 24 hours until colorless substances appeared and then concentrated with a vacuum rotary evaporator to get a consistent paste [12].

- RAW 264.7 Cell Culture

Dulbecco's Modified Eagle Medium was used to culture RAW 264.7 cells and 10 % fetal bovine serum and 1% are supplemented ABAM and then cultivated at 37 °C and 5% CO2 until the cells were confluent. The cells then washed using PBS and lastly centrifuged at 1600 rpm speed for 5 minutes, then cultivated at 37 °C and 5% CO2 [20].

- Cell Viability Analysis

The cell viability was examined using MTS Cell Proliferation Assay Kit. The cell was cultivated at 37 °C and 5% CO2 for 24 hours; they were placed with a density of 5 × 103 cells on each well. The medium was later discarded and supplemented with a 180 µL new medium. A 20 µL of various *P. edulis* pulp extract concentrations (12.5, 25, 50, 100 µg/mL) were placed into each well-plate then cultivated for 24 hours at 37°C, 5% CO2. Subsequently, 20 µL MTS was added to each well-plate. The well-plate was cultivated for 3 hours at 37°C, 5% CO2. A spectrophotometer was used to measure the absorbance at 490 nm [21].

- Proinflammatory Cytokine Analysis

Total protein was measured by placing 20 µl Bovine Serum Albumin (BSA) standard and each sample solution into well-plate. Then, 200 µL Quick Start Dye Reagen 1X was added to each well-plate and was reconstituted until thoroughly mixed. Absorbance was measured utilizing a spectrophotometer at 595 nm after 5 min of incubation at room temperature [22].

The activation of the pro-inflammatory situation of the RAW 264.7 macrophage cell was being done. The cell was counted using a hemocytometer and then cultivated at 37°C with a density of 1x106 cells/well in a 6-well plate for 24 hours, 5% CO2. The culture medium was later discharged and substituted with 1,600 µL of the fresh medium and 200 µl of *P. edulis* extract sample at each well, then cultivated for 1-2 hours at 37 °C and CO2 in 5%. For the next 24 hours, cultivation from 200 µl of LPS (1 µg/ml) was augmented at 37 °C and CO2 5% for 10 minutes. The 2000xg conditioned medium was then centrifuged to get supernatant before proceeding to the following assessment.[11].

Quantification examination of IL-1β, TNF-α, and PGE-2 was being done using a Mouse ELISA kit, and the absorbance was measured at 450 nm. The anti-inflammatory efficacy toward IL-1β, TNF-α, and PGE-2 was shown in the percentage (%) of each extracted sample compared to the negative and positive control [21]. The negative control used in this study was the pre-treated RAW 264.7 cells, positive control was the RAW 264.7 cells induced with LPS that simulated the inflammation condition.

- Cell Morphology Analysis

The cell morphology analysis was performed using an inverted microscope with 40x magnification. The microscopic features of the macrophage cells were being analyzed.

- Statistical Analysis

Data of cell viability, quantification of IL-1β, TNF-α, and PGE-2 and cell histologic features were recorded from the experiments. The data obtained were being analyzed using SPSS software (version 20.0). Significant comparison between each group was shown using the analysis of variance test followed by the Duncan *post hoc* test. [20] Data were shown as mean ± SD; superscript letter (a,b,c,d) among each concentration indicated significant differences ($p<0.05$) based on the Duncan *post hoc* test.

III. Results & Discussion

A. Cell viability analysis of P. edulis pulp extract (PEPE) in various concentration

Table 1. Cell viability of *Passiflora edulis* pulp extract in various concentration

Sample	Control	PEPE 12,5	PEPE 25,0	PEPE 50,0	PEPE 100,0
1	98,91	129,06	128,42	132,82	73,84
2	102,81	124,64	130,60	127,85	79,91
3	98,28	132,66	133,33	126,95	79,16
Mean ± SD	100.00 ± 2.45	128.79 ± 4.01 ^{b}	130.78 ± 2.46 ^{b}	129.20 ± 3.16 ^{b}	77.64 ± 3.31 ^{a}

RAW 264.7 cell viability examination was an essential examination to assess the safety effect of *P.edulis* pulp extract towards RAW 264.7 cells on various concentrations. The cell viability of PEPE 12.5, 25, 50, and 100 µg/mL groups were being compared with the control group (100.00 ± 2.45). *P. edulis* pulp extract with the final concentration of 12.5, 25, 50, and 75 µg/mL have higher viability (>90%) and is proven to be non-toxic. Hence, PEPE 12.5 and 75 µg/mL were used as the minimum and maximum

Authorized licensed use limited to: UNIVERSITY OF NOTTINGHAM. Downloaded on September 20,2021 at 09:28:27 UTC from IEEE Xplore. Restrictions apply.

concentrations analyzed in this research due to significant differences between the groups, as seen in “Table. 1”.

B. Effects of P. edulis pulp extract on IL-1β, TNF-α, and PGE-2 level in LPS-induced RAW 264.7 cell

TABLE 2. EFFECTS OF *PASSIFLORA EDULIS* PULP EXTRACT ON IL-1B LEVEL IN LPS-INDUCED RAW 264.7 CELL

Treatment Group		IL-1β concentration (pg/mL)	% Inhibition activity over control group
Positive Control	1	451,48	-3,42
	2	403,67	7,54
	3	454,58	-4,12
	Mean ± SD	436.58 ± 28.54 ^{a}	0.00 ± 6.54 ^{a}
PEPE 12.5 µg/mL	1	358,76	17,82
	2	359,20	17,72
	3	359,56	17,64
	Mean ± SD	359.17 ± 0.40 ^{b}	17.73 ± 0.09 ^{b}
PEPE 75 µg/mL	1	207,70	52,43
	2	207,61	52,45
	3	211,00	51,67
	Mean ± SD	208.77 ± 1.93 ^{c}	52.18 ± 0.44 ^{c}
Negative control	1	132,64	69,62
	2	135,06	69,06
	3	148,68	65,94
	Mean ± SD	138.79 ± 8.65 ^{d}	68.21 ± 1.98 ^{d}

Investigations of passion fruit pulp extract towards IL-1β level in LPS-induced RAW 264.7 cells were analyzed in four experimental groups: positive control, PEPE 12.5 µg/mL, PEPE 75 µg/mL, and negative control (“Table. 2”). Each group has experimented three times. In terms of concentration level, there was a significant decrease from 436.58 ± 28.54 pg/mL (in the positive control group) to 208.77 ± 1.93 pg/mL when treated with PEPE 75 µg/mL. Nonetheless, the concentration of IL-1β observed in PEPE 75 µg/mL group was still a significant difference (40% higher) than the negative control.

TABLE 3. EFFECTS OF *PASSIFLORA EDULIS* PULP EXTRACT ON TNF-α LEVEL IN LPS-INDUCED RAW 264.7 CELL

Treatment Group		TNF-α concentration (pg/mL)	% Inhibition activity over control group
Positive Control	1	617,81	-4.79
	2	582,25	1,24
	3	568,69	3.54
	Mean ± SD	589,58 ± 25.37 ^{a}	0.00 ± 4.30 ^{a}
PEPE 12.5 µg/mL	1	429,81	27,10
	2	481,00	18,42
	3	444,69	24,58
	Mean ± SD	451.83 ± 26.33 ^{b}	23.36 ± 4.47 ^{b}
PEPE 75 µg/mL	1	230,25	60,95
	2	300,81	48,98
	3	301,75	48,82
	Mean ± SD	277.60 ± 41.01 ^{c}	52.92 ± 6.96 ^{c}
Negative control	1	243,00	58,78
	2	258,00	56,24
	3	242,13	58,93
	Mean ± SD	247.71 ± 8.92 ^{c}	57.99 ± 1.51 ^{c}

The impact of *P. edulis* pulp extract on TNF-α level in LPS-induced RAW 264.7 cells was shown in “Table. 3”. Compared to the positive control group, the amount of TNF-α concentration level in the PEPE 75 µg/mL group decreased by approximately 50%. The good news is that there was no significant difference in TNF-α concentration level when comparing the PEPE 75 µg/mL to the negative control group in this experimental study.

TABLE 4. EFFECTS OF *PASSIFLORA EDULIS* PULP EXTRACT ON PGE-2 LEVEL IN LPS-INDUCED RAW 264.7 CELL

Treatment Group		PGE-2 concentration (pg/mL)	% Inhibition activity over control group
Positive Control	1	850,58	-0,07
	2	923,16	-4,90
	3	836,26	4,97
	Mean ± SD	880.00 ± 43.45 ^{a}	0.00 ± 4.94 ^{a}
PEPE 12.5 µg/mL	1	824,77	6,28
	2	780,97	11,25
	3	738,61	16,07
	Mean ± SD	781.45 ± 43.08 ^{b}	11.20 ± 4.90 ^{b}
PEPE 75 µg/mL	1	552,74	37,19
	2	516,55	41,30
	3	503,84	42,75
	Mean ± SD	524.38 ± 25.37 ^{c}	40.41 ± 2.88 ^{c}
Negative control	1	405,97	53,87
	2	390,48	55,63
	3	423,35	51,89
	Mean ± SD	406.60 ± 16.44 ^{d}	53.80 ± 1.87 ^{d}

Likewise, as shown in “Table. 4”, the level of PGE-2 in LPS-induced RAW 264.7 cells was declining when treated with PEPE 12.5 µg/mL for almost 20%. The level reduced to almost 40% of the positive control group level when treated with PEPE 75 µg/mL. These results showed that both 12.5 µg/mL and 75 µg/mL of the *P. edulis* pulp extract could reduce PGE-2 levels significantly related to positive control and opposing control groups ($p>0.05$).

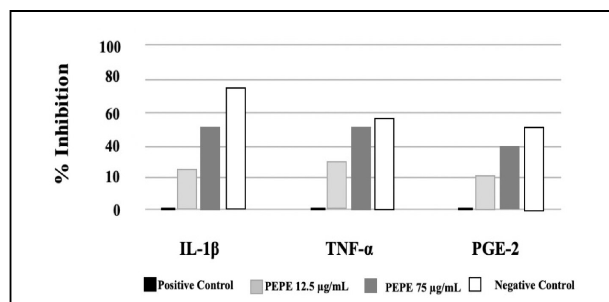

Fig. 1. IL-1β, TNF-α and PGE-2 % Inhibition activity over control group (Mean ± SD)

The inhibition power of PEPE 12.5 µg/mL and PEPE 75 µg/mL groups were illustrated in “Fig. 1”. The inhibition capacity of PEPE 75 µg/mL over positive control was almost similar for both IL-1β and TNF-α (more than 50%) and around 40% for PGE-2. In comparison with negative control, there was no significant difference noticed in TNF-α inhibition percentage. Inhibition of IL-1β, TNF-α, and PGE-2 played a vital role in displaying an anti-inflammatory effect. The study results revealed a great potential of the

Authorized licensed use limited to: UNIVERSITY OF NOTTINGHAM. Downloaded on September 20,2021 at 09:28:27 UTC from IEEE Xplore. Restrictions apply.

PEPE 75 μg/mL group in reducing the pro-inflammatory cytokines and mediators in inflamed cells.

In this study, *P. edulis* pulp extract was found to reduce IL-1β, TNF-α, and PGE-2 levels in LPS-induced RAW 264.7 cells. Phytonutrients found in *P. edulis* pulp extract were flavonoid, saponin, tocopherol, carotene, and ascorbic acid compounds [23]. These bioactive chemical compounds have natural anti-oxidants, anti-microbial, and anti-inflammatory agents [24]. Naturally activated macrophages release excessive pro-inflammatory cytokines, NO, and COX-2, resulting in damaging effects on tissues and cells. TNF-α, interleukin-6, and IL-1β are pro-inflammatory cytokines that deal with infections and thus promote Th1 responses [25]. Natural flavonoids and saponin appeared to suppress cytokine production, including that of IL-1β, IL-6, and TNF-α, in several cell lines through the MAPK signaling pathway [26]. The NF-κB signaling pathway in regulating the transcription factors that control the expression of iNOS and COX-2, therefore, suppressed NO and PGE-2 production [26]. Another study identified that isoorientin, a compound originated from flavonoids, inhibits COX-2 expression via cytokines mediated communication that catalyzes arachidonic acid into PGE-2 [26, 27]. In cell culture models, the phenotypic changes are reversed by treatment with a highly selective COX-2 inhibitor to prevent adhesion to the extra-cellular matrix [28]. Our results indicated that *P. edulis* pulp extract downregulated the expression of COX-2 and the activation of IL-1β, TNF-α, and PGE-2 in LPS-stimulated macrophage cells, the critical marker for anti-inflammatory responses. This research has proven that *P. edulis* pulp extract has anti-inflammatory activity in LPS-activated RAW 264.7 cells.

B. RAW 264.7 Cells Histologic Feature

"Fig. 2" displayed the healthy RAW 264.7 cells microscopically (a) compared with post-treated cells. At the same time, the LPS-induced (pro-inflammatory) RAW 264.7 cells were seen invaded by macrophages (b). Post-treatment with 12.5 μg/mL of *P. edulis* pulp extract, the number of macrophages decreased slightly (c). Along with the rise of concentration to 75 μg/mL, *P. edulis* pulp extract demonstrated superior anti-inflammation activity, proven with the declining number of macrophages (d).

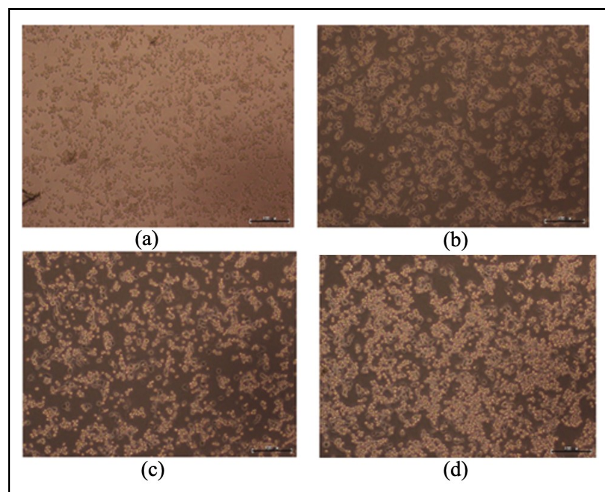

Fig. 2. Study of Cell morphology (magnification 40X): (a) Negative Control RAW 264.7; (b) Positive Control RAW 264.7; (c) RAW 264.7+PEPE 12.5μg/mL; (d) RAW 264.7+PEPE 75μg/mL

IV. CONCLUSION

This study concluded that >90% cell viability was observed in *Passiflora edulis* pulp extract (PEPE) 12.5 to 75 μg/mL concentration. In addition, PEPE was effective in reducing interleukin-1β, Tumor Necrosis Factor-a, and Prostaglandin levels. The efficacy of 75 μg/mL *P. edulis* pulp extract doubled the 12.5 μg/mL concentration in reducing IL-1β and TNF-α level and tripled the % inhibition of PGE-2 level. The post-treated group with 75 μg/mL *P. edulis* pulp extract significantly decreased the TNF-α concentrations ($p<0.5$), closely approaching the amount of the negative control concentrations. Histologically, the cells displayed a considerable restoration post-treated with the *P. edulis* pulp extract, in which higher concentrations enhance the potential to inhibit inflammation activity. Consequently, advanced researches were required to reinforce the specific pathway of *P. edulis* in decreasing inflammation in oral diseases.

ACKNOWLEDGMENT

The authors gratefully acknowledge Aretha Medika Utama Biomolecular and Biomedical Research Center, Bandung, Indonesia, for facilities used in this research.

REFERENCES

[1] A. Cekici, A. Kantarci, H. Hasturk, & T E Van Dyke, "Inflammatory and immune pathways in the pathogenesis of periodontal disease," Periodontol 2000, 2014, vol. 64, no. 1, pp. 57–80.

[2] D R Laksmitawati, A P Prasanti, N. Larasinta, G A Syauta, R. Hilda, Ramadaniati, W. Widowati, " Anti-Inflammatory Potential of Gandarusa and Soursoup Extracts in LPS Stimulated-Macrophage Cell RAW264.7," Journal of Natural Remedies, 2016, vol. 16, no. 2, pp. 73-81.

[3] O. Khoury, A. Atala, S V Murphy, "Stomal Cells from Perinatal and Adult sources modulate the inflammatory Immune Response in vitro by decreasing Th1 cell proloferation and cytokine secretion," STEM CELLS Trans Med, 2020, vol. 9, pp. 661-73.

[4] V P Kale, P J Gilhooley, S. Phadtare, A. Nabavizadeh, M K Pandey, "Role of Gambogic Acid in Chemosensitization of Cancer in Cancer Sensitizing Agents for Chemotherapy," Vol. 2, pp. 151-67, 2018.

[5] G. Massimiliano, Y. Tamara, Forbes-Hernandez., Maurizio Battino, "Anti-Inflammatory effect of strawberry extract againts LPS-induced stress in RAW 264.7 macrophages," Food and Chemical Toxicology, 2017.

[6] Yan-Ru Li., Chun-Sheng Fu.,Wen-Jing Yang., Xiao-Ling Wang., Dan Feng., Tao Shen, " Investigation of constituents from Cinnamomum Camphora L. J. Presl and evaluation of their anti Inflammatory properties in lipopolysaccharide-stimulated RAW 264.7 macrophanges," Journal of Ethnopharmacology, 2018.

[7] G A Nasser, "*Circuma longa L* as Anti-Inflammation Agent," Wellness and Healthy Magazine, 2020, Vol. 2, no. 1, pp. 147-158.

[8] R I Andriyono, " *Kaempferia galanga L.* as Anti-Inflammatory and Analgesi," Healthy Journal, 2019, Vol. 10, no. 3, P 495-502.

[9] L. Wilvia, L. Novelya, " Sitotoxic Test and Anti-Inflammation from (*Pachyrizus erous (L.) Urb.*) Towards RAW 265.7 Cells stimulated with Lipopolisacharide," eBiomedik, 2020, vol. 8, no. 2, pp. 188-195.

[10] N M Sandhiutami, M. Moordiani, D R Laksmitawati, F. Nurul, M. Maesaroh, & W. Widowati, " In vitro assesment of anti-inflammatory activities of coumarin and Indonesian cassia extract in RAW264.7 murine macrophage cell line," Iran. J. Basic. Med. Sci, 2017, vol. 20, pp. 99-106.

[11] V. Krusty, A. Raif, & R. Ikhtiari, "Antioxidant and Anti-elastase Activity of Seed and Peel Extract of P.edulis," ASRJETS, 2019, vol. 53, no. 1, pp. 43-8.

[12] Anabel, D W Cindy, L. Shieny, " Antibacterial Effectiveness Test of Purple Passion Fruit Peel Againts *Staphylococcus aureus,"* Healthy Tadulako Journal, 2020, vol. 6, no. 3, pp. 79-85.

[13] H O Fachril, W. Adriatmak, P. Astuti, " Effect of Yellow Markisa Fruit Extract *P. Edulis* Sims as Antiinflamation Seen from The

Authorized licensed use limited to: UNIVERSITY OF NOTTINGHAM. Downloaded on September 20,2021 at 09:28:27 UTC from IEEE Xplore. Restrictions apply.

Number of Monocyte on Male Wistar Rat," Stomatognatic. J. K. G. Unej, 2019, vol. 15, no. 2, pp. 34-36.

[14] A. Sunny, V. Perumal, & V. Chandy, " Leaves of *Passiflora Edulis*," World Journal of Pharmaceutical Research, 2020, vol. 9, no. 5, pp. 1513-1522.

[15] M L Zeraik, D. Serteyn, G. Deby-Dupont, J N Wauters, M. Tits, J H Yariwake, T. Franck, " Evaluation of the antioxidant activity of passion fruit (*Passiflora edulis)* and Passiflora," Food Chemistry, 2011, no. 128, pp. 259-265.

[16] Mei Xiao-Dan., Cao Yan-Feng., Che Yan-Yun., Li Jing, Zhang Jia-Yu, " A phytichemical and Pharmacological overview." Chinese Journal of Natural Medicines, 2019, vol. 17, no. 1, pp. 0059-0080.

[17] J C Soares, P L Rosalen, J G Lazarini, A P Massarioli, C. Fortunato da Silva, B D Nani, M. Franchin, S. Matias de Alencar, "Comprehensive Characterization of Bioactive Phenols from New Brazilian Superfruits by LC-ESI-QTOF-MS, and their ROS and RNS scavenging Effects and Anti-inflammatory Activity," Food Chemistry, 2019, vol. 281, pp. 178-188.

[18] J C Soares, P L Rosalen, J G Lazarini, A P Massarioli, C. Fortunato da Silva, B D Nani, M. Franchin, S. Matias de Alencar, "Comprehensive Characterization of Bioactive Phenols from New Brazilian Superfruits by LC-ESI-QTOF-MS, and their ROS and RNS scavenging Effects and Anti-inflammatory Activity," Food Chemistry, 2019, vol. 281, pp. 178-188.Activity," Food Chemistry, 281, 178-188. 2019.

[19] Q C Thanh Nguyen, Tran Duy Binh, L A Tuan Phan., D H Yen Nguyen, Dai Thi Xuan Trang, Trong Tuan Nguyen, Kenji Kanaori and Kaeko Kamei, " Anti-Inflammatory Effects of Lasia Spinosa Leaf Extract in Lipopolysaccharide-Induced RAW 264.7 Macrophages." International Journal of Molecular Sciences, 2020, vol. 21, pp. 34-39.

[20] D R Laksmitawati, A. Widyastuti, N. Karami, E. Afifah, D D Rihibiha, H. Nufus, & 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.

[21] A. Novilla, D S Djamhuri, B. Nurhayati, D D Rihibiha, E. Afifah, & 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.

[22] W. Widowati, S. Prahastuti, N L Ekayanti, U Z Munshy, H S Kusuma, S H Wibowo, R. Rizal, "Anti-Inflammation Assay of Black Soybean Extract and Its Compounds on Lipopolysaccharide-Induced RAW 264.7 Cell," Journal of Physics Conference Series, 2019.

[23] M. Johnson , M. Maridass, & V. Irudayaraj, "Preliminary Phytochemical and Anti-Bacterial Studies on *Passiflora edulis*," Ethnobotanical Leaflets, pp. 425-432, 2008.

[24] P B Pertuzatti, M. Sganzerla, A C Jacques, M T Barcia, & R C Zambiazi, " Carotenoids, tocopherols and ascorbic acid content in yellow passion," LWT, Food Science and Technology, vol. 64, pp. 259-263, 2015.

[25] P. B. Pertuzatti, M. Sganzerla, A. C. Jacques, M. T. Barcia, & R. C.

[26] F S Joseph, E T Ichim, M S Prince, Santosh Kesari, M. Francesco, Anton Rolando Escobedo, Amir Jafri, "Unleashing endogenous TNF-alpha as a cancer immunotherapeutic," Journal of Translation Medicine 16, no. 242, 2018.

[27] K. Anilkumar, G V Reddy, R. Azad, N S Yarla, G. Dharmapuri, A. Srivastava, R. Pallu, "Evaluation of Anti-Inflammatory Properties of Isoorientin Isolated from Tubers of Pueraria tuberosa," Hindawi-Oxidative Medicine and Celular Longevity, pp. 1-10, 2017.

[28] Rashida Ginwala, Raina Bhavsar, De Gaulle I. Chigbu, Pooja Jain, Zafar.K. Khan, " Potential Role of Flavonoids in Treating Chronic Inflammatory Diseases with Special Focus on the Anti-Inflammatory Activity of Apigenin," Antioxidants. vol 8, no. 2, 2018.

[29] Ji-Won Park, Ok-Kyoung Kwon, Hyung Won Ryu, Jin-Hyub Paik, Imam Paryanto, Prasetyawan Yuniato , Sangho Choi, Sei-Ryang Oh, Kyung-Seop Ahn, " Anti-inflammatory effects of Passiflora Foetida L. in LPS-stimulated RAW264,7 macrophages," International Journal of Molecular Medicine," vol. 41, no. 6, 2018.

Authorized licensed use limited to: UNIVERSITY OF NOTTINGHAM. Downloaded on September 20,2021 at 09:28:27 UTC from IEEE Xplore. Restrictions apply.