narra j

Original Article

Antioxidant and immunomodulatory activities of ethanol extracts from *Syzygium cumini* L. Skeels and *Pogostemon cablin* Benth

Meutia Faradilla[1,2,3], Irda Fidrianny[4] and Maria I. Iwo[1*]

[1]Department of Pharmacology-Clinical Pharmacy, School of Pharmacy, Institut Teknologi Bandung, Bandung, Indonesia; [2]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia; [3]PUI-PT Atsiri Research Center, Universitas Syiah Kuala, Banda Aceh, Indonesia; [4]Department of Pharmaceutical Biology, School of Pharmacy, Institut Teknologi Bandung, Bandung, Indonesia

*Corresponding author: mariaiwo77@gmail.com

Abstract

Syzygium cumini and *Pogostemon cablin* are mostly cultivated in tropical climates for culinary and perfumery purposes, yet their potential medicinal properties remain underreported. The aim of this study was to examine the antioxidant and immunomodulatory activities of ethanol extracts from *S. cumini* (EESC) and *P. cablin* (EEPC). Reflux extraction was carried out using 96% ethanol on the collected plant specimens to produce EESC and EEPC. Secondary metabolites of each extract were identified using gas chromatography-mass spectrometry (GC-MS). The extracts were measured for total phenol and flavonoid levels and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. The immunomodulatory activity test was carried out in vivo by assessing several parameters, including the phagocytic index via the carbon clearance method, organ indices, antibody titers, and delayed-type hypersensitivity (DTH) response, using sheep red blood cells (SRBC) as antigens. The extracts were also examined for their anti-inflammatory activity in acute and chronic inflammation models. In the DPPH antioxidant test, EESC and EEPC had IC_{50} values of 12.33 µg/mL and 182.17 µg/mL, respectively. Both extracts showed immunosuppressant activity, marked by a phagocytic index of <1. EESC yielded lower organ indices for the liver (p=0.025 at 200 mg/kg BW), spleen (p=0.028 at 100 mg/kg BW), and thymus (p=0.032 at 200 mg/kg BW) compared to the control group. For EEPC, lower organ indices were observed in the liver at 100 mg/kg BW (p=0.005) and 200 mg/kg BW (p=0.031). In the primary antibody titer and DTH tests, both EESC and EEPC showed immunosuppressant activity at 200 and 400 mg/kg BW (p<0.05). The extracts suppressed both the innate and adaptive immune systems. Both EEPC (p=0.004) and EESC at 100 mg/kg BW (p=0.03) significantly reduced serum TNF-α levels. In conclusion, EESC and EEPC have the potential as immunosuppressive and anti-inflammatory agents.

Keywords: Antioxidant, immunomodulator, immunosuppressant, *Pogostemon cablin*, *Syzygium cumini*

Introduction

Immunomodulators are compounds that can modulate (increase or decrease) immune responses in innate and adaptive immunity to encounter diseases related to the immune system [1]. These agents can be categorized as immunosuppressants, immunostimulants, and adjuvants. Immunosuppressants have a significant role in suppressing immune responses, particularly in

Received: June 7, 2024 - Accepted: August 22, 2024 - Published online: October 9, 2024

the context of autoimmune disorders. Immunomodulators influence a range of cellular processes, including apoptosis, protein production, antigen presentation, and the regulation of various transcription and immune signaling factors [2].

Existing studies have tried to find the potential to use natural materials as immunomodulators based on the content of secondary metabolites owned by these natural materials. Herbal formulations derived from medicinal plants, such as "Jamu" in Indonesia or Ayurvedic medicine in India, have shown the ability to change the function of the immune system and provide extensive activities as immunomodulators, for example, by modulating the activity of macrophages through its phagocytosis and cytokine production [3]. For many years, herbal remedies have been utilized to reduce the impact of illnesses by adjusting the reaction of the immune system. Numerous plants have been tested for their activity to regulate the immune system [4].

Several studies have shown the potential of *Syzygium cumini* as an immunomodulator. For instance, *S. cumini* leaf powder mixed in shrimp larvae feed has been shown to increase phagocytic and lysozyme activities [5]. Additionally, *S. cumini* seed extract has been reported to inhibit inflammatory reactions in animal models [6,7]. On the other hand, methanol extracts of *Pogostemon cablin* have shown anti-inflammatory effects in an animal study [8]. Patchouli alcohol, isolated from *P. cablin* leaves, has been reported to have immunomodulatory activity and the ability to increase macrophage phagocytosis activity, humoral immune responses, and cellular immune responses [9]. These studies show that medicinal plants have potential as immunomodulators, especially as immunosuppressants. However, to this day, no study has examined the immunomodulatory activities of ethanol extracts from *S. cumini* and *P. cablin* leaves. Therefore, the aim of the study was to observe the immunomodulatory effects of the two extracts by assessing their immunomodulatory impact on parameters such as phagocytosis activity via reactive oxygen species (ROS), antibody titers, and delayed-type hypersensitivity (DTH) responses.

Methods

Plant specimens

Plant specimens of *S. cumini and P. cablin* were collected from the Geunteut District, Lhong, Aceh Besar, Indonesia. The plants were identified at the Biology Research Center of the National Research and Innovation Agency in Bogor, Indonesia. The collected plants were air-dried and ground into a fine powder for further use.

Extraction process

The extraction of the dried fine powder of *S. cumini* and *P. cablin* was carried out separately. The extraction was performed using the reflux method with 96% ethanol, with a plant-to-solvent ratio of 1:10. The reflux extraction was run in three cycles, each lasting two hours. The extract solution was then concentrated using the rotary evaporator on the Rotary Evaporator Buchi at 40°C and 50 rpm. The resulting thick extract paste was evaporated in a water bath at 65°C to remove the solvent completely. The extracts were labelled EESC and EEPC for those derived from *S. cumini* and *P. cablin* leaves, respectively.

Determination of total phenolic content

The quantification of total phenolic content in both extracts was conducted using a slightly modified Folin-Ciocalteu method. A diluted sample of each plant extract or gallic acid (a standard phenolic compound) was mixed with Folin-Ciocalteu reagent and aqueous Na_2CO_3. After allowing the mixtures to stand for 15 minutes, the total phenols were determined using a spectrophotometer at 675 nm. To establish a standard reference, a calibration curve was generated using solutions of gallic acid in methanol: water (50:50, v/v) at concentrations of 0, 50, 100, 150, 200, and 250 mg/L. The total phenolic content is expressed in milligrams per gram of dry mass, equivalent to gallic acid [10].

Determination of total flavonoid content

The total flavonoid content was quantified using a modified aluminum chloride colorimetric assay, with quercetin serving as the standard reference. An extract was prepared in methanol (1

g/10 mL), and a standard curve for quercetin was established by dissolving 10 mg of quercetin in 80% ethanol and diluting it to 25, 50, and 100 µg/mL concentrations. For the analysis, 0.5 mL of either the extract or quercetin solution was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water. The mixture was then incubated for 30 minutes, and absorbance was measured at 415 nm, with distilled water replacing $AlCl_3$ in the blank control [11].

Screening and characterization of plants and extracts

Phytochemical screening and characterization were performed on the dried plants and concentrated extracts. Phytochemical screening included the examination of alkaloids, tannins, saponins, flavonoids, and steroids/triterpenoids. The characterizations analyzed from the extracts include water content, ash content, water-soluble content, ethanol-soluble content, residual solvent levels, and aflatoxin contamination (B1, B2, G1, and G2), following the procedures outlined in the Farmakope Herbal Indonesia and the World Health Organization (WHO) guidelines for quality control for medicinal plants material [12].

2,2-diphenyl-1-picrylhydrazyl assay

The examination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was conducted according to the method used in a previously published report [13]. The determination of radical scavenging activity was calculated using the following formula = radical scavenging activity (%) = (A–B)/A × 100, where, A represents the absorbance of the negative control (DPPH plus ethanol) and B corresponds to the absorbance of the sample (DPPH, ethanol plus test substance). The relationship between various concentrations and their respective scavenging percentages was established by plotting a correlation, and the IC_{50} value was derived through interpolation. The activity is expressed as IC_{50} (the inhibitory concentration of each extract that scavenges 50% of DPPH radicals).

Examination of extract composition using gas chromatography-mass spectrometry (GC-MS)

Examination of compound content in both extracts was conducted in a GC-MS system operated using a J&W capillary column with dimensions of 30 meters in length, 0.25 millimeters in internal diameter, and a film thickness of 0.25 micrometers. The interface temperature was set at 280°C, with an injection volume of 1 µL. The carrier gas flow rate was maintained at 1 mL/min, and the column temperature was programmed to range from 60°C to 240°C.

Test animals

The test animals used in this study were female BALB/c mice (*Mus musculus*) aged 6–8 weeks (three mice per group) for the immunomodulatory activity test and female Wistar rats (*Rattus novergicus*) aged 6–8 weeks for the anti-rheumatoid arthritis activity test (three rats per group). The sample size was calculated using Federer's formula. All animals were obtained from CV Kencana, West Java, Indonesia, in a healthy condition and had not been used in prior studies. Initially, all animals were acclimatized in the Quarantine Room of the Animal Laboratory of the School of Pharmacy, Institut Teknologi Bandung, Bandung, Indonesia, for seven days, under a 12-hour light-dark cycle. The animals were given a standard pellet diet and water ad libitum. Randomization and blinding procedures were not applied in this study. The use of test animals in this research had received approval from the ethics committee, and its reporting followed the ARRIVE guidelines [14].

Macrophage phagocytosis assay via carbon clearance method

The test was performed in accordance with the previous recommendation with several modifications [15,16]. The animals were divided into nine groups: a negative control group, reference drug groups (levamisole 4 mg/kg BW and methylprednisolone 15 mg/kg BW), and test groups receiving ethanol extracts of *S. cumini* (100, 200, and 400 mg/kg BW) and *P. cablin* (100, 200, and 400 mg/kg BW). A schematic diagram illustrating the group assignments is presented in **Figure 1**. The extracts and reference drugs were administered orally once daily for seven consecutive days. All groups were intravenously injected with ink suspended in gelatin 1% (dose: 0.1 mL/10 g BW) through the tail vein on the eighth day. Blood samples were collected at 0, 5,

and 15 minutes following the injection of carbon suspension. Each blood sample (50 µL) was treated with 4 mL of 0.1% sodium carbonate solution, and their optical densities were measured using a spectrophotometer at 675 nm. The phagocytic index (K), representing the rate of carbon clearance, was calculated using the following equation = (ln(OD1)–ln(OD2))/t2–t1, where OD1 is the optical density at time t1 (0 minutes after blood collection) and OD2 is the optical density at time t2 (15 minutes after blood collection). The immunomodulatory activity was determined by comparing the extract's K value or comparison group to the control group. If K>1, the substance has immunostimulant activity. Meanwhile, if K<1, the substance has immunosuppressive activity [17].

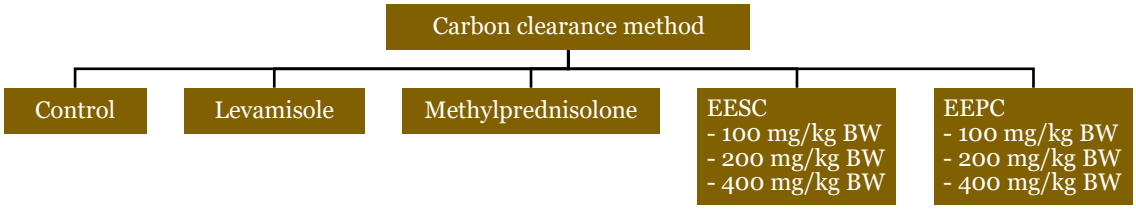

Figure 1. Grouping of animals in the carbon clearance test. EESC: ethanol extract from *S. cumini*; EEPC: ethanol extract from *P. cablin*.

Humoral antibody response

Nine groups were used in this test with the following assignments: a negative control group, reference drug groups (levamisole 4 mg/kg BW and methylprednisolone 15 mg/kg BW), and ethanol extract test groups for *S. cumini* (100, 200, and 400 mg/kg BW) and *P. cablin* (100, 200, and 400 mg/kg BW) (**Figure 2**). Animals were immunized with sheep red blood cells (SRBC) (0.1 mL/10 g BW, 10% suspension in saline) on days 3 and 8 after the last administration of the reference drugs or extracts. Upon the collection, the blood was then centrifuged at 12,000 rpm for 10 minutes to obtain the serum sample. The antibody titer was assessed using “V” microtiter plates with each well containing twofold dilutions of the antibodies present in the serum. To these wells, SRBCs were added, and then observed for hemagglutination. The highest dilution that displayed hemagglutination was recorded as the antibody titer. The antibody titer determined on days 8 and 13 following the challenge with SRBCs was categorized as the primary and secondary humoral immune responses, following the previous recommendation [18].

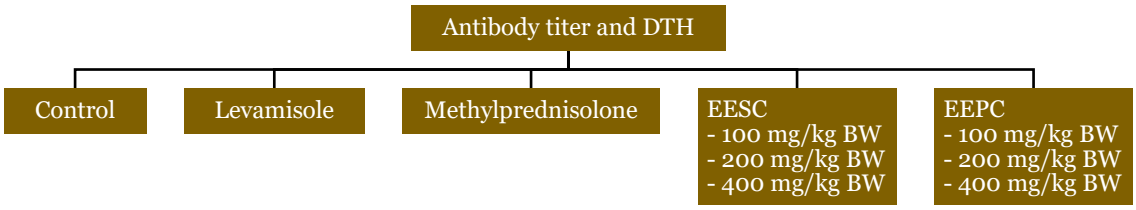

Figure 2. Grouping of animals in the antibody titer and delayed-type hypersensitivity (DTH) tests. EESC: ethanol extract from *S. cumini*; EEPC: ethanol extract from *P. cablin*.

Delayed-type hypersensitivity response

Each animal received immunization through an intraperitoneal injection of a 0.1 mL/10 g BW of SRBCs suspension on the third day after the last administration of the reference drugs or extracts. On the eighth day, the thickness of their paws was measured using a plethysmometer. Subsequently, the animals were subjected to a re-challenge by injecting 0.05 mL of SRBCs into the left hind footpad. The thickness of the paw was measured before the injection, 24 hours, and 48 hours after the challenge. The alteration in footpad thickness following the SRBC challenge was regarded as an indicator of the delayed-type hypersensitivity (DTH) response [16,19].

Acute anti-inflammatory test

An acute anti-inflammatory test was performed according to a published protocol [20]. Wistar rats were given extracts (100, 200, and 400 m/kg BW), dexamethasone 0.1 mg/kg BW, and the

control group (CMC-Na 0.5%) one hour before induction (**Figure 3**). Animals were then induced with 1% Carrageenan 0.05 mL/paw. Carrageenan-induced inflammation rats would show persistent paw swelling. Measurements of the development of rat foot swelling, including foot volume, joint circumference, and foot thickness, were observed for 30 minutes until six hours after induction. The percentage of inhibition was measured using the formula = 1 – (ΔVt/ΔVc) × 100, where ΔVt represents the delta paw volume of the group treated with the drug/extracts, and ΔVc represents the delta paw volume of the control group.

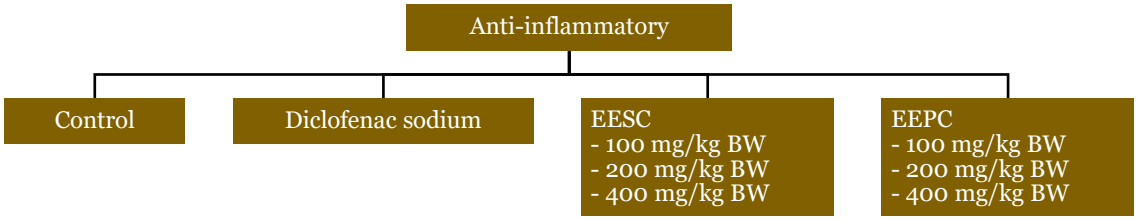

Figure 3. Grouping of animals in the acute anti-inflammatory test. EESC: ethanol extract from *S. cumini*; EEPC: ethanol extract from *P. cablin*.

Chronic anti-inflammatory test

The chronic anti-inflammatory test in this experiment was modified from a previously reported procedure [21]. Animals (rats) were divided into eight groups consisting of negative groups, an immunosuppressant group (dexamethasone 0.1 mg/kg BW), and ethanol extract test groups *S. cumini* and *P. cablin* (100, 200, and 400 mg/kg BW) (**Figure 4**). Wistar rats were induced with complete Freund's adjuvant (CFA) 0.05 mL/paw. CFA-induced rats would show persistent leg swelling. Measurements of the development of rat foot swelling, including joint circumference and foot thickness, were observed every three days from day 1 to day 30.

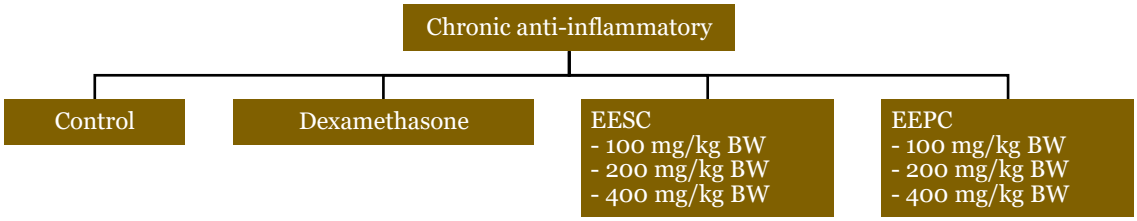

Figure 4. Grouping of animals in the chronic anti-inflammatory test. EESC: ethanol extract from *S. cumini*; EEPC: ethanol extract from *P. cablin*.

Statistical analysis

Data were checked for homogeneity (Levene's test) and normality (Shapiro-Wilk test). The data were then analyzed using one-way ANOVA with a post-hoc Fisher's multiple comparison test on Minitab Software (license provided by Institut Teknologi Bandung, Bandung, Indonesia).

Results

Plant characterization and phytochemical screening

The ethanol extract of *S. cumini* leaves (EESC) and *P. cablin* leaves (EEPC) yielded extraction percentages of 35.75% and 25.01%, respectively. The extraction reduced the total ash content for both *S. cumini* and *P. cablin* (**Table 1**). The water-soluble content increased for both *S. cumini* and *P. cablin* following the extraction. The EESC had lower ethanol-soluble content, but the EEPC had higher soluble content than their respective raw material. The solvent residual levels in EESC and EEPC were 1.31% and 0.02%, respectively. All samples were negative for the presence of aflatoxin contaminations (**Table 1**).

The phytochemical screening results are presented in **Table 2**. Following the extraction in both plants, alkaloids became undetectable. Flavonoids, steroids, and triterpenoids were

observable in plants and their respective extracts. After the extraction, *P. cablin* increased in tannins and saponins.

Table 1. Plant characterization and extracts from *S. cumini* (EESC) and *P. cablin* (EEPC)

Parameters	Plants		Extract	
	S. cumini	*P. cablin*	EESC	EEPC
Total ash content (% w/w)	6.17	5.70	1.86	4.36
Loss on drying (% w/w)	4.16	2.63	26.46	47.46
Water-soluble content (% w/w)	15.75	15.00	22.59	32.38
Ethanol-soluble content (% w/w)	27.64	20.00	22.45	32.38
Solvent residual level (%)	NA	NA	1.31	0.02
B1 aflatoxin contamination	NA	NA	Undetected	Undetected
B2 aflatoxin contamination	NA	NA	Undetected	Undetected
G1 aflatoxin contamination	NA	NA	Undetected	Undetected
G2 aflatoxin contamination	NA	NA	Undetected	Undetected

NA: not applicable

Table 2. Phytochemical screening of plants and extracts from *S. cumini* (EESC) and *P. cablin* (EEPC)

Secondary metabolites	Plants		Extracts	
	S. cumini	*P. cablin*	EESC	EEPC
Flavonoids	+	+	+	+
Alkaloids	+	+	-	-
Tannins	+	-	+	+
Saponins	+	-	+	+
Steroids/triterpenoids	+	+	+	+

Total phenolic content, total flavonoid content, and antioxidant activity

The total phenolic content of EESC (40.39±2.18 mg gallic acid equivalent (GAE)/g dried extract) was higher than EEPC (8.17±0.28 mg GAE/g dried extract). Accordingly, the results of the DPPH antioxidant activity assay suggested that EESC had higher activity (IC_{50}=12.33±0.00 µg/mL) as compared to EEPC (IC_{50}=182.17±90.89 µg/mL) (**Table 3**).

Table 3. Total phenolic content, total flavonoid content, and antioxidant activity

Sample	TPC (mg GAE/ g dried extract)	TFC (g QE/100 g dried extract)	DPPH scavenging activity	
			IC_{50} (µg/mL)	AAI
EESC, mean±SD	40.39±2.18	2.15±0.07	12.33±0.00	4.05
EEPC, mean±SD	8.17±0.28	1.89±0.11	182.17±90.89	0.27
Ascorbic acid, mean±SD	Not applicable	Not applicable	5.38±0.02	9.29

AAI: antioxidant activity index; DPPH: 2,2-diphenyl-1-picrylhydrazyl; GAE: gallic acid equivalent; QE: quercetin equivalent; TFC: total flavonoid content; TPC: total phenolic content

Compounds in the ethanol extracts

The chromatograms generated from the GC-MS analysis are presented in **Figure 5**. The GC-MS analysis revealed that EESC contained several compounds such as guaiol; β-eudesmol; 2-(4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydro-2-naphthalenyl)-2-propanol; myristic acid; and hexadecenoic acid. Meanwhile, the EEPC was observed to contain seychellene, α-patchoulene, (-)-globulol, dihydro-costunolide, patchouli alcohol, methyl isomyristate, santalcamphor, and methyl palmitate (**Table 4**).

Table 4. Gas chromatography-mass spectrometry (GC-MS) results from the extracts

Identified compound	Retention time (minutes)	Molecular weight (g/mol)
Ethanol extract from *S. cumini* (EESC)		
Guaiol	18.446	222
β-eudesmol	19.104	222
2-(4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydro-2-naphthalenyl)-2-propanol	19.127	222
Myristic acid, methyl ester	19.670	242
Hexadecanoic acid, methyl ester	21.854	270
Ethanol extract from *P. cablin* (EEPC)		

Identified compound	Retention time (minutes)	Molecular weight (g/mol)
Seychellene	16.592	204
α-patchoulene	16.765	204
(-)-globulol	19.133	222
Dihydro-costunolide	19.179	234
Patchouli alcohol	19.283	222
Methyl isomyristate	19.670	242
Santalcamphor	21.652	236
Methyl palmitate	21.854	270

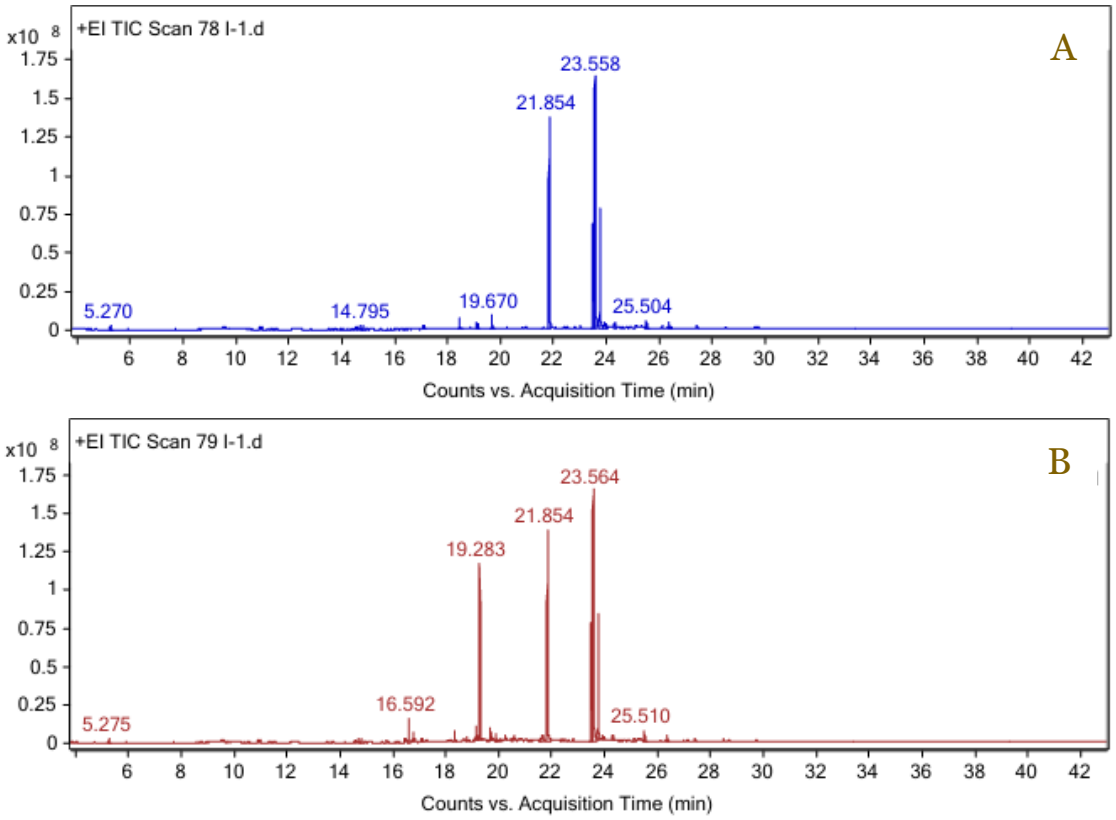

Figure 5. Gas chromatography-mass spectrometry (GC-MS) chromatograms for ethanol extracts from *S. cumini* (EESC) (A) and ethanol extracts from *P. cablin* (EEPC) (B).

Immunomodulatory activity

The result of the carbon clearance test showed that both extracts at all dosages had immunosuppressive activity, as indicated by K<1 (**Table 5**). The effects of the extracts on organ indices are presented in **Figure 6**. Compared to the control, a significantly lower liver index was observed in EESC (p=0.025) and EEPC (p=0.031) at the same dosage of 200 mg/kg BW. EEPC 100 mg/kg BW had the lowest liver index (p=0.005) compared to control. EESC 100 mg/kg BW had a significantly lower spleen index than control (p=0.028). Thymus indices were lower in EESC at 200 (p=0.032) and 400 mg/kg BW (p=0.041) compared to the control group.

Table 5. Effect of extracts from *S. cumini* (EESC) and *P. cablin* (EEPC) on macrophage phagocytic index

Groups	Dose (mg/kg BW)	Phagocytic index (K)	Category
Levamisole	4	1.237	Immunostimulant
Methylprednisolone	15	0.762	Immunosuppressant
EESC	100	0.733	Immunosuppressant
	200	0.731	Immunosuppressant
	400	0.722	Immunosuppressant
EEPC	100	0.689	Immunosuppressant
	200	1.020	Immunosuppressant
	400	0.517	Immunosuppressant
Control	Not applicable	1	Not applicable

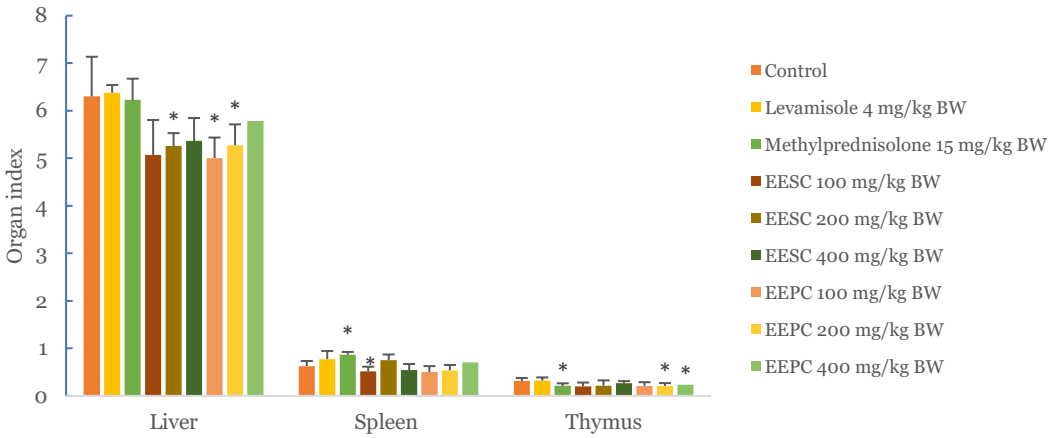

Figure 6. Immunosuppressive effects of ethanol extracts from *S. cumini* (EESC) and *P. cablin* (EEPC) based on organ indices. *Statistically significant at $p<0.05$ as compared to control. Data was presented in mean±SD (n=3) and analyzed using one-way ANOVA, followed by Fisher's multiple comparison test.

Both EESC and EEPC showed potential as immunomodulators. In the primary antibody test, the three doses of EESC gave lower agglutination results than the control, while EEPC gave the same results at a dose of 100 and 200 mg/kg BW (**Table 6**). The effect of EESC and EEPC treatment on mice paw volume is presented in **Figure 7**. A decline in T lymphocyte activity was observed in a significant reduction of mice paw volume 24 hours post-induction of levamisole (p=0.044) and EEPC doses of 200 (p=0.037) and 400 mg/kg (p=0.048). As the paw volume in the control group experienced a decrease in the next 24 hours, the difference between the extract and control group was no longer significant in the extract groups (p>0.05).

Table 6. Effect of extracts from *S. cumini* (EESC) and *P. cablin* (EEPC) on humoral antibody response

Groups	Primary antibody titer	Secondary antibody titer
Levamisole 4 mg/kg BW	1:1,024	1:8,192
Methylprednisolone 15 mg/kg BW	1:512	1:512
EESC 100 mg/kg BW	1:1,024	1:4,096
EESC 200 mg/kg BW	1:512	1:8,192
EESC 400 mg/kg BW	1:1,024	1:8,192
EEPC 100 mg/kg BW	1:512	1:4,096
EEPC 200 mg/kg BW	1:512	1:8,192
EEPC 400 mg/kg BW	1:2,048	1:8,192
Control	1:1,024	1:4,096

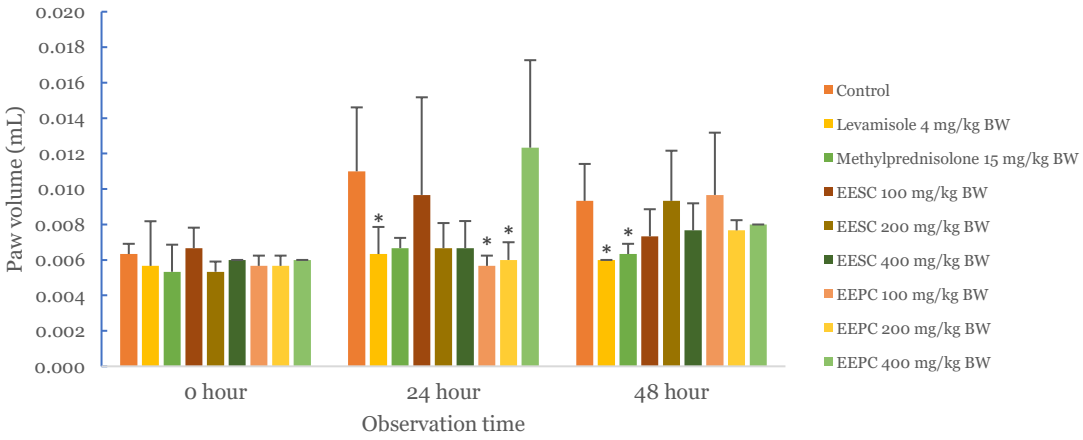

Figure 7. Effects of ethanol extracts from *S. cumini* (EESC) and *P. cablin* (EEPC) on inflammation rat model based on delayed-type hypersensitivity (paw volume). *Statistically significant at $p<0.05$ compared to control. Data was presented in mean±SD (n=3) and analyzed using one-way ANOVA, followed by Fisher's multiple comparison test.

Acute anti-inflammatory activity

From the test, both EESC and EEPC extracts showed inhibition on the formation of inflammation after the injection of carrageenan (**Table 7**). The inhibitory effect is observable three hours after carrageenan injection on each animal's hind paw, and it reached maximum inhibition six hours after the induction (**Figure 8**). Six hours after induction, both EESC and EEPC at 400 mg/kg BW had the highest inhibitory effect, close to the diclofenac sodium group.

Table 7. Inhibitory effects of ethanol extracts from *S. cumini* (EESC) and *P. cablin* (EEPC) on carrageenan-induced inflammatory reaction

Group	Inhibition (%)			
	3 hours	4 hours	5 hours	6 hours
Control	0	0	0	0
Diclofenac sodium (5 mg/kg BW)	43.83	25.76	26.67	50.98
EESC 100 mg/kg BW	17.90	4.54	6.67	19.61
EESC 200 mg/kg BW	17.90	64.65	42.22	43.14
EESC 400 mg/kg BW	39.51	15.15	26.67	47.06
EEPC 100 mg/kg BW	35.18	25.76	37.78	15.69
EEPC 200 mg/kg BW	17.90	29.29	40.00	29.41
EEPC 400 mg/kg BW	48.15	36.36	60.00	45.10

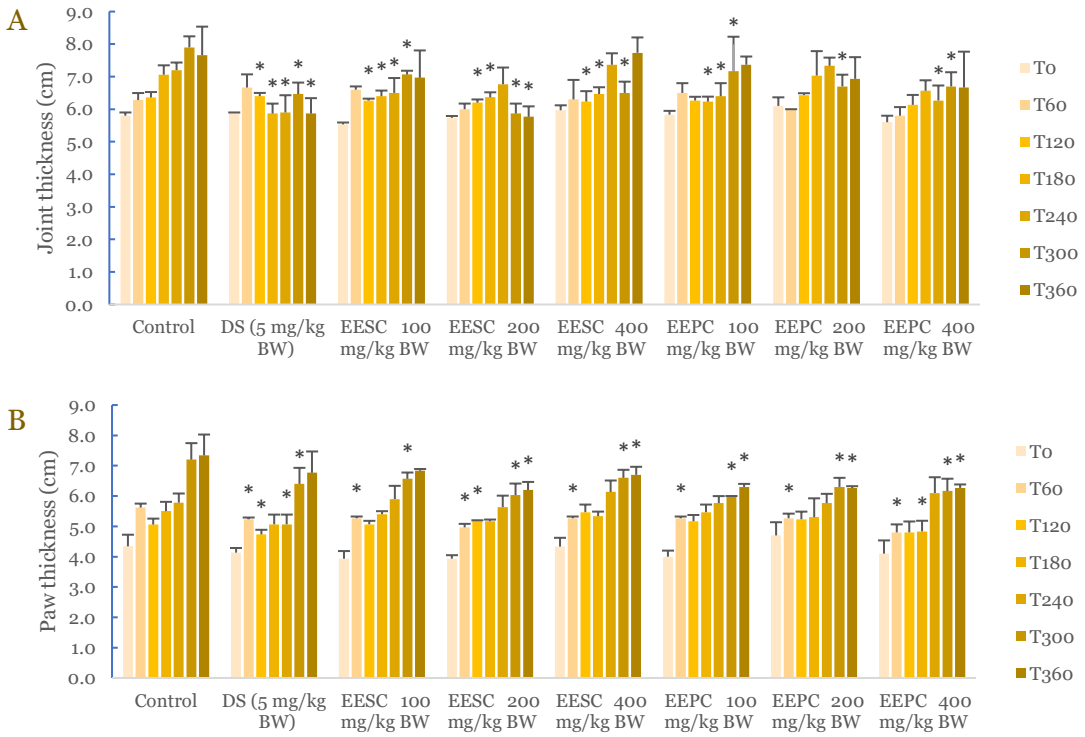

Figure 8. Joint (A) and paw thickness (B) of hind paw rats of acute inflammation test. *Statistically significant at $p<0.05$ as compared to control. Data was presented in mean±SD (n=3) and analyzed using one-way ANOVA, followed by Fisher's multiple comparison test. DS: diclofenac sodium. Legend indicated time (minutes).

From the test results, it was found that EESC and EEPC were able to reduce inflammation that appeared in experimental animals. Both EESC and EEPC at doses 200 and 400 mg/kg BW reduced the joint edema formed in the experimental animals induced by CFA (**Figure 9**). The effect of the extract on the serum TNF-α concentrations is presented in **Figure 10**. The TNF-α concentrations are lower in EEPC at concentrations of 100 (p=0.004), 200 (p=0.003), and 400 mg/kg BW (p=0.004) than in control. In animals receiving EESC 100 mg/kg BW, the TNF-α was reduced significantly (p=0.03).

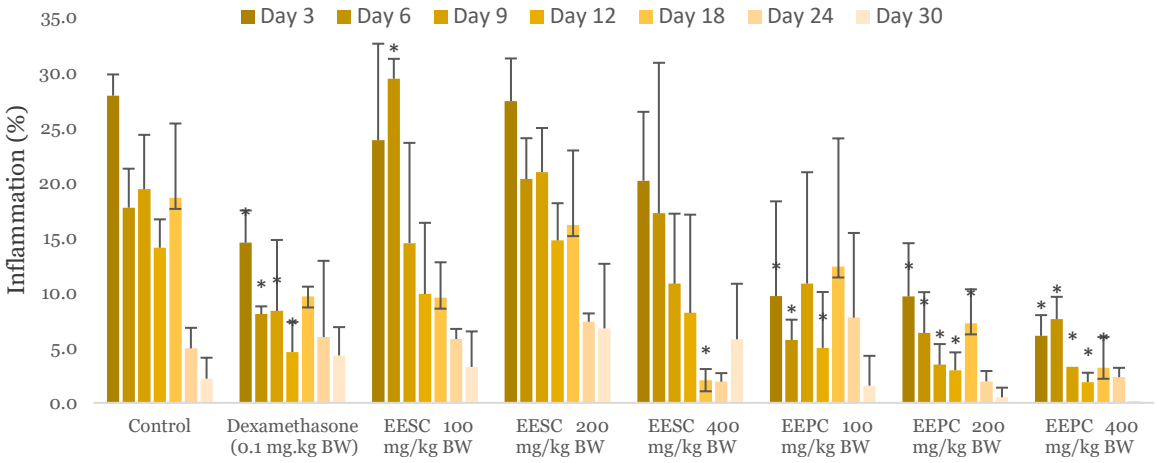

Figure 9. Percentage of inflammation on the joint of rats on rheumatoid arthritis test. *Statistically significant at $p<0.05$ as compared to control. Data was presented in mean±SD (n=3) and analyzed using one-way ANOVA, followed by Fisher's multiple comparison test.

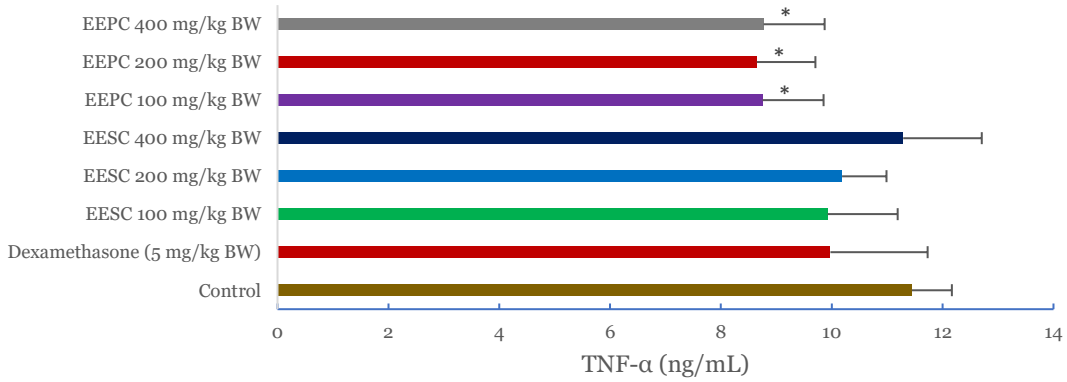

Figure 10. TNF-α concentration of rats induced by complete Freund's adjuvant (CFA) after 30 days of treatment. *Statistically significant at $p<0.05$ as compared to control. Data was presented in mean±SD (n=3) and analyzed using one-way ANOVA, followed by Fisher's multiple comparison test.

Discussion

Findings from the present study suggest that both extracts had antioxidant activity, indicated by their antioxidant activity indices. These extracts also had immunosuppressive activity, where a phagocytic index of less than one was observed. Moreover, animals receiving the extract treatment had lower organ indices than the control, suggesting the ability of EESC and EEPC to inhibit T-cell activity. Both extracts were also observed to provide anti-inflammatory activity in acute and chronic conditions. These findings imply that both extracts may exert immunosuppressant activity because of their ability to attenuate chronic inflammation. In autoimmune disease, the underlying pathomechanism is the chronic inflammation. This is in line with reported studies, where plants from the genus *Syzygium* were reported to have antioxidant, immunosuppressive and anti-inflammatory activities that are beneficial for autoimmune disease treatment [22].

Despite the absence of clinical evidence, the use of *S. cumini* had been traditionally used for diabetes, poisoning, centipede bites, gastric issues, and kidney disorders. Based on previous in vitro studies, *S. cumini* leaves were found to have anti-inflammatory and antioxidant activities [23-26]. The leaf extract was positive for flavonoids, tannins, saponins, and steroid/triterpenoids. In addition to the results from the qualitative screening, the GC-MS analysis suggested the presence of terpenoids, including β-eudesmol. As previously reported, the leaves of *S. cumini* are also rich in flavonol glycosides, quercetin, myricetin, myristicin, and triterpenoids [27,28]. In human mast cells, β-eudesmol had been observed to inhibit the production and expression of

interleukin (IL)-6, along with the decrease in IgE, IL-1β, IL-4, IL-5, IL-16, IL-13, and vascular endothelial growth factor serum levels [29]. Furthermore, β-eudesmol was reported to reduce the levels of serum histamine, IgE, thymic stromal lymphopoietin, IL-1β, tumor necrosis factor-alpha (TNF-α), and macrophage inflammatory protein-2 (MIP-2) in ovalbumin-induced allergic rhinitis mice [30].

As for the EEPC extract, the present study suggested that the extract contained patchouli alcohol, Seychellene, α-patchoulene, (-)-globulol, dihydro-costunolide, (E)-atlantone, hexadecenoic acid; methyl isomyristate, santalcamphor, and methyl palmitate. Patchouli alcohol is the main component and also acts as a marker in *P. cablin*. Chronic anti-inflammatory tests in the present study revealed that the EEPC could reduce serum TNF-α levels. Previously, patchouli alcohol (20 mg/kg BW) was found to repress delayed-type hypersensitivity reactions in Kunming mice significantly [9]. Patchouli alcohol also had the ability to reduce the production and expression of several pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [31-33].

A previous study reported that *Terminalia catappa*, a plant belonging to the same order as *S. cumini* (Myrtales), had immunosuppressive activity targeting pro-inflammatory cytokines and chemokines such as TNF-α, IL-1β, IL-23, IL-6, and cytokine-induced neutrophil chemoattractant-1 as observed in rats with colitis [34]. Another study revealed that the isolate from *Eucalyptus globulus* Labill (a plant from order Myrtales) could form a binding with phosphoinositide 3-kinase gamma that consequently inhibits the differentiation of spleen cells from a mouse model of allergic contact dermatitis [35]. In addition, compounds isolated from *P. cablin* had been reported to inhibit T-cell proliferation, suppress early- and mid-stage activation of T-cells, reduce serum IL-6 levels in a DTH model [36].

The use of phytotherapy could offer potential benefits for addressing diseases stemming from immune system dysfunction. This approach involves the dynamic regulation of informational molecules like hormones, cytokines, chemokines, neurotransmitters, and other peptides. Alternative therapies rely on medicinal plants that can modulate diverse biomolecules, thereby facilitating favorable biochemical responses that impact the immune system and help maintain homeostasis. Consequently, incorporating these plant-based components in future immunomodulation strategies could be valuable in enhancing overall health [37].

The present study is primarily limited by the lack of randomization and blinding procedures, which may introduce bias and affect the validity and reliability of the results. Additionally, while the study provided evidence of the immunosuppressive and anti-inflammatory activities of the extracts, it did not establish a direct causal relationship between the observed effects and the specific compounds identified. The study also did not explore potential side effects or toxicity. Furthermore, the research relied heavily on animal models, and the findings might not be directly applicable to humans without further clinical trials. Moreover, the study's coverage is limited to basic parameters of the immune system, such as phagocytic activity and humoral and cellular immune responses. There are many other specific parameters that could better elucidate the mechanisms of action of *S. cumini* and *P. cablin* extracts in modulating the immune system, such as a more comprehensive examination of pro-inflammatory cytokine levels and a direct examination of T-cell levels. Additionally, the present study did not investigate the long-term effects of the extracts, which are crucial for understanding their potential use in chronic conditions.

Conclusion

In conclusion, EESC and EEPC at doses 200 and 400 mg/kg BW have the potential immunomodulatory activity as indicated by the attenuation of macrophage and T-cell activity. In addition, both extracts also showed strong and weak DPPH antioxidant activities for EESC and EEPC, respectively. Both extracts also showed anti-inflammatory activity both for acute inflammation and chronic inflammation. Further research should be conducted to confirm the utility of these extracts as immunosuppressants in autoimmune disease models, such as systemic lupus erythematosus. Future studies should examine various cytokines and chemokines to determine the mechanisms of action of both extracts.

Ethics approval

This study has received the approval of the Institut Teknologi Bandung Animal Experiment Ethics Committee with the numbers KEP/I/2022/XII/H171122MF/INJS; KEP/I/2022/XII/H171122MF/INJK; and KEP/I/2022/XII/H171122MF/INJH.

Acknowledgments

The authors would like to thank the Center for Education Services (Pusat Layanan Pendidikan) under the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia, the Indonesia Endowment Funds for Education (LPDP) (grant number: 202101121420), and PUI-PT Atsiri Research Center, Universitas Syiah Kuala, Banda Aceh, Indonesia.

Competing interests

All the authors declare that they have no conflict of interest.

Funding

This research was funded by Higher Education Directorate General for the PDD Grant with grant number 083/E5/PG.02.00.PT/2022 (330/IT1. B07.1/SPP-LPPM/V/2022).

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

Faradilla M, Fidrianny I, Iwo MI. Antioxidant and immunomodulatory activities of ethanol extracts from *Syzygium cumini* L. Skeels and *Pogostemon cablin* Benth. Narra J 2024; 4 (3): e918 - http://doi.org/10.52225/narra.v4i3.918.

References

1. Behl T, Kumar K, Brisc C, *et al.* Exploring the multifocal role of phytochemicals as immunomodulators. Biomed Pharmacother 2021;133:110959.
2. Nair A, Chattopadhyay D, Saha B. Plant-derived immunomodulators. In: Khan MSA, Ahmad I, Chattopadhyay D, editors. New look to phytomedicine: Advancements in herbal products as novel drug leads. London: Academic Press; 2019.
3. Catanzaro M, Corsini E, Rosini M, *et al.* Immunomodulators inspired by nature: A review on curcumin and echinacea. Molecules 2018;23(11):2778.
4. Siddiqui M, Aslam A, Khan T. Comparison and evaluation of different seed extracts of *Trachyspermum ammi* for immunomodulatory effect on cell-mediated immunity through delayed-type hypersensitivity assay skin thickness method. J Pharm Bioallied Sci 2019;11(1):43-48.
5. Prabu DL, Chandrasekar S, Ambashankar K, *et al.* Effect of dietary *Syzygium cumini* leaf powder on growth and non-specific immunity of *Litopenaeus vannamei* (Boone 1931) and defense against virulent strain of *Vibrio parahaemolyticus*. Aquaculture 2018;489:9-20.
6. Kumar A, Ilavarasan R, Jayachandran T, *et al.* Anti-inflammatory activity of *Syzygium cumini* seed. Afr J Biotechnol 2008;7(8):941-943.
7. Modi DC, Patel JK, Shah BN, *et al.* Antiinflammatory activity of seeds of syzygium cumini linn. J Pharm Educ Res 2010;1:68-70.
8. Swamy M, Sinniah U. A Comprehensive review on the phytochemical constituents and pharmacological activities of *Pogostemon cablin* Benth.: An aromatic medicinal plant of industrial importance. Molecules 2015;20(5):8521-8547.
9. Liao J, Wu D, Peng S, *et al.* Immunomodulatory potential of patchouli alcohol isolated from *Pogostemon cablin* (Blanco) Benth (Lamiaceae) in mice. Trop J Pharm Res 2013;12(4):559-565.
10. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol 2006;5(11):1142-1145.
11. Chang CC, Yang MH, Wen HM, *et al.* Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002;10(3):178-182.

12. World Health Organization. Quality control methods for medicinal plant materials. Geneva: World Health Organization; 1998.
13. Salazar-Aranda R, Pérez-López LA, López-Arroyo J, *et al.* Antimicrobial and antioxidant activities of plants from northeast of Mexico. Evid Based Complement Alternat Med 2011;2011:536139.
14. Percie du Sert N, Hurst V, Ahluwalia A, *et al.* The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. J Cereb Blood Flow Metab 2020;40(9):1769-1777.
15. Ghule BV, Murugananthan G, Nakhat PD, *et al.* Immunostimulant effects of *Capparis zeylanica* Linn. leaves. J Ethnopharmacol 2006;108(2):311-315.
16. Sahu M, Mali P, Waikar S, *et al.* Evaluation of immunomodulatory potential of ethanolic extract of *Roscoea procera* rhizomes in mice. J Pharm Bioallied Sci 2010;2(4):346-349.
17. Wagner H, Jurcic K. Immunological studies of Revitonil, a phytopharmaceutical containing *Echinacea purpurea* and *Glycyrrhiza glabra* root extract. Phytomedicine 2002;9(5):390-397.
18. Ganeshpurkar A, Saluja AK. Experimental animal models used for evaluation of potential immunomodulators: A mini review. Bull Fac Pharm Cairo Univ 2017;55(2):211-216.
19. Puri A, Saxena R, Saxena RP, *et al.* Immunostimulant activity of *Nyctanthes arbor-tristis* L. J Ethnopharmacol 1994;42(1):31-37.
20. Karim N, Khan I, Khan W, *et al.* Anti-nociceptive and Anti-inflammatory Activities of Asparacosin A involve selective cyclooxygenase 2 and inflammatory cytokines inhibition: An in-vitro, in-vivo, and in-silico approach. Front Immunol 2019;10:581.
21. Ih H, Kusharyanti I, Iwo MI. Antiarthritic activity of pacar air (*Impatiens balsamina* Linn.) herb extract in animal model of rheumatoid arthritis - an autoimmune disease. Int J PharmTech Res 2016;9(3):131-137.
22. Cock IE, Cheesman M. The potential of plants of the genus *Syzygium* (Myrtaceae) for the prevention and treatment of arthritic and autoimmune diseases. In: Watson RR, Preedy VR, editors. Bioactive food as dietary interventions for arthritis and related inflammatory diseases. London: Academic Press; 2019.
23. Machado RRP, Jardim DF, Souza AR, *et al.* The effect of essential oil of *Syzygium cumini* on the development of granulomatous inflammation in mice. Rev Bras Farmacogn 2013;23(3):488-496.
24. Ruan ZP, Zhang LL, Lin YM. Evaluation of the antioxidant activity of *Syzygium cumini* leaves. Molecules 2008;13(10):2545-2556.
25. Jain PLB, Patel SR, Desai MA. Patchouli oil: An overview on extraction method, composition and biological activities. J Essent Oil Res 2022;34(1):1-11.
26. Franco RR, Ribeiro Zabisky LF, Pires de Lima Júnior J, *et al.* Antidiabetic effects of *Syzygium cumini* leaves: A non-hemolytic plant with potential against process of oxidation, glycation, inflammation and digestive enzymes catalysis. J Ethnopharmacol 2020;261:113132.
27. Ayyanar M, Subash-Babu P. *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. Asian Pac J Trop Biomed 2012;2(3):240-246.
28. Dos Santos MM, De Souza Prestes A, De Macedo GT, *et al. Syzygium cumini* leaf extract protects macrophages against the oxidized LDL-induced toxicity: A promising atheroprotective effect. Biomed Pharmacother 2021;142:111196.
29. Acharya B, Chaijaroenkul W, Na-Bangchang K. Therapeutic potential and pharmacological activities of β-eudesmol. Chem Biol Drug Des 2021;97(4):984-996.
30. Costa WK, Oliveira JRSD, Oliveira AMD, *et al.* Essential oil from *Eugenia stipitata* McVaugh leaves has antinociceptive, anti-inflammatory and antipyretic activities without showing toxicity in mice. Ind Crops Prod 2020;144:112059.
31. Jeong JB, Choi J, Lou Z, *et al.* Patchouli alcohol, an essential oil of *Pogostemon cablin*, exhibits anti-tumorigenic activity in human colorectal cancer cells. Int Immunopharmacol 2013;16(2):184-190.
32. Wang HT, Wang ZZ, Wang ZC, *et al.* Patchouli alcohol attenuates experimental atherosclerosis via inhibiting macrophage infiltration and its inflammatory responses. Biomed Pharmacother 2016;83:930-935.
33. Hu G, Peng C, Xie X, *et al.* Availability, pharmaceutics, security, pharmacokinetics, and pharmacological activities of patchouli alcohol. Evid Based Complement Alternat Med 2017;2017:1-9.
34. Abiodun OO, Rodríguez-Nogales A, Algieri F, *et al.* Antiinflammatory and immunomodulatory activity of an ethanolic extract from the stem bark of *Terminalia catappa* L. (Combretaceae): In vitro and in vivo evidences. J Ethnopharmacol 2016;192:309-319.
35. Hu XL, Shen W, Wang R, *et al.* Discovery of Eucalyptin C, derived from the fruits of *Eucalyptus globulus* Labill., as a novel selective PI3Kγ inhibitor for immunosuppressive treatment. Chin J Nat Med 2021;19(11):844-855.

36. Su J, He J, Su Z, *et al.* T cell inhibition by pogostone from *Pogostemon cablin* (Blanco) Benth: In vitro and in vivo immunosuppressive analysis. Mol Med Rep 2017;16(4):4511-4520.

37. Khandelwal V, Choudhary PK, Goel A, *et al.* Immunomodulatory activity of *Neolamarckia cadamba* (Roxb.) Bosser with reference to IL-2 induction. Indian J Tradit Knowl 2018;17(3):451-459.