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# Metabolite Profiling and Inhibitory Effects of Nitric Oxide on *Andrographis paniculata* Burm. F Nees Extract using Different Solvents as a Potential Candidate in COVID-19 Therapy

EVUL WINOTO LUKITO[1], DYAH ISWANTINI[1,*], BUDHI ANTARIKSA[2], MOHAMAD RAFI[1] and SETYANTO TRI WAHYUDI[3]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, Indonesia
[2]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Indonesia, Depok, Indonesia
[3]Department of Physics, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, Indonesia

*Corresponding author: E-mail: dyahis@apps.ipb.ac.id

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*Andrographis paniculata* Burm. F Nees used to treat COVID-19 in several Asian countries because it has metabolites with pharmacological activity that may decrease the pathogenesis features of COVID-19. The extraction solvent significantly affected the composition and concentration of the metabolites. Therefore, the inhibitory effects of nitric oxide of *A. paniculata* extract based on differences in extraction solvent concentrations were investigare and also identified its metabolite profile. The leaves of *A. paniculata* were extracted with three different solvents like water solvent, 50% ethanol and pure ethanol. Nitrite oxide (NO) inhibitory activity on LPS-activated RAW 264.7 macrophage cells was used to evaluate the anti-inflammatory activity of the extract, the identification of metabolite profile using LC-MS/MS. Extract pure ethanol had the highest inhibition potential against NO at 91.02%, whereas water and 50% ethanol solvents had inhibitory potentials of 21.35% and -8.13%, respectively. The metabolites 14-deoxyandrographoside and 14-deoxy-17-hydroxyandrographolid were only identified in the pure ethanol extract. This study shows that differences in solvent concentrations play an essential role in enhancing the pharmacological potential of *A. paniculata* as an anti-inflammatory candidate for COVID-19 treatment.

**Keywords: *Andrographis paniculata*, Nitrite oxide, COVID-19, Extraction, LC-MS/MS.**

## INTRODUCTION

Inflammation is a biological response of the immune system that functions as the human body defense against specific stimuli such as microbial infections and tissue damage. Organ malfunction and a systemic inflammatory response not under control are the primary causes of many chronic diseases [1]. Infection of macrophage cells in the body causes a persistent inflammatory response by releasing nitric oxide (NO), reactive oxygen species and pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) [2]. Acute respiratory distress syndrome (ARDS) is a respiratory system inflammation found to be the leading cause of death among COVID-19 patients [3,4]. Inhibition of pro-inflammatory mediators in the early stages of COVID-19 is an effective therapeutic strategy to prevent the development of more severe ARDS [5-7].

*A. paniculata*, known in Indonesia as sambiloto, is the most commonly reported plant with metabolite compounds with biological and pharmacological functions that can reduce COVID-19 pathogenesis features, such as anti-inflammatory [8], immunomodulatory [9] and antiviral activities [10-12]. *A. paniculata* has been used to treat COVID-19 in several Asian countries [13]. *A. paniculata* has been extensively studied as an anti-inflammatory. Andrographolide, dehydroandrographolide and neoandrographolide extracted from *A. paniculata* have been shown to inhibit IL-6 and TNF-α production in RAW 264 macrophage cells activated by lipopolysaccharide (LPS) [14]. Andrographolide has also been shown to reduce the development of nitric oxide (NO) in LPS-stimulated RAW 264 macrophage cells by inhibiting the synthesis of the inducible nitric oxide synthase (iNOS) protein [15]. Furthermore, *A. paniculata* crude extract inhibited the activity of proinflammatory mediators such as NO, IL-1 and IL-6 [16]. Nitric oxide is a pro-inflam-

matory mediator that is involved in the inflammatory process and tissue damage. In endothelial cells, NO is produced and released by inducible nitric oxide synthase (iNOS), which converts arginine to citrulline. Inhibiting NO generation and secretion could be used to avoid inflammatory disorders [17]. The composition and concentration of metabolite compounds in the plant influence the pharmacological and biological activities of *A. paniculata*. Such a composition is influenced by genetics, growth environmental conditions, harvest conditions and post-harvest ages [18]. Tajidin *et al.* [19] observed that young leaves of *A. paniculata* harvested before flowering or pre-flowering contained more metabolites than older leaves. The most abundant diterpenoid lactones found in *A. paniculata* leaves are andrographolide, neoandrographolide and deoxyandrographolide [20].

Extraction is a common method for isolating plant metabolites and the use of appropriate solvents in extraction will produce extracts with the optimal pharmacological activity. The type of solvent and the solvent concentrations used for extraction are essential factors in optimizing the composition and concentration of extracted metabolite compounds [21]. The most extensively described anti-inflammatory metabolites in *A. paniculata* extract are andrographolide, dehydroandrographolide and neoandrographolide [14-16]. However, the influence of the extraction solvent concentration on the metabolite profile and anti-inflammatory activity for *A. paniculata* has not been reported. Therefore, this study aimed to investigate the profiles of metabolite and anti-inflammatory activity of *A. paniculata* extract based inhibitory on nitric oxide using differences in extraction solvent concentrations and with potential anti-inflammatory agents. The investigation was conducted *in vitro* utilizing RAW 264.7 macrophage cells stimulated by lipopolysaccharides (LPS) to examine their anti-inflammatory potential based on the generation of the pro-inflammatory mediator nitric oxide (NO) and LC-MS/MS to determine the profile of their metabolite.

## EXPERIMENTAL

Pre-flowering *Andrographis paniculata* leaves were collected from Tropical Biopharmaca Research Center, IPB University and the RAW 264.7 cell culture from Primate Research Center, IPB University, Bogor, Indonesia. Other chemicals and reagents included ethanol (Merck, Germany), fetal bovine serum (FBS) (Biosera), Roswell Park Memorial Institute Medium and penicillin streptomycin, trypsin-EDTA, all procured from Sigma-Aldrich, Germany. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Bio Basic), Dulbecco's modified eagle medium (DMEM) (Sigma-Aldrich, Germany) and LPS (Sigma-Aldrich, Germany). Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), Thermo-Scientific Vanquish Flex UHPLC tandem Q Exactive Plus Orbitrap-High Resolution Mass Spectrometer (Thermo-Scientific, Germany), Unscrambler X 10.4 (CAMO, Norwegia), ThermoX Calibur (Thermo Scientific, Germany) and Compound Discoverer 2.1 (Thermo Scientific, Germany) were used in the identification and analysis of secondary metabolite profiles of *A. paniculata* extract. The rotary evaporator was utilized for extract concentration. In addition, a cell viability assay was performed using a universal microplate reader (Bio-Tek ELX 800).

**Plant material and extraction:** *A. paniculata* leaves were harvested before flowering and were washed with water thoroughly. The samples were dried in the sun and then crushed into 80-mesh powder. Approximately 25 g of powder was added to 250 mL of extraction solvent, soaked with continuous stirring for 6 h and finally left for 12 h without stirring. Distilled water, 50% ethanol and pure ethanol were the extraction solvents and the application of each was repeated five times. The filtrate was collected, concentrated using a rotary evaporator at 40 ºC and the extract was then stored in a freezer at -80 ºC [22].

**LC-MS/MS analysis:** A 5 mg of extract was dissolved in 1 mL of LC-MS grade methanol followed by sonication for 30 min. The solution was filtered using a 0.22 µm PTFE membrane and the filtrate was analyzed using UHPLC-Q-Orbitrap HRMS to separate and identify metabolites. Accucore $C_{18}$ (100 × 2.1 mm, 1.5 µm) was used as the column. The mobile phase used consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution was formatted as follows: 15% (B) from 0 to 1 min, 15–55% (B) from 1–20 min, 55–95% (B) from 20–23 min, 95% (B) from 23–28 min, then 15% (B) from 28–30 min. The flow rate used was 0.2 mL/min with an injection volume of 2.0 µL. The ionization source used was ESI, with positive and negative ionization modes in the *m/z* range of 100–1500 amu. The capillary temperature used was 320 ºC, the spray voltage was 3.8 kV and the sheath and auxiliary gas flow rates were 15 and 3 mL/min, respectively. The injection time was set to 100 ms and the automatic gain control at $3 \times 10^6$. The scan type employed was full MS/dd MS2 and a full scan dataset with a resolving power of 70,000 FWHM. The collision energies used were 18, 35 and 53 eV. Compound Discoverer 2.2 was used to process data from UHPLC-Q-Orbitrap HRMS with a database built from metabolite data in *A. paniculata.*

**Preparation of RAW 264.7 macrophages cell culture:** Cells were grown in DMEM with 10% FBS and 1% penicillin-streptomycin. The cells were incubated at 37 ºC with 5% $CO_2$ humidity until confluence and then harvested with trypsin-EDTA [23].

**Cell viability assay:** The cell viability assay was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. RAW 264.7 cells containing $1 \times 10^4$ cells per well were planted in 96-well plates and incubated for 24 h. The discarded medium was replaced with a new one and added to the test sample at 25, 50 and 100 µg/mL. The medium was then removed and the cells were given PBS wash after being incubated for a second time for 24 h. Afterward, 10 µL of 5 mg/mL MTT solution was added to each well and incubated for 4 h. The formed formazan crystals were dissolved in 100 µL DMSO. The absorbance was measured using a microplate reader at a wavelength of 570 nm.

**Anti-inflammatory activity:** The anti-inflammatory activity of each extract was determined by measuring the production of nitric oxide (NO) in LPS-stimulated RAW 264.7 cells. In this work, the anti-inflammatory activity approach based on Marques *et al.* [24] and Sandhiutami *et al.* [25] was followed.

RAW 264.7 cells of $1 \times 10^3$ cells per well were planted in 96-well plates and incubated for 24 h. The medium was discarded, replaced with a new one and added to test samples at concentrations of 15, 20, 25, 30, 35 and 40 µg/mL, then incubated for 1-2 h. The cells were then stimulated with 1 µg/mL LPS and incubated for another 24 h. The medium was removed, centrifuged for 10 min at 2000 rpm and then the collected supernatant was added to the Griess reagent (1:1). Cell nitric oxide levels were measured using a microplate reader at a wavelength of 540 nm. Calibration curves were prepared using sodium nitrite as standard.

## RESULTS AND DISCUSSION

**Extract yields:** Maceration at room temperature was used to extract the metabolites from *A. paniculata*. The results showed that extraction with variations in solvent concentration produced slightly different extract yields (Fig. 1). The extract with 50% ethanol solvent produced the highest yield (11.02% ± 1.20) and the extract with water solvent produced the lowest (8.61% ± 1.25). Meanwhile, the pure ethanol solvent reached 10.23% ± 0.88. These results additionally indicated that solvent concentration variations during extraction influence metabolite quantity and composition [21].

The different concentrations of the extraction solvents used led to different levels of polarity, resulting in differences in the concentration and composition of the extracted metabolites. This condition also affects their toxicity and anti-inflammatory activity. Andrographolide, neoandrographolide and deoxyandrographolide are the most abundant diterpenoid lactones in *A. paniculata* leaves. Andrographolide is the most frequently reported metabolite with anti-inflammatory properties [14,26-28] and also easily soluble in ethanol and slightly soluble in water [20].

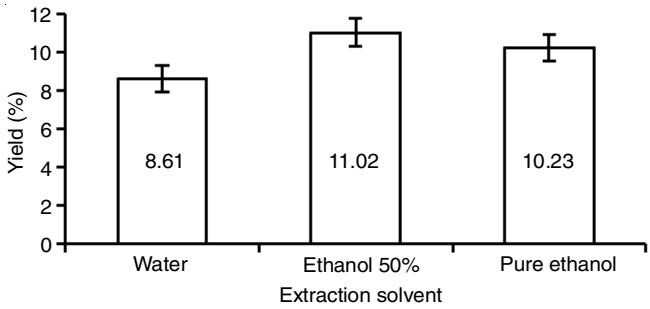


Fig. 1. Average yield from the *A. paniculata* leaf extracts of five replicates

**Profile of metabolite compounds:** The metabolite compounds of each *A. paniculata* extract were separated and identified using UHPLC-Q-Orbitrap HRMS to determine the effect of the concentration of the extraction solvent on the composition of the extracted metabolites. The LC-MS/MS chromatograms of each showed a different pattern. The 50% ethanol solvent produced significantly more peaks than the pure ethanol solvent and the water solvent, this condition shows that there is more metabolite diversity in the 50% ethanol extract compared to the pure ethanol extract and water extract (Fig. 2).

The differences in chromatograms between the two ionization methods indicates that each extract has a different metabolite profile. The metabolite profile in the chromatogram showed the presence of 32 metabolites, 13 metabolites were identified in the water extract, 26 were identified in the 50% ethanol extract and 20 were identified in pure ethanol extract. All metabolites were identified using the full scan technique, data was obtained using Compound Discoverer 3.2 software. Stages of spectrum selection, metabolite detection, alignment, prediction of metabolite formula. In addition, metabolite identification was determined by confirming MS-1 based on

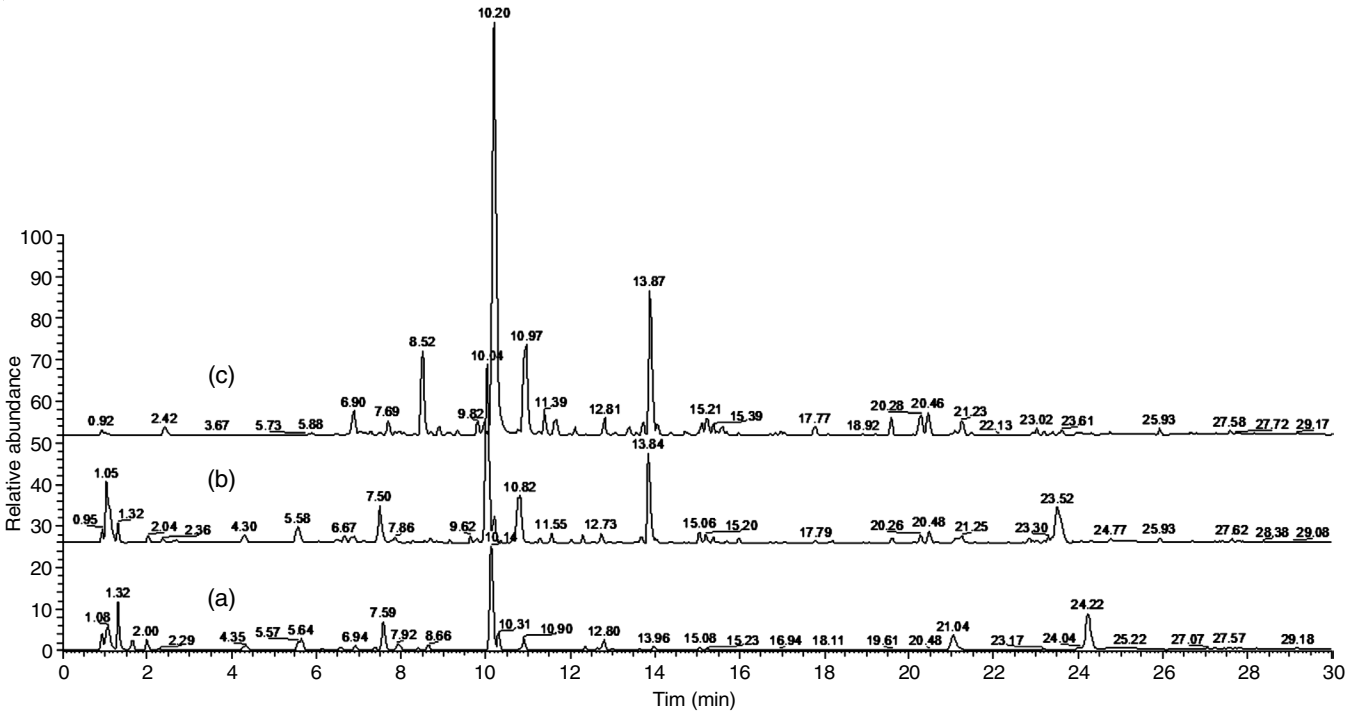


Fig. 2. Representative chromatograms of water extracts (a), 50% ethanol extracts (b) and pure ethanol extracts (c)

TABLE-1
METABOLITE COMPOUNDS IN *A. paniculata* LEAF EXTRACTS AS IDENTIFIED BY UHPLC-Q-Orbitrap HRMS

| No. | Name of metabolites | RT (min) | m.f. | m.w. | Error mass (ppm) | MS-MS |
|---|---|---|---|---|---|---|
| | **Diterpene lactone** | | | | | |
| 1 | 12S-Hydroxyandrographolide | 4.01 | $C_{20}H_{32}O_6$ | 368.2192 | -1.88 | 369, 351, 333 |
| 2 | 14-Acetylandrographolide | 15.16 | $C_{22}H_{32}O_6$ | 392.2199 | -0.05 | 391, 345, 301 |
| 3 | 14-Deoxy-11,12-didehydroandrographolide | 10.11 | $C_{20}H_{28}O_4$ | 332.1978 | -2.99 | 333, 315, 297, 285, 257 |
| 4 | 14-Deoxy-11-oxo-andrographolide | 10.17 | $C_{20}H_{28}O_5$ | 348.1927 | -2.70 | 349, 331, 313, 285 |
| 5 | 14-Deoxy-17-hydroxyandrographolide | 7.76 | $C_{20}H_{32}O_5$ | 352.2238 | -3.32 | 353, 317, 299, 287 |
| 6 | 14-Deoxyandrographolide | 15.28 | $C_{20}H_{30}O_4$ | 334.2132 | -3.75 | 335, 317, 299, 287, 259 |
| 7 | 14-Deoxyandrographoside | 10.83 | $C_{26}H_{40}O_9$ | 496.2656 | -3.34 | 497, 299, 287, 259 |
| 8 | 3,14-Dideoxyandrographolide | 13.92 | $C_{20}H_{30}O_3$ | 318.2183 | -3.83 | 319, 301, 289 |
| 9 | Andrographolactone | 15.57 | $C_{20}H_{24}O_2$ | 296.1768 | -2.80 | 297, 269, 255 |
| 10 | Andrographic acid | 6.58 | $C_{20}H_{28}O_6$ | 364.1879 | -1.82 | 365, 347, 329 |
| 11 | Andrographiside | 6,75 | $C_{26}H_{40}O_{10}$ | 512.2613 | -1.63 | 513, 351, 333, 315, 297, 285, 257 |
| 12 | Andrographolide | 7.58 | $C_{20}H_{30}O_5$ | 350.2082 | -3.10 | 351, 333, 315, 297, 285, 257 |
| 13 | Bisandrographolide A | 15.57 | $C_{40}H_{56}O_8$ | 664.3960 | -2.23 | 665, 315, 297, 285 |
| 14 | Diterpene II (Lactone) | 10.28 | $C_{20}H_{26}O_5$ | 346.1769 | -3.22 | 347, 329, 283 |
| 15 | Ethyl caffeate | 11.01 | $C_{11}H_{12}O_4$ | 208.0733 | -1.48 | 179, 161, 135 |
| 16 | Neoandrographolide | 13.93 | $C_{26}H_{40}O_8$ | 480.2712 | -2.30 | 481, 319, 301, 289 |
| 17 | Paniculide-A | 2.76 | $C_{15}H_{20}O_4$ | 264.1359 | -1.12 | 265, 247 |
| | **Flavonoids** | | | | | |
| 18 | 5-Hydroxy-7,8-dimethoxyflavanone | 21.01 | $C_{17}H_{16}O_5$ | 300.0986 | -3.78 | 301, 197 |
| 19 | 7-O-metilwogonin | 21.97 | $C_{17}H_{14}O_5$ | 298.083 | -3.65 | 299, 285 |
| 20 | Apigenin | 7.58 | $C_{15}H_{10}O_5$ | 270.0517 | -4.06 | 271, 153, 145 |
| 21 | Apigenin-7-O-glucuronide | 7,58 | $C_{21}H_{18}O_{11}$ | 446.0839 | -2.36 | 447, 271, 153 |
| 22 | Luteolin-7-O-glucuronide | 5.88 | $C_{21}H_{18}O_{12}$ | 462.0799 | 0.09 | 463, 287 |
| 23 | Scoparin | 8.37 | $C_{22}H_{22}O_{11}$ | 462.1155 | -1.57 | 463, 301, 287 |
| | **Phenolic acid** | | | | | |
| 24 | 4-O-feruloylquinic acid | 3.24 | $C_{17}H_{20}O_9$ | 368.1108 | 0.12 | 367, 193 |
| 25 | 5-Hydroxyjasmonic acid 5-O-hexoside | 2.45 | $C_{19}H_{30}O_8$ | 386.1931 | -2.47 | 387, 351, 207, 149 |
| 26 | Coumaroylquinic acid | 2.74 | $C_{16}H_{18}O_8$ | 338.1003 | 0.42 | 337, 191, 173, 163 |
| 27 | Dihydro-caffeic acid | 2.42 | $C_9H_{10}O_4$ | 182.0575 | -2.20 | 181, 137, 121, 109 |
| 28 | Dihydroferulic acid-4-O-glucuronide | 3.47 | $C_{16}H_{20}O_{10}$ | 372.1060 | 0.90 | 371, 245, 195 |
| 29 | *p*-Coumaric acid | 4.49 | $C_9H_8O_3$ | 164.0467 | -3.88 | 163, 119 |
| 30 | *p*-Coumaric acid-glucoside | 1.95 | $C_{15}H_{18}O_8$ | 326.1005 | 1.08 | 325, 163, 119 |
| 31 | Protocatechuic acid | 1.74 | $C_7H_6O_4$ | 154.0260 | -4.15 | 153, 109, 95 |
| 32 | Quinic acid | 1.11 | $C_7H_{12}O_6$ | 192.0629 | -2.68 | 191, 147, 87, 85 |

the in-house database and MS-2 fragmentation patterns based on the literature (Table-1).

The composition of the bioactive compounds extracted is affected by the type of solvent and concentration utilized in the extraction process [21,29]. The differences in the composition of each extract are in line and directly proportional to the results shown in the extraction yield, the ethanol extract shows a greater number of metabolites than pure ethanol extract and water extract. Some specific metabolites identified from each extract are shown in Table-2.

Andrographolide, 14-deoxy-11,12-didehydroandrographolide andrografolactone andrographic acid, 14-deoxy-11-oxo-andrographolide, 14-deoxyandrographolide and 3,14-dideoxyandrographolide were diterpenoid compounds identified in each sample extract. Dihydro-caffeic acid, paniculide-A and protocatechuic acid compounds were suspected to only be found in aqueous extracts. Meanwhile, 4-O-feruloylquinic acid, apigenin, coumaroylquinic acid, dihydroferulic acid-4-O-glucuronide, *p*-coumaric acid, luteolin-7-O-glucuronide, ethyl caffeate and *p*-coumaric acid-glucoside were evidently found only in the 50% ethanol extract. 14-Deoxy-17-hydroxyandrographolide and 14-deoxyandrographoside belong to the diterpene lactone group, which are thought to have only been identified in pure ethanol extracts. 14-Deoxyandrographoside compound was identified in the positive ionization mode with a retention time of 10.83 min and fragmented at *m/z* 497.27469 $[M+H]^+$, 335.185 $[M+H\text{-}Glc]^+$, 317.174 $[M+H\text{-}Glc\text{-}2H_2O]^+$, 299.164 $[M+H\text{-}Glc\text{-}2H_2O]^+$ and 287.16382 $[M+H\text{-}Glc\text{-}2H_2O\text{-}C]^+$. 14-Deoxy-17-hydroxyandrographolide compound was identified in the positive ionization mode with a retention time of 7.76 min and was fragmented at *m/z* 353.23282 $[M+H]^+$, 335.22214 $[M+H\text{-}H_2O]^+$, 317.21164 $[M+H\text{-}2H_2O]^+$, 299.20032 $[M+H\text{-}3H_2O]^+$ and 271.20468 $[M+H\text{-}3H_2O\text{-}CO]^+$.

**Viability assay:** The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method was applied to cell viability tests to determine the concentration of extracts that were not toxic to RAW 264.7 cells by measuring the number of living cells after they were administered the extract. Metabolites can be toxic to cells through several mechanisms, such as preventing protein synthesis and enzymatic processes [30].

TABLE-2
PUTATIVE IDENTIFICATION OF METABOLITE COMPOUNDS IN THE WATER, 50% ETHANOL AND PURE ETHANOL

| No. | Name of metabolites | Solvent | | |
|---|---|---|---|---|
| | | Water | 50% Ethanol | Pure ethanol |
| 1 | 12S-Hydroxyandrographolide | ✓ | ✓ | × |
| 2 | 14-Acetylandrographolide | × | ✓ | ✓ |
| 3 | 14-Deoxy-11,12-didehydroandrographolide | ✓ | ✓ | ✓ |
| 4 | 14-Deoxy-11-oxo-andrographolide | ✓ | ✓ | ✓ |
| 5 | 14-Deoxy-17-hydroxyandrographolide | × | × | ✓ |
| 6 | 14-Deoxyandrographolide | ✓ | ✓ | ✓ |
| 7 | 14-Deoxyandrographoside | × | × | ✓ |
| 8 | 3,14-Dideoxyandrographolide | ✓ | ✓ | ✓ |
| 9 | 4-O-feruloylquinic acid | × | ✓ | × |
| 10 | 5-Hydroxy-7,8-dimethoxyflavanone | × | ✓ | ✓ |
| 11 | 5-Hydroxyjasmonic acid 5-O-hexoside | × | ✓ | ✓ |
| 12 | 7-O-metilwogonin | × | ✓ | ✓ |
| 13 | Andrographolactone | ✓ | ✓ | ✓ |
| 14 | Andrographic acid | ✓ | ✓ | ✓ |
| 15 | Andrographiside | × | ✓ | ✓ |
| 16 | Andrographolide | ✓ | ✓ | ✓ |
| 17 | Apigenin | × | ✓ | × |
| 18 | Apigenin-7-O-glucuronide | ✓ | × | ✓ |
| 19 | BisandrographolideA | × | ✓ | ✓ |
| 20 | Coumaroylquinic acid | × | ✓ | × |
| 21 | Dihydro-caffeic acid | ✓ | × | × |
| 22 | Dihydroferulic acid-4-O-glucuronide | × | ✓ | × |
| 23 | DiterpeneII(Lactone) | ✓ | ✓ | ✓ |
| 24 | Ethyl caffeate | × | ✓ | × |
| 25 | Luteolin-7-O-glucuronide | × | ✓ | × |
| 26 | Neoandrographolide | × | ✓ | ✓ |
| 27 | Paniculide-A | ✓ | × | × |
| 28 | *p*-Coumaric acid | × | ✓ | × |
| 29 | *p*-Coumaric acid-glucoside | × | ✓ | × |
| 30 | Protocatechuic acid | ✓ | × | × |
| 31 | Quinic acid | ✓ | ✓ | ✓ |
| 32 | Scoparin | × | ✓ | ✓ |

The results of the viability assay showed a decrease in the number of living cells along with an increase in the concentration of the extract given (Fig. 3).

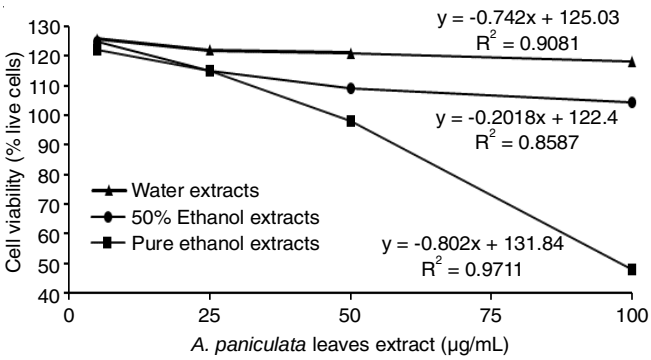


Fig. 3. Viability result test of RAW264.7 cells on water, 50% ethanol and pure ethanol extracts by MTT assay

Extracts derived from water and 50% ethanol at test doses of up to 100 µg/mL are safe against RAW 264.7 cells. This condition differs from extracts derived from pure ethanol which, at a test concentration of 100 µg/mL, causes cell death of up to 57%, leaving 43% of the cells viable [30]. The maximum test concentration with a viability of 100% can be calculated by the equation of the linear line of pure ethanol extract: $y = -802x + 131,84$; an antilog $x$ value of 40 µg/mL was obtained. This concentration was used to evaluate the activity inhibiting NO production.

**Evaluation of anti-inflammatory activity:** The anti-inflammatory activity of each extract was evaluated by measuring the production of NO in LPS-stimulated RAW 264.7 cells (Table-3). The assay results for the inhibition of NO production in LPS-stimulated RAW 264.7 cells showed that at an assay dose of 40 µg/mL pure ethanol extract had an NO inhibitory activity of 91.02%. This result was the highest when compared to aqueous extracts and 50% ethanol, which had inhibitory activities of 21.35% and -8.13%, respectively. Inhibitory activity against NO production was shown in the 50% and pure ethanol extracts. Different results were revealed by extracts with water solvents, which did not inhibit NO production. The pharmacological and biological activity of *A. paniculata* depends on the composition of its metabolites. The difference in the solvent concentration used in each extraction process has been proven to influence the yield, metabolite profile, toxicity and anti-inflammatory activity.

TABLE-3
EFFECT OF SOLVENT CONCENTRATION ON LPS-INDUCED NITRIC OXIDE (NO) RELEASE OF MACROPHAGES

| Samples | NO level (μg/mL ± SD) | | | NO inhibition (%) | | |
|---|---|---|---|---|---|---|
| | Water extracts | 50% Ethanol extracts | Pure ethanol extracts | Water extracts | 50% Ethanol extracts | Pure ethanol extracts |
| Negative control (cells only) | 0.323 ± 0.11 | 0.323 ± 0.11 | 0.323 ± 0.11 | – | – | – |
| Positive control (LPS only) | 1.531 ± 0.03 | 1.531 ± 0.03 | 1.531 ± 0.03 | – | – | – |
| LPS/extract (15 μg/mL) | 1.557 ± 0.09 | 1.300 ± 0.05 | 0.910 ± 0.05 | -1.74 | 15.09 | 40.54 |
| LPS/extract (20 μg/mL) | 1.634 ± 0.06 | 1.333 ± 0.02 | 0.702 ± 0.08 | -6.75 | 12.93 | 54.17 |
| LPS/extract (25 μg/mL) | 1.602 ± 0.03 | 1.338 ± 0.02 | 0.482 ± 0.08 | -4.66 | 12.59 | 68.49 |
| LPS/extract (30 μg/mL) | 1.591 ± 0.08 | 1.287 ± 0.03 | 0.280 ± 0.04 | -3.96 | 15.92 | 81.70 |
| LPS/extract (35 μg/mL) | 1.613 ± 0.05 | 1.259 ± 0.05 | 0.233 ± 0.02 | -5.35 | 17.73 | 84.76 |
| LPS/extract (40 μg/mL) | 1.655 ± 0.02 | 1.204 ± 0.06 | 0.138 ± 0.03 | -8.14 | 21.35 | 91.02 |

## Conclusion

The metabolite profile and inhibition of the pro-inflammatory mediator nitric oxide (NO) by *Andrographis paniculata* leaves were significantly influenced by variations in three types of extraction solvents *viz*. water solvent, 50% ethanol solvent and pure ethanol solvent. The ability of pure ethanol extract from *A. paniculata* leaves to reduce NO production by 91% indicated promising suppression of nitrite oxide activity. This condition is thought to be triggered by 14-deoxyandrographoside and 14-deoxy-17-hydroxyandrographolide that is present in the pure ethanol extract. The ability of apure ethanol extract of *A. paniculata* leaves to inhibit NO production could be utilized as a potential therapeutic for inflammatory diseases such as adverse drug reactions (ADRS) and COVID-19. However, the inhibitory activity of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 from the pure ethanol extract of *A. paniculata* leaves should be determined for future studies.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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