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Original Research Article

Anti-Plasmodial Activities of Ethanol Extract of *Eucheuma cottonii* and *Sargassum polycystum* Seaweeds Against *Plasmodium falciparum*

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Abstract: ***Introduction:*** The increasingly widespread resistance of malaria parasites to currently available anti-malarial drugs has encouraged researchers to search for natural ingredients that are safe and effective in eradicating malaria. Previously, the search for natural medicinal ingredients was performed by exploiting organisms on land. This trend has changed, and marine-derived medicinal ingredients have become increasingly in demand. This study was conducted to investigate the potential of red seaweed (*Eucheuma cottonii*) and brown seaweed (*Sargassum polycystum*) as antiplasmodial agents against *Plasmodium falciparum*. ***Methodology:*** Ethanol extracts of the two seaweeds were qualitatively tested for the presence of saponins, alkaloids, flavonoids, tannins, and terpenoids. Gas chromatography–mass spectrometry was performed to determine the metabolite profiles of the extracts. The antiplasmodial properties of the extracts against malaria parasites were determined by calculating the percentage of parasitemia inhibition, which was then used as a variable in probit analysis to determine the IC_{50} values of the extracts. ***Results:*** Saponins, alkaloids, flavonoids, tannins, and terpenoids were identified in the extracts. Hexadecanoic acid (CAS) palmitic acid, and heptadecene-(8)-carbonic acid-(1) were found in both extracts, where asazulene (CAS) cyclopentacycloheptene,1,2-benzenedicarboxylic acid, and dioctyl ester (CAS) dioctyl phthalate were identified only in the brown seaweed extract. Probit analysis in the anti-plasmodial assays revealed that the IC_{50} values of the red and brown seaweed extracts were 11.067and 10.214µg/mL, respectively. ***Conclusions:*** Given their IC_{50} values, the red and brown seaweed extracts can be categorized as active anti-plasmodials. Thus, they could be developed as anti-malarial ingredients.

Keywords: Seaweeds; *Eucheuma cottonii*; *Sargassum polycystum*: *Plasmodium falciparum*; antimalarial; antiplasmodial.

INTRODUCTION

In the five years since 2016, malaria cases have increased globally, with an estimated 247 million cases in 2021 in 84 malaria-endemic countries. The largest increase in the number of malaria cases occurred in countries in the WHO African Region, where the estimated number was 232 million in 2020 and 234 million in 2021. In 2021, in nine malaria-endemic countries in the WHO Southeast Asia Region, including Indonesia, 5.4 million cases were recorded, contributing 2% of the global burden of malaria cases [1].

The high incidence of malaria is a major health problem in humans. Another challenge related to efforts to eradicate malaria is the increasing resistance of malaria parasites to the most available anti-malarial drugs. *Plasmodium* resistance to modern synthetic drugs was first observed in the early 1930s. However, this resistant case did not present a serious challenge to anti-malaria programs because the malaria parasites continued to be highly susceptible to 4-aminoquinolines, especially chloroquine and amodiaquine. However, in the 1960s, *Plasmodium falciparum* resistant to 4-aminoquinolines was reported [2].

In the 1980s, malarial resistance to chloroquine continued to spread, becoming a major health challenge worldwide [3,4]. In the late 1990s, when chloroquine was no longer effective, the artemisinin-based combination therapy (ACT) was used to treat malaria [5]. While containment efforts to stop the spread of resistant *Plasmodium* were underway in the 2010s, resistance to ACT had emerged in various countries [6].

The rapid emergence and spread of resistant malaria parasites, especially *P. falciparum*, to nearly all available anti-malarial drugs pose a threat to malaria control and eradication efforts. This anti-plasmodial drug resistance has prompted the search for novel, effective, and affordable anti-malarial drugs to continue[7]. Infectious disease researchers in malaria-endemic countries are relentlessly conducting research to find natural active ingredients to be developed into anti-malarial drugs. Various active ingredients from plants [8,9], fungi [10], bacteria [11], and animals [12] with anti-plasmodial properties against malaria parasites have been identified.

This study was conducted to determine whether the active substances present in commercial red seaweed (*Eucheuma cottonii* Weber Bosse) and wild brown seaweed (*Sargassum polycystum* C.Agardh) have anti-plasmodial properties against *P.falciparum*. The research idea for this study was based on results of previous studies that red macroalgae (Rhodophyta) and brown macroalgae (Phaeophyta) contain substances with antibacterial [13,14], antioxidant [15], and anticancer[16] properties. Chemical screening and assays have shown that seaweeds are rich in bioactive substances, including tannins, saponins, alkaloids, flavonoids, and terpenoids [17].

METHODOLOGY

Seaweed samples and extraction

Fresh samples of *E.cottonii* and *S.polycystum* were collected from Ruguk Village in the Ketapang District, South Lampung Regency, Lampung Province, Indonesia. The samples were washed with running water, cut into pieces, and then air-dried for 4 days. Furthermore, the drying process was carried out using an oven at 35–40°C until completely dry. After drying, the samples were mechanically ground into simplicia powder by using an electrical blender. Next, 500 g of each simplicia was macerated separately using 96% ethanol (5 L) until exhaustion. After filtration, the extracts were evaporated using a rotary evaporator until a thick extract was obtained. The stock extract was stored at 4°C until subsequent assays.

Metabolite identification

Standard phytochemical tests for total saponins, alkaloids, flavonoids, tannins, and terpenoids were conducted to determine the presence or absence of secondary metabolites in each seaweed extract.

Saponins were detected using a foam test. A0.5 mL aliquot of each extract was added with 5 mL of distilled water. The mixture was shaken until the extract was completely dissolved. Foam indicated the presence of saponins.

Alkaloids were identified using Mayer's test. Each sample was added with 5 drops of chloroform and 5 drops of Mayer's reagent (1 g of KI dissolved in 20 mL of distilled water containing 0.271 g of $HgCl_2$). The presence of alkaloids was indicated by a white–brownish color.

For flavonoids, a 0.5 mL sample was added with Mg powder (0.5 g) and 5 mL of HCl drop wise. The presence of flavonoids was indicated by the foam and red or yellow color of the solution.

For tannins, a ferric chloride test was performed. A 1 mL sample was added with 3 drops of $FeCl_3$. The change in the color of the solution to black–bluish indicated the presence of tannins.

Terpenoids were identified by performing Salkowski's test. Each extract was mixed with 2 mL of chloroform and 3 mL of concentrated H_2SO_4. A reddish-brown coloration of the interface indicated the presence of terpenoids.

Gas chromatography–mass spectrometry (GC–MS) of the extract

The two ethanol extracts were measured through GC–MS using a Shimadzu QP 2010 SE system equipped with Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (polyethylene glycol) standard columns. The components were identified based on molecular structure, molecular mass, and calculated fragments. Datainterpretation was based on mass spectral matching with standard reference compounds in *Wiley Spectral Library*version 7.

Assay for antiplasmodial activity

Both extracts were evaluated for their anti-malarial activities against the chloroquine-sensitive *P.falciparum* strain 3D7. *P. falciparum*isolates were obtained from the Center-Natural Product Medicine Research and Development, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.

Before being used in testing, the isolates were subjected to continuous cultivation and maintained using type O-positive human erythrocytes. An anti-plasmodial assay was initiated by diluting 1 mg of each extract in 100 mL of *dimethyl sulfoxide*. Serial concentrations (100, 10, 1, 0.1, and 0.01µg/mL) of each seaweed extract were prepared.

The extract (2 µL) was added to a microplate well containing 198 µL of parasite suspension with 1% initial parasitemia. The test plates were placed in a chamber containing a gas mixture (5% O2, 5% CO_2, and 90% N_2) under controlled conditions and then incubated at 37°C for 48 h. After incubation, the cultures were harvested to prepare thin blood smears stained with20% Giemsa dye on glass slides.

Data analyses

For parasitaemia measurement, the slides were observed under a microscope to determine the number of parasites in the blood smear. Ten fields on each slide were observed to calculate the percentage of parasitemia using the following formula:

$$\text{Parasitaemia } (\%) = \frac{\sum \text{parasitizied RBC}}{\sum \text{ of RBC}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

The data obtained from Equation 1 were then used to calculate the percentage of parasitemia growth (Equation 2) and parasitemia suppression (Equation 3) induced by the extracts.

$$\text{Growth } (\%) = \%\ \text{parasitaemia} - \text{Do}\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

where D_0 represents initial 1% parasitaemia prior to incubation.

$$\text{Suppression}(\%) = 100 - \frac{\text{Treated parasitaemia}}{\text{Control parasitaemia}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots(3)$$

A probit analysis in the Minitab statistical software package*was used to determine the seaweed extract concentration that causes half-maximal inhibition of P. falciparum (*IC_{50} *values).* The IC_{50} valueswere determined by plotting the extract concentration on the X-axis and the suppression percentage on the Y-axis with dose–response curves. The anti-plasmodial activities of the extracts were categorized into four groups based on the IC_{50} values: very active (IC_{50} < 5 µg/mL); active (5 < IC_{50} < 50 µg/mL); weakly active (50 < C_{50} < 100 µg/mL); and inactive (IC_{50} >100 µg/mL)[18].

RESULTS

Secondary metabolite profile

Qualitative analysis results of the extracts are presented in Table 1 and Fig.1. All methods commonly used to identify secondary metabolites in plants applied in this study positively indicated the presence of saponins, alkaloids, flavonoids, tannins, and terpenoids.

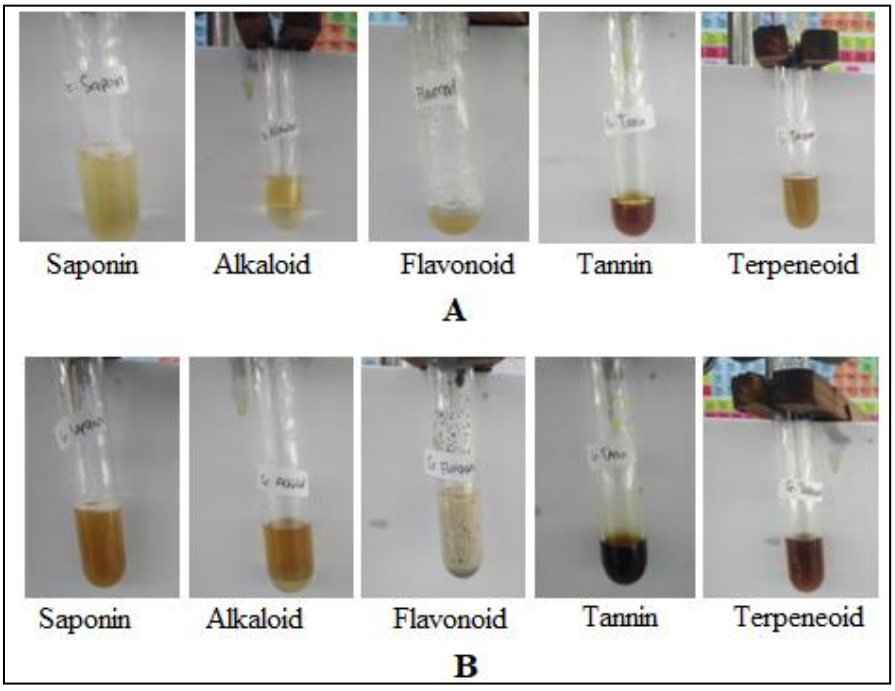

Figure 1: Qualitative analysis results of (A) red seaweed (*Eucheuma cottonii*) and (B) brown seaweed (*Sargassum falciparum*)

Table 1: Secondary metabolites identified from ethanol extracts of red (*Eucheuma cottonii*) and brown (*Sargassum polycystum*) seaweed

Endah Setyaningrum	Endah Setyaningrum	Endah Setyaningrum	
		Endah Setyaningrum	Endah Setyaningrum
Saponin	Distilled water in foam test	+	+
Alkaloid	Mayer's reagent	+	+
Flavanoid	Mg powder and concentrated HCl	+	+
Tannin	Ferric chloride ($FeCl_3$)	+	+
Terpenoid	Concentrated H_2SO_4	+	+
*(+) means the metabolites are present, (-) means absent			

GC–MS metabolite profiles

The GC–MS metabolite profile of the red seaweed extract is shown in a chromatogram in Fig.2.The two peaks in the chromatogram indicated the presence of secondary metabolites hexadecanoic acid (CAS) palmitic acid and heptadecene-(8)-carbonic ccid-(1) (Table 2).

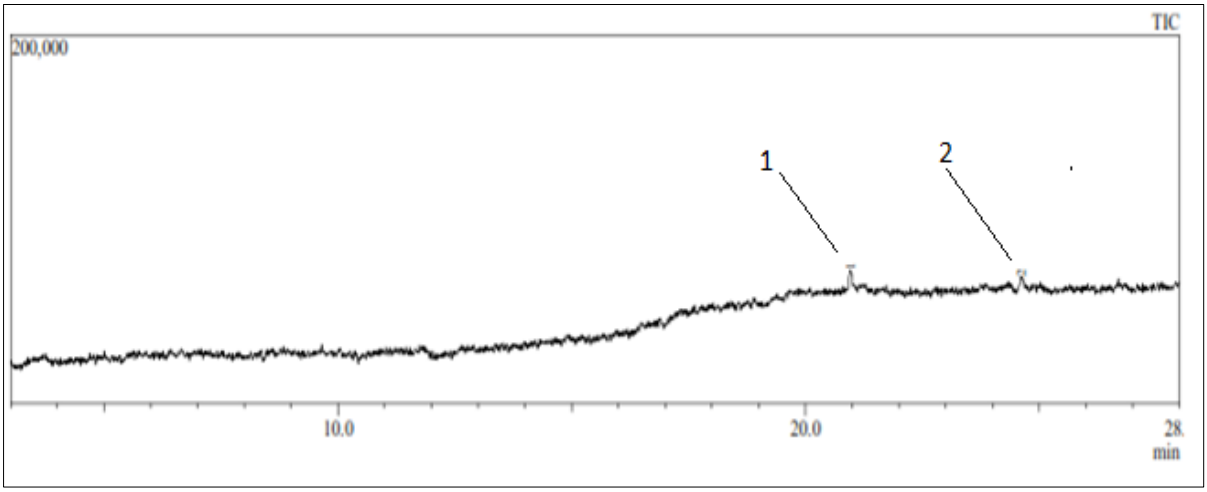

Figure 2: GC–MS chromatogram of ethanol extract of the red seaweed *Eucheuma cottonii*

Table 2: Metabolite constituents of the ethanol extract of Eucheuma cottonii identified by GC-MS analysis

Peak	Compounds analyzed	Retention time (min)	% of peak area	Molecular formula
1	Hexadecanoic acid (CAS) Palmitic acid	20.966	61.11	$C_{16}H_{32}O_2$
2	Heptadecene-(8)-carbonic acid-(1)	24.614	38.89	$C_{18}H_{34}O_2$

The GC–MS profile of the brown seaweed extract is shown in Fig.3. The curve peaks of the chromatogram indicated the presence of metabolites azulene (CAS) cyclopentacycloheptene, hexadecanoic acid (CAS) palmitic acid, heptadecene-(8)-carbonic acid-(1), and 1,2-benzenedicarboxylic acid, dioctyl ester (CAS) dioctyl phthalate (Table 3).

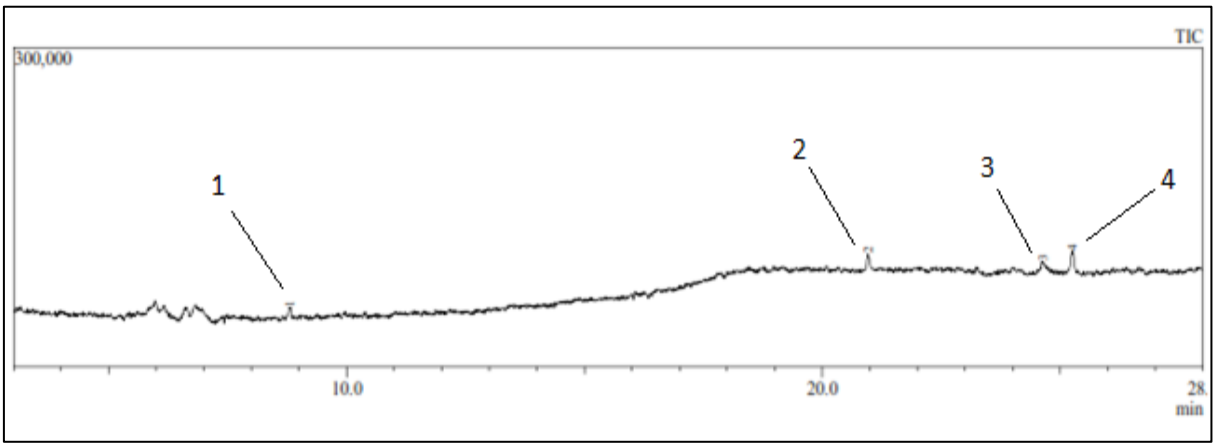

Figure 3: GC–MS chromatogram of ethanol extract of the brown seaweed *Sargassum polycystum*

Table 3: Metabolite constituents of ethanol extract of *Sargassum polycystum* identified by GC-MS analysis

Peak	Compounds analyzed	Retention time (min)	% of peak area	Molecular formula
1	Azulene (CAS) Cyclopentacycloheptene	8.795	16.01	$C_{10}H_8$
2	Hexadecanoic acid (CAS) Palmitic acid	20.963	21.10	$C_{16}H_{32}O_2$
3	Heptadecene-(8)-carbonic acid-(1)	24.633	15.53	$C_{18}H_{34}O_2$
4	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) dioctyl phthalate	25.250	47.36	$C_{24}H_{38}O_4$

Antiplasmodial activity

The anti-plasmodial properties of the extracts against *P.falcifarum* were indicated by their suppression percentage and IC_{50} values (Table 4).

Table 4: Suppression percentage and IC_{50} values of ethanol extract of red (Eucheuma cottonii) and brown (Sargassumpolycystum) seaweeds assayed against P. falciparum

Seaweeds	Extract concentration(µg/mL)	% of suppression	IC_{50}(µg/mL)
Eucheuma cottonii	0.01 0.1 1 10 100	2.76 12.40 23.45 44.83 75.87	11.067
Sargassum polycystum	0.01 0.1 1 10 100	4.14 13.79 24.83 42.76 77.94	10.214

The anti-plasmodial test results of the extracts in Table 4 showed that their IC_{50} values were in the range of 5–50 µg/mL. Thus, both extracts were categorized as active metabolites. However, the IC_{50} value of the brown seaweed extract (10.214µg/mL) was lower than that of the red seaweed extract (11.067 µg/mL), indicating that the secondary metabolites of the former were more active than those of the latter.

DISCUSSION

Qualitative analysis of the extracts indicated that both red and brown seaweeds contained saponins, alkaloids, flavonoids, tannins, and terpenoids. These data confirm the results of previous studies that the extract of *E.cottonii* contains alkaloids, flavonoids, steroids, and terpenoids [19], whereas the extract of *S.polycystum* contains alkaloids, saponins, flavonoids, tannins, and terpenoids [20]. GC–MS analysis also confirmed the metabolites, including hexadecanoic acid, palmitic acid, n-hexadecanoic acid, and carboxylic acid, found in previous research by Hassan and Shobier [21] and Prasasty *et al.,* [22].

The main findings of this experiment indicated that the brown seaweed extract showed better anti-plasmodial activity than the red seaweed extract. This result may be ascribed to the fact that although the two extracts contained the same group of metabolites, their GC–MS profiles were still different. Only two metabolites, namely, hexadecanoic acid (CAS) palmitic acid and heptadecene-(8)-carbonic acid-(1), were identified in the red seaweed extract, whereas four metabolites, namely, azulene (CAS) cyclopentacycloheptene, hexadecanoic acid (CAS) palmitic acid, heptadecene-(8)-carbonic acid-(1), 1,2-benzenedicarboxylic acid, and dioctyl ester (CAS) dioctyl phthalate, were found in the brown seaweed extract.

The bioactivities of secondary metabolites in seaweeds are relatively broad. Prasedya *et al.,* (2018) reported that *Sargassum polycystum* extract exertscytoprotective effects in human cervical cancer (HeLa) and human umbilical vein endothelialcells by reducing nuclear DNA damage induced by UV-B radiation [23]. This brown seaweed also has relatively strong antioxidant properties, with an IC_{50} value of 0.16 ± 0.03 mg/mL [24]. Aside from *Sargassum*, other seaweed species, including *Halimeda tuna, Turbinaria conoides*, and *Gracilaria foliifera, possess* antioxidant properties [25].

As shown in Table 3, brown seaweed extract contains azulene (CAS) cyclopenta-cycloheptene. This compound and its derivatives exert various bioactivities, including anti-inflammatory (e.g., on peptic ulcers), antineoplastic (e.g.,onleukemia), antidiabetic, antiretroviral (e.g., on HIV-1), antifungal, and antimicrobial [26]. Furthermore, GC–MS analysis revealed that the red and brown seaweed extracts contained n-hexadecenoic acid. This metabolite constituent has potential antifungal properties [27]. Other suggested bioactivities of hexadecenoic acid includeantioxidant, hypocholesterolemic nematicidal, pesticidal, anti-androgenic flavor, hemolytic, and 5-alpha reductase inhibitor [28].

The anti-plasmodial properties of the two extracts in this study can be ascribed to their secondary metabolites that are similar to conventional anti-malarial drugs. Qualitative screening and GC–MS metabolite profiling indicated that the extracts contained alkaloids, terpenoids and hexadecanoic acid. Plant extracts containing these metabolites, such as those from *Zea mays*, possess anti-malarial activities against *P.falciparum* [29]. Quinine, the first effective malarial drug, is an alkaloid. Additionally, some classes of alkaloids, such as terpenoidal, indole, bisindole, quinolone, and isoquinoline alkaloids,have beensuggested aspromising antimalarials [30]. Alkaloid extracts of African plants (*Tridax procumbens, Ipomoea purpurea, Sida acuta, Senna alata, Phyllanthus amarus, Azadirachta indica, Nauclea latifolia*, and *Polyalthia longifolia*) exhibit antimalarial activitiesagainst 3D7 [31].

Saponins are bioactive substances qualitatively identified in *E.cottonii* and *S.polycystum.* These secondary metabolites exhibit anti-plasmodial activities. Total saponins extracted from *Terminalia avicennioides* leaves have anti-malarial activities against *Plasmodium berghei* up to 56.95% [32]. Additionally, saponins extracted from *Glycyrrhiza glabra* show anti-malarial activities against *P. falciparum* [33].

Furthermore, the secondary metabolites suggested to be related to the antimalarial properties of the red and brown seaweed extracts were tannins. Tannins extracted from *Punica granatum* exhibit anti-malarial activities against *P.falciparum* D6 and W2 clones [34]. In addition to tannins, flavonoids and saponins were also found in the red and brown seaweed extracts used in the present study. Flavonoids, saponins, tannins, and terpenoids extracted from the stem bark of *Gardenia ternifolia* have anti-malarial properties against *P.berghe I* [35].The leaf extract of *Pappea capensis*, which is rich in flavonoids, also exhibits anti-malarial properties against 3D7 [36]. Some structurally related flavonoids, such as quercetin, rutin, eriodictyol, eriodictyl chalcone, and catechin, exhibit anti-plasmodial activities against *P. falciparum.* Furthermore, flavonoids, particularly quercetin, affect the potent anti-plasmodial activity of artemisinin, leading to a pronounced synergistic effect [37].

CONCLUSION

Ethanol extracts of red (*E.cottonii*) and brown (*S. polycystum*) seaweeds contain saponins, alkaloids, flavonoids, tannins, and terpenoids. Parasite growth inhibition assays of the extracts against *P.falciparum* indicated that their IC_{50} values are sufficient for them to be considered as active anti-plasmodials. Thus, the extracts could be developed as anti-malarial ingredients.

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Conflict of Interest: Authors declare there is no conflict of interest.

Author's Contribution: Endah Setyaningrum (ES) and Jeany Audina Suryanngkunti (JAS) conceptualized and designed the study. Endang Linirin Widiastuti (ELW) performed the data analysis; and Mohammad kanedi (MK) prepared the first draft of the manuscript.

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