



## ARTICLE

### IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM PEDADA (*Sonneratia caseolaris*) FRUIT EXTRACT TO PREVENT STUNTING

Azarine Ahnafia Damayanti<sup>1</sup>, Wienta Diarsvitri<sup>2\*</sup>, Eva Pravitasari Nefertiti<sup>3</sup>, Indri Ngesti Rahayu<sup>4</sup>

<sup>1</sup>Fakultas Kedokteran, Universitas Hang Tuah, Surabaya, Indonesia

<sup>2</sup>Departemen Kedokteran Komunitas, Fakultas Kedokteran, Universitas Hang Tuah, Surabaya, Indonesia

<sup>3</sup>Departemen Patologi Anatomi, Fakultas Kedokteran, Universitas Hang Tuah, Surabaya, Indonesia

<sup>4</sup>Departemen Fisiologi, Fakultas Kedokteran, Universitas Hang Tuah, Surabaya, Indonesia

\*Correspondence email: [wienta.diarsvitri@hangtuah.ac.id](mailto:wienta.diarsvitri@hangtuah.ac.id)

#### ABSTRACT

Stunting is a problem that still cannot be resolved throughout the world, especially in Indonesia. Children who are malnourished show high levels of blood oxidative stress biomarkers as well as inhibition in growth. To understand the prevention of stunting, research was conducted using pedada (*Sonneratia caseolaris*) fruit which is a mangrove plant taken from Wonorejo Mangrove Ecotourism, Rungkut District, Surabaya City, East Java. The research revealed there are bioactive compounds in pedada fruit extract that has the potential to prevent stunting. This research used One Shot Experimental Study. Data were accrued by screening the phenolic and flavonoid antioxidant metabolite compounds at the Pharmacy Laboratory of Hang Tuah University; and screening for Cu, Fe, and Zn mineral content at the UPT East Java Nutrition Laboratory. Based on the results of qualitative phytochemical screening, pedada fruit contained phytochemical compounds, namely phenolics, and flavonoids. Mineral screening of pedada fruit was carried out using the Atomic Absorption Spectrophotometry (AAS) method, and showed zinc (Zn) of 1.057 mg, while copper (Cu) and iron (Fe) of 0 mg.

**Keywords:** Stunting; Antioxidants; Pedada fruit; Minerals; Zinc

#### АБСТРАКТ

Задержка роста - проблема, которую до сих пор не удается решить во всем мире, особенно в Индонезии. У детей, которые недоедают, наблюдается высокий уровень биомаркеров окислительного стресса в крови, а также замедление роста. Чтобы понять, как предотвратить отставание в росте, было проведено исследование с использованием плодов педады (*Sonneratia caseolaris*) - мангрового растения, взятого в мангровом экотуризме Wonorejo, район Rungkut, город Сурабая, Восточная Ява. Исследование показало, что в экстракте плодов педады есть биоактивные соединения, которые способны предотвратить задержку роста. В данном исследовании использовался одномоментный эксперимент. Данные были получены путем скрининга фенольных и флавоноидных антиоксидантных метаболитов в фармацевтической лаборатории Университета Ханг Туах, а также путем скрининга содержания минералов Cu, Fe и Zn в лаборатории питания UPT East Java. По результатам качественного фитохимического скрининга, плоды педады содержат фитохимические соединения, а именно фенолы и флавоноиды. Минеральный скрининг плодов педады был проведен методом атомно-абсорбционной спектrophотометрии (ААС) и показал, что содержание цинка (Zn) составляет 1,057 мг, а меди (Cu) и железа (Fe) - 0 мг.

**Ключевые слова:** Задержка роста; Антиоксиданты; Плоды педады; Минералы; Цинк

## INTRODUCTION

Stunting is a health problem worldwide that affect 149.2 million toddlers<sup>1</sup>, including in Indonesia. Based on the 2021 Indonesian nutrition status survey (*Survey Status Gizi Indonesia*), 24.4% of Indonesian children experienced stunting.<sup>2</sup> The impacts of stunting include susceptibility to infections and parasites, the risk of long-term chronic diseases, and even death.<sup>3</sup>

Based on the second edition of *Warta Kesmas* in 2022, the Ministry of Health of the Republic of Indonesia built Five Movements to Prevent Stunting through collaboration between the community and multi-sectors, namely the Nutrition Action Movement, the Healthy Pregnant Women Movement, the Active Posyandu Movement, the Cadre Jamboree Movement, and the *Cegah Stunting Itu Penting* as a campaign movement.<sup>4</sup> The nutritional action movement is one of the intervention efforts to reduce morbidity in stunting, including anemia.

Anemia is generally caused by iron deficiency due to certain conditions, causing growth disorders through the decrease of IGF-1 secretion.<sup>5</sup> The contribution of iron deficiency anemia to stunting is due to a long-term reduction in food intake rich in iron, vitamin A, and zinc, thereby disrupting iron metabolism, especially in the production of red blood cells.<sup>6</sup>

The Presidential Regulation (Perpres) number 72 the year 2021, has an important role in accelerating the reduction of stunting in Indonesia to 14% by 2024.<sup>7</sup> The National Movement for Reduction of Stunting provides supplementation for children to reach the standard daily source of nutrition.<sup>2</sup>

Stunting is a condition of growth failure that causes body length or height to be less than age, and it is caused by an interaction of various factors.<sup>8</sup> All nutrients, especially protein, and other micronutrients, are essential for a child's growth and development. Moreover, the quality and diversity of nutrients should always be considered, to warrant all nutritional needs are met.<sup>9</sup>

Antioxidants inhibit oxidation and can produce free radicals, causing cell damage.<sup>10</sup> The bond between proteins and antioxidants will inhibit cell oxidation through several pathways, including the inactivation of reactive oxygen species (ROS), reduction of free radicals, removal of pro-oxidative metal bonds, and reduction of hydroperoxidase. The main role of antioxidants is in reducing free radicals and pro-oxidative metal bonds.<sup>11</sup> The need for antioxidants in the body can be met through food intake that is high in antioxidants, such as phenolic compounds and flavonoids.<sup>12</sup>

Stunting is one markers of chronic malnutrition in children. Children who are considered to be nutritionally stunted have significantly lower levels of biomarkers for blood oxidative stress, such as catalase, superoxide, and total plasma protein, as well as copper, zinc, and vitamin C.<sup>13</sup>

One of the micronutrients is minerals. Minerals are classified into two groups, namely, metal trace elements (MTE) and essential metal trace elements (EMTE)<sup>13</sup>, Essential metal trace elements (EMTE) are micronutrients composed of essential minerals that function in the body's physiology and play a role in enzymatic reaction signaling pathways. EMTE consists of zinc, copper, iron, and selenium.<sup>13</sup>

Efforts to prevent stunting cases are needed, especially related to daily nutritional needs (macronutrients and micronutrients), and exogenous antioxidant needs.<sup>14</sup> The Indonesian coastal communities utilize food sources from natural products, one of which is the fruit of mangrove plants, namely pedada (*Sonneratia caseolaris*) fruit.<sup>15</sup> In Indonesia, pedada is commonly found in coastal areas. Several studies reported that almost all parts of the plant such as leaves, stems, and fruit have properties such as astringent, antiseptic, analgesic, anti-inflammatory, antimicrobial, antidiabetic, and antioxidant, and secondary metabolite compounds such as flavonoids, phenolics, terpenoids, steroids and alkaloids.<sup>6,16</sup>

In Dr Duke's Phytochemical and Ethnobotanical database, there is no data related to bioactive compounds of pedada (*Sonneratia caseolaris*) fruit. To support the marine and maritime vision and mission of Hang Tuah University, this research aimed to identify bioactive compounds of pedada (*Sonneratia caseolaris*) fruit extract that has the potential to prevent stunting.

## MATERIAL AND METHODS

This study used One Shot Experimental Study based on qualitative screening of phenolic and flavonoid antioxidant metabolite compounds of pedada (*Sonneratia caseolaris*) fruit.

Pedada (*Sonneratia caseolaris*) fruit was obtained from Wonorejo Mangrove Ecotourism, Rungkut District, Surabaya City, East Java, with the criteria that the fruit was ripe, marked by the fruit falling to the ground, in good condition (not rotten, not rounded). This study used 2.6 kg of pedada fruit that produced 100 grams of pedada (*Sonneratia caseolaris*) fruit extract.



**Figure 1.** Pedada (*Sonneratia caseolaris*) fruits

The screening of the phenolic and flavonoid antioxidant metabolite compounds was conducted at the Pharmacy Laboratory of Hang Tuah University. Screening for Cu, Fe, and Zn mineral content was conducted at the UPT East Java Nutrition Laboratory, Surabaya, using the Atomic Absorption Spectrophotometry (AAS) method.

## RESULT

**Table 1.** Phytochemical Screening Results of Pedada Fruit Extracts

Screening Test	Result	Descriptions
Flavonoid	+	reddish color
Phenolik	+	blue discoloration

The pedada fruit extract was divided into three test tubes. The first tube of flavonoid and phenolic tests was used as a control tube, and the second and third tubes were added NaOH and H<sub>2</sub>SO<sub>4</sub>. In contrast, in the second and third tubes of the phenolic test were added with the Folin-Ciocalteu reagent and Na<sub>2</sub>CO<sub>3</sub>. The color of each tube was compared with the control tube. The result was a red-to-red-purple color change that indicated the presence of flavonoids. Flavonoids are compounds with Alpha-benzopyran cores that result in the reaction in the form of dark red flavylum salts. In the phenolic test, a blue-black color change is produced due to the formation of a phosphotungstic-phosphomolybdate complex between phenolic-hydroxyl groups with a Folin-Ciocalteu reagent.<sup>17</sup>

The mineral content of pedada fruit extract was carried out at the East Java Nutrition Lab with the following results:

**Table 2.** Proximate and Mineral Screening Results of Pedada Fruit Extracts

Screening Test		Total
Proximate Test	Water	43,93 %
	Ash	5,55 %
Mineral Test (ASS)	Zinc	1,057 mg
	Iron	0 mg
	Copper	0 mg

## DISCUSSION

From the findings of the qualitative phytochemical screening, it was determined that pedada fruit did, in fact, contain phytochemical components. These compounds included flavonoids and phenolic elements. The presence of phenolics was indicated by a change in the color of the light green solution to blue-black due to Fe<sup>3+</sup> ions reacting with phenolic keto-groups that had metal chelating properties. In comparison, flavonoids were

indicated by a change in the color of the pedada fruit extract solution, which was reacted with a strong base (NaOH) to form red acetophenone.<sup>18</sup>

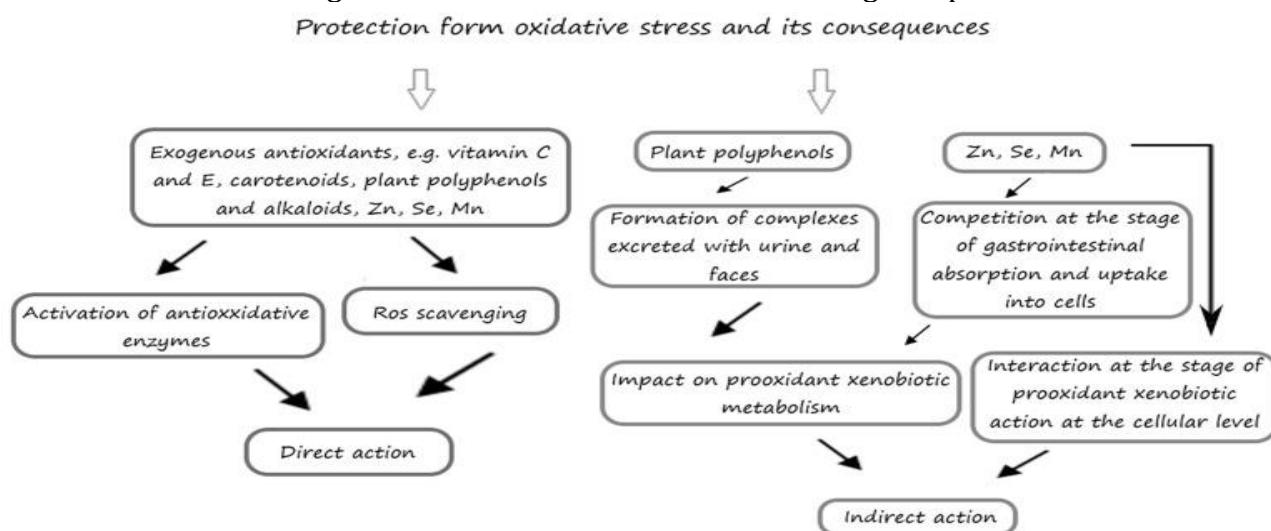
The results of this study were correlated with research conducted<sup>16</sup>, through qualitative phytomic analysis. Pedada (*Sonneratia casseolaris*) fruit with 70% ethanol solvent positively contained phenolics, flavonoids, saponins, and tannins with antioxidant activity obtained through 2,2-diphenyl-1-picrylhydrazyl, which is 0.08%.<sup>19</sup> Total phenolic content was one of the most significant antioxidant capacity parameters and was used extensively to evaluate antioxidant extracts.<sup>20</sup> In a previous study, the TPC of pedada fruit extract was  $119.44 \pm 3.99$  mg GAE/g. The DPPH method determined antioxidant activity and reduced capacity with an IC<sub>50</sub> value of 119 µg/mL extract.<sup>21</sup>

In previous research, the total phenolic and flavonoids of pedada fruit were 104 mg/EAG and 5.6 mg/EAG.<sup>22</sup> Several studies reported that an assessment using the DPPH method to

identify antioxidant activity in pedada fruit showed a high activity of 32.58 - 96.02 ppm, indicating strong to very strong antioxidant activity. The solvent compound content influenced antioxidant activity. The value of the ethanol extract and ethyl acetate fraction provided high activity as an antioxidant against DPPH radiation. Further research into the phytochemicals of pedada fruit and other benefits was needed.<sup>19,23</sup>

A recent study conducted by Riyadi et al. (2022) reported that the best antioxidant activity was obtained in pedada fruit vinegar with a 30% sugar concentration that resulted in a pH value of 3.10, which contained flavonoids, tannins, terpenoids, and saponins.<sup>24</sup>

The metabolite components in pedada fruits may vary following their environmental conditions, such as high salinity, high temperature, nutrients, and radiation exposure. So, differences in metabolite components may occur depending on the habitat of mangrove plants.<sup>21,22</sup>



**Figure 2.** Protection against oxidative stress<sup>24</sup>

Mieczan *et al.*<sup>13</sup> "reported there are three ways in which antioxidant activity occurs in the body as shown in Figure 2 and Figure 3". The first is that it prevents reactive oxygen species from forming, traps oxygen, and binds pro-oxidative metals by oxidation reactions. Examples of substances that can trap oxygen include ascorbic acid and ascorbyl palmitate. Other secondary antioxidants include

flavonoids, amino acids, beta-carotene, and selenium are metal-binding compounds.<sup>25</sup>

The second is that it limits the generation of reactive oxygen species by preventing oxidative enzymes and chelating elements activities by providing hydrogen atoms or electrons, which converts radicals into more stable compounds.<sup>25</sup>



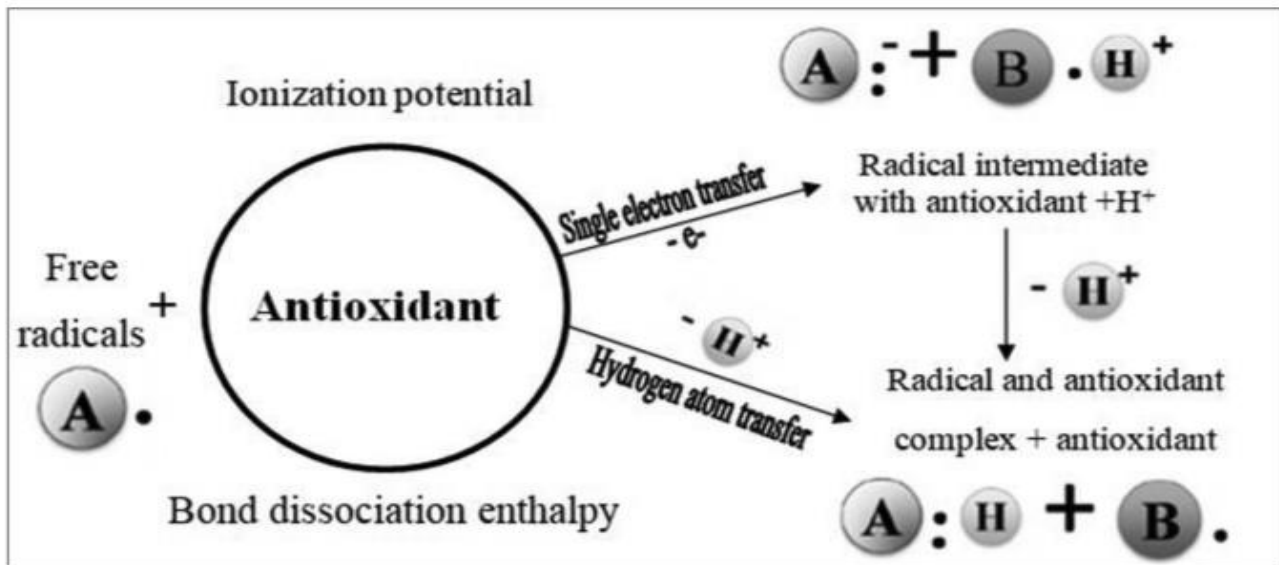


Figure 3. Protection against oxidative stress<sup>26</sup>

The third is that it enhances the effectiveness of endogenous antioxidants by mixing radicals and antioxidant reaction mechanisms, for example, involving several transfers of electron and proton, or proton-couple and electron. Phenolics, such as hydroquinone, gallate, tocopherol, and trihydroxy-butyrophenone are some examples of compounds that have the third mechanism.<sup>27</sup> Thus, the effect of exogenous antioxidants was multifactorial. Phenolics and flavonoids as antioxidants played a role in reducing oxidative stress, so they could reduce the risk of metabolic deficiencies, susceptibility to infections, risk of degenerative diseases, and prevent stunting.

Mineral screening of pedada (*Sonneratia caseolaris*) fruit at Wonorejo Mangrove Ecotourism area, Rungkut District, Surabaya City, East Java Province, was carried out using the atomic absorption spectrophotometry (AAS) method. Pedada fruit extract contained zinc (Zn) of 1.057 mg, while copper (Cu) and iron (Fe) of 0 mg.

In previous studies, the results of the total zinc concentration in pedada fruit were 5.9 µg/g, and copper (Cu) was 26.8 µg/g in the waters of Rembau Linggi, Negeri Sembilan, Malaysia. The study also explained the differences in heavy metal concentrations in pedada fruit with sediments, such as lead (Pb)

and cadmium (Cd). Sediments in the region contained Cd of 0.8 µg/g and Pb of 83.1 µg/g, with total concentrations of Cd in pedada fruit 1.0 µg/g, and Pb was 35.5 µg/g.<sup>28</sup> Meanwhile, another study found that the zinc content of pedada fruit was 19.58-23.51 mg and copper was 0.59-0.99 mg in Blanakan River waters, Subang Regency, West Java. Differences in zinc and copper content in pedada roots were correlated with mangrove environmental and metabolism factors. *Sonneratia caseolaris* has supporting roots connected, forming a similar cage that can hold more sediment. The more sediment retained, the more material, including metals, will be retained (Figure 4).<sup>29</sup>

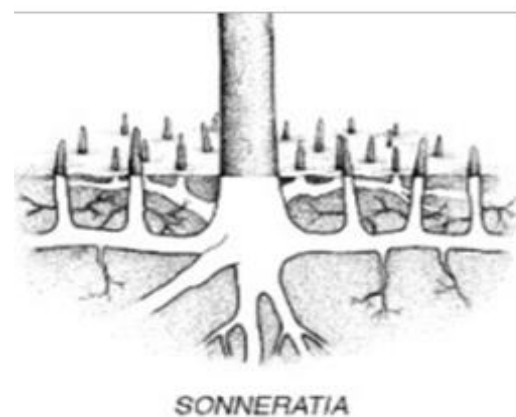


Figure 4. Mangrove plant root species of *Sonneratia caseolaris* (cone root)<sup>29</sup>

The selectivity of heavy metal buildup in soil is influenced by soil pH. Precipitates are formed when soil pH values reach above 4 or 5. The most commonly found heavy metals in soil are lead, copper, zinc, and cadmium. Heavy metals in the environment where a plant grows will trigger oxidative stress, that leads to the destruction of the plant cells.<sup>30,31</sup> Further, heavy metals in pedada fruits can lower the uptake of all mineral ions, including sodium, potassium, calcium, phosphor, iron, magnesium, zinc, and copper.<sup>32</sup> Research conducted by Collin *et al.* reported that food crops were good collectors of heavy metals. The array of heavy metals commonly found in plants is in the leaf, root or stem, tuber or leaf, fruit, and stem.<sup>33</sup>

Essential metal trace elements (EMTE) are micronutrients composed of essential minerals that function in the body's physiology and contribute to the enzymatic reactions signaling pathways. EMTE consists of zinc, copper, selenium, and iron. Zinc is a mineral identified in pedada fruit extract. Zinc contributes to DNA and RNA synthesis, creates immunity, and serves as a covariate for more than 200 body enzymes. So zinc is complex to enzymatic processes at the cellular level.<sup>34</sup>

However, the insufficiency of zinc and iron can prevent growth and fetal development in pregnancy, susceptibility to infection, growth retardation in infants and young children, oligospermia, hypogonadism, dysgeusia, night blindness, immune dysfunction, wound healing disorder, and acrodermatitis enteropathica, characterized by periorificial dermatitis, diarrhea, and alopecia.<sup>35</sup>

Minerals account for about 5% of the usual human diet, yet are important for normal health and function. Macrominerals are minerals required by the human body. An adult requires EMTE, including zinc, copper, and iron, in amounts of 1-100 mg/day.<sup>36</sup> Zinc in pedada fruit acts as an element involved as a growth factor for children, especially in bone growth, body metabolism, regulation of growth hormone, and insulin-like growth factor I. Zinc in pedada fruit (*Sonneratia caseolaris*) regulates the ability of growth

hormone signaling in its dimeric form and prolongs the binding of growth hormone with growth hormone receptors. However, zinc-deficient stunting may affect Zn-protein targets of insulin-like growth factor I action in the pituitary, liver, and adipose tissues. The potential of zinc as an anabolic effect of insulin-like growth factor I in osteoblastic cells with a complex system is also important for bone matrix calcification and as an indication of bone growth disorders caused by decreased zinc availability in bone cells.<sup>37</sup> So, it is necessary to supplement zinc in children with growth disorders, such as stunting. The provision of 40 mg zinc supplementation or the equivalent of 2-3 times is the recommended daily requirement dose to cause a significant increase in height and weight in children and will show improvement in growth status within 6-12 months.<sup>38</sup>

Heavy metals can cause nutritional deficiencies in pedada fruit and in the human body, especially in those living in developing countries with malnutrition problems.<sup>33</sup> To reduce the amount of heavy metal content in pedada fruit, initial treatment is needed to reduce the heavy metal content so that it meets the threshold value of the heavy metal content, for example, the adsorption method to break down heavy metal groups with adsorbent.<sup>39</sup>

However, pedada (*Sonneratia caseolaris*) is a mangrove plant that grows on the coast and can contain heavy metal accumulation from waste discharged into the sea, so it is necessary to apply a pretreatment before processing the pedada (*Sonneratia caseolaris*) fruit, to prevent possible neurotoxic effects. Further research is needed to ensure the safety and effectiveness of pedada fruit extract in preventing stunting. The research can be in silico to predict the bioavailability, absorption, distribution, metabolism, excretion, and toxicity; as well as in vivo in animal research to assess the effect of pedada fruit extract on the body.

Based on the results of the study, there is potential for bioactive compounds of pedada (*Sonneratia caseolaris*) fruit extract to prevent stunting, which consists of antioxidants in the form of phenolics and flavonoids to reduce

oxidative stress, as well as the mineral zinc needed for child growth.

## CONCLUSION

Based on the results of the identification of bioactive compounds in pedada (*Sonneratia caseolaris*) fruit extract carried out by researchers, it contained phenolics and flavonoids as antioxidants, and zinc 1,057 mg/100 grams of pedada fruit as mineral content. The antioxidants in pedada fruit are a beneficial factor that can reduce oxidative stress to prevent stunting in children. Zinc in pedada fruit is an essential metal trace element that has a role in growth, immunity, and digestive system function, so hopefully, it can prevent stunting.

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## DECLARATION

Author contribution. AAD: conceptualization, laboratory work, data analysis, initial draft, and final draft. WD: conceptualization, study design, data analysis, manuscript preparation, and final draft. EPN: initial draft, manuscript final draft. INR: initial draft, manuscript final draft.

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No additional information is available for this paper.

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