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# Research Article
# Evaluation of Nutritional and Antioxidant Capacity of Marine Bean grown in coastal areas, Southeast Sulawesi, Indonesia

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## Abstract
**Background and Objective:** The marine bean (*Vigna marina*) is one of tropical legumes grown in coastal areas and can be used as renewable food source. The present study aimed to assess the nutritional and antioxidant capacity of marine bean as renewable food source for the coastal communities. **Materials and Methods:** The leaves and fruits of marine bean were sampled from sandy area, shaded area and on the tree canopy. The samples then labeled and kept in the box contained dry ice and brought to the laboratory. All samples were washed thoroughly in tap and distilled water and then separated for the fresh and drying samples analysis. The protein, fat, carotenoid and micronutrients contents in leaves and seeds of marine beans were determined. **Results:** The results showed that the protein content in leaves of *Vigna marina* (*V. marina*) ranges from 1.73±0.37-3.59±0.88 mg $g^{-1}$, while the protein content in its seeds ranges from 4.98±0.16-5.10±1.44 mg $g^{-1}$. However, the fat content in leaves of *V. marina* ranges from 4.94±0.13-6.22±0.61 mg $g^{-1}$, while it ranges from 6.92±1.32-8.54±1.33 mg $g^{-1}$ in seeds. The carotenoid content ranges from 233.32±27.27-287.35±4.45 mg/100 g) and ranges from 18.14±2-29 25.06±0.91 mg/100 g in seeds of *V. marina*. Moreover, the ascorbic acid content in leaves of *V. marina* ranges from 5.56±0.17-6.27±0.25 ppm and it ranges from 5.07±0.02-5.17±0.04 ppm in its seeds. The mean of micronutrients of Na, Fe, Mn, Cu, Zn contents in leaves were estimated as 4.6, 0.32, 0.051, 0.062 and 0.38 mg $g^{-1}$, respectively. In addition, micronutrients of Na, Fe, Mn, Cu, Zn contents in seeds were 6.6, 0.25, 0.044, 0.048 dan 0.27 mg $g^{-1}$, respectively. **Conclusion:** The leaves and seeds of *V. marina* contained higher nutrient and antioxidant contents.

Key words: Antioxidant content, marine bean, nutrition content, renewable food source, Southeast Sulawesi

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Legume plants are known to have critical part in most of developing countries of Asia, Africa and South America of the world[1]. Although, more than 1,000 species of legumes are known till now but only 20 species have been cultivated by farmers for use as food. It is well known that legumes are source of nutrition, mineral and antioxidant[2]. In many regions of the world, legume seeds are the unique protein supply in the diet and became supplement to other protein sources[3] and therefore legumes have an important role in human nutrition[4]. Meanwhile, the importance of legumes in sustainable cropping systems has been extensively documented[5]. Nevertheless, developing countries have high demand for protein-rich food due to their teeming population, cereal-based diet and scarcity of fertile land[6,7]. Wild legumes are important for food security, nutrition, agricultural development and rotation of crops to improve the nation's economy in developing countries and many wild legumes are known to contain an expensive proteins, calorific value, essential amino acids, essential fatty acids, fiber and vitamins[8]. However, these information mention only for legumes growing in the terreterial land, while little is known regarding potential of legumes that growing in the coastal area.

The marine bean (*Vigna marina*) is a tropical legume that is widely distributed in the coastal areas of the tropical and subtropical regions[9]. In traditional ways, many coastal peoples in that region use this plant for multiple purposes; the seeds use as substituted of coffee in West Africa, Tanzania, Mozambique and South Africa, while in Maladewa and Malaysia, the seeds consume as a food. However, there was no information about nutritional potential of *Vigna marina* till now. In addition, the medical uses of *Vigna marina* has also been reported in Indonesia such as heal skin burns of babies in the Marshall Islands, urinary tract infection, vomiting blood and puerperal infection in Polynesian and use for blocking the blood. In spite of these usefulness, there is no information concerning antioxidant capacity of *Vigna marina* to be used as new food source for the coastal community until now. Therefore, this study aimed to determine the nutrition and antioxidant contents in the vegetative and generative organs of marine bean (*V. marina*) and to know the potentiality of marine bean (*V. marina*) as renewable food source for coastal community in Southeast Sulawesi, Indonesia.

## MATERIALS AND METHODS

**Study site:** The plant material of marine bean (*Vigna marina*) were collected from Toronipa beach, which is located at the northern part of Kendari city, Southeast Sulawesi, Indonesia (Fig. 1). The *Vigna marina* grow along the beach with sandy and climbed on tress and far away from tourism activities. This plant covered about 1000 m$^2$ and show nice panorama in the

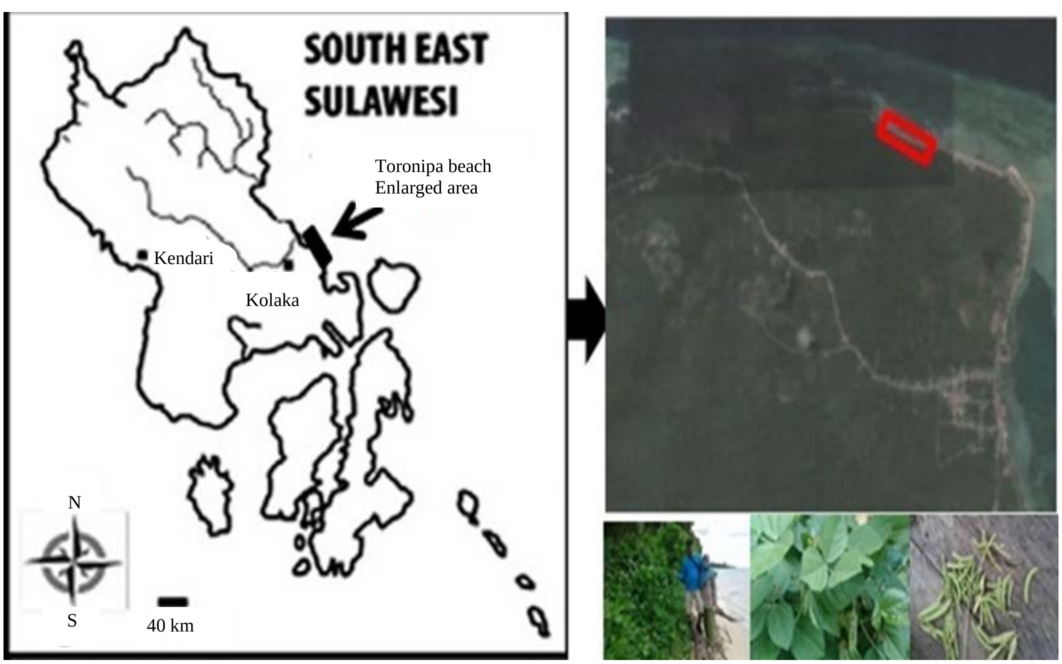


Fig. 1: Study site of Toronipa beach at Southeast Sulawesi (left), sampling sites (upper right) and marine bean plant with leaves and fruits (lower right)

beach. This plant produce abundant of leaves and fruits at rainy season, while they have few leaves only at dry season.

**Sampling and preparation of marine bean:** The young leaves and fruits of marine bean were sampled from three site namely sun area, shaded area and on the trees. The samples then labelled according to their sites and kept in the box contained dry ice and brought to the laboratory Forensic and Molecular at Halu Oleo University. In Laboratory, all samples were washed thoroughly in tap and distilled water and then separated for the fresh and drying samples analysis.

**Nutritional analysis**

**Extraction and estimation of total protein:** The fresh young leaves and fruits of marine bean were taken about 100 mg each and homogenized with pre-chilled mortar and pestle in ice-cold protein extraction buffer. The homogenate samples then centrifuged at 10,000 rpm at 4°C for 30 min and pellets washed with 10% TCA and incubated overnight at 40°C. Pellets were suspended in 2 mL of 0.1N NaOH. Estimation of total protein was made according to Lowery *et al.*[10]. Proteins in the unknown samples were estimated at 750 nm using bovine serum albumin (fraction V) as standard and calculated using standard curve and expressed as mg per g fresh weight basis.

**Extraction and estimation of ascorbic acid content:** Ascorbic acid content in young leaves and fruits of marine bean was estimated following the method of Harris and Ray[11]. Sample extraction of leaves and seeds of marine bean was done by grinding 0.5 g of sample material in 6% oxalic acid solution followed by centrifugation at 3000 rpm for 10 min. Transferred the aliquot and made up the volume to 100 mL. 5 mL of supernatant was added to 10 mL of 0.6% oxalic acid solution and it was titrated against dye solution (standard indophenol solution) until pale pink colour was seen. Standardization of dye was done with standard ascorbic acid (1 mg $mL^{-1}$). Total ascorbic acid content (mg/100 g) of leaves and seeds was calculated by using the following formula

$$\begin{array}{l}\text{Total ascorbic acid}\\ \text{content (mg/100 g)}\end{array} = \left(\frac{0.5\,\text{mg}}{\text{volume}\,1}\right)\times\left(\frac{\text{volume}\,2}{5\,\text{mL}}\right)\times\left(\frac{100\,\text{mL}}{\text{wt. of the sample}}\right)\times 100$$

where, volume 1 is burette reading of titration of dye against standard ascorbic acid and volume 2 is burette reading of titration of dye against sample.

**Extraction and estimation of fat content:** The fat content in vegetative and reproductive organs of marine bean was extracted by using method of Conway and Adams[12]. Fat was extracted from 1 g sample of each organ by heating in alcoholic HCl, followed by the addition of 95% ethanol. The sample was allowed to cool, ether and sodium sulphate were added and the sample was shaken. Petroleum ether was added and the sample was shaken again. The acidic ethanol layer was re-extracted twice more with a mixture of ether and petroleum ether. The combined, recovered supernatants were allowed to evaporate in a ventilated area.

**Extraction and estimation of carotenoid content:** Carotenoid was evaluated following standard method ofArnon[13]. About 0.5 g of the sample was weighed and homogenized in 80% acetone. The volume was then made up to 50 mL. The sample was centrifuged at 5000 rpm for 20 min till the supernatant became transparent. The supernatant was taken and absorbance was measured at 480, 645 and 663 nm.

**Micronutrient analysis:** The micronutrients analysis of young leaves and seeds of marine bean were done by using the methods of Rout *et al.*[14]. About 0.5 g of fine powdered sample of leaves and seeds of marine bean were digested with wet digestion procedures using concentration $HNO_3$ and 30% $H_2O_2$. The digested samples were used for elemental analysis of Iron (Fe), Copper (Cu), Manganese (Mn) and Zinc (Zn), which were determined by using Atomic Absorption Spectrophotometer (AAS Z-2000, Hitachi Ltd., Chiyoda, Tokyo, Jepan), while elements of Sodium (Na), Potassium (K) and Calcium (Ca) were determined by using Flame photometer.

**Data analysis:** The data were expressed as means±standard error. All of the data were analysed using one way ANOVA and followed by post hoc of Tukey Test at significance level of 95%. All of the statistical analyses were performed by using software of Kaleidagraph 4.0.

## RESULTS

**Nutrition and antioxidants:** The nutrition (protein and fat) and antioxidant (carotenoid and ascorbic acid) contents of *V. marina* leaves and seeds grown in a variety of habitat types at Toronipa Beach, Southeast Sulawesi is presented in Table 1. The protein content of *V. marina* leaves creeping under the tree canopy, (3.59±0.88 mg $g^{-1}$) and on tree canopy (3.57±1.35 mg $g^{-1}$) were significantly higher than the protein content of *V. marina* leaves creeping on the sandy beach (1.73 ±0.37 mg $g^{-1}$) (F = 23,12, p = 0,000; Tukey Test). On the other hand, the protein content of *V. marina* leaves that climbed on the canopy and under the canopy of trees was not significantly different. Meanwhile, the protein content of

Table 1: Nutritional and antioxidant contents of leaves and seeds (Mean±SE) of marine bean (*Vigna marina*) growing in Southeast Sulawesi, Indonesia. Similar letters in the same column indicate no significant difference (Tukey test)

| Marine bean organs | Protein content (mg $g^{-1}$) | Fat content (mg $g^{-1}$) | Carotenoid (mg/100 g) | Ascorbic acid (ppm) |
|---|---|---|---|---|
| Sandy leaves | 1.73±0.37$^{a}$ | 6.22±0.61$^{a}$ | 240.46±19.28$^{a}$ | 6.27±0.25$^{a}$ |
| Canopy leaves | 3.57±1.35$^{b}$ | 4.94±0.13$^{b}$ | 287.35±4.45$^{ab}$ | 5.84±0.10$^{b}$ |
| Shaded leaves | 3.59±0.88$^{bc}$ | 5.52±0.46$^{abc}$ | 233.32±27.27$^{abc}$ | 5.56±0.17$^{c}$ |
| Sandy seeds | 5.10±1.44$^{d}$ | 6.92±1.23$^{cd}$ | 25.06±0.91$^{d}$ | 5.17±0.04$^{d}$ |
| Canopy seeds | 4.98±0.16$^{de}$ | 8.54±1.33$^{e}$ | 18.14±2.29$^{de}$ | 5.07±0.20$^{de}$ |

Table 2: Trace elements contents in leaves and seeds (Mean± standard error SE mg $g^{-1}$) of marine bean (*Vigna marina*), grown in Southeast Sulawesi, Indonesia. Similar letters in the same column indicate no significantly different at 5% by one-way ANOVA and advanced Tukey Test

| Organs | Na | K | Ca | Fe | Zn | Mn | Cu |
|---|---|---|---|---|---|---|---|
| Sandy leaves | 5.52±0.35$^{a}$ | 4.26±0.02$^{a}$ | 3.83±0.68$^{a}$ | 0.29±0.04$^{a}$ | 0.34±0.03$^{a}$ | 0.05±0.01$^{a}$ | 0.052±0.01$^{a}$ |
| Leaves on the canopy | 3.77±0.21$^{b}$ | 4.27±0.15$^{ab}$ | 3.50±0.01$^{b}$ | 0.32±0.01$^{ab}$ | 0.37±0.01$^{ab}$ | 0.05±0.01$^{a}$ | 0.063±0.01$^{a}$ |
| Leaves under the canopy | 4.79±0.07$^{abc}$ | 4.21±0.08$^{abc}$ | 3.33±0.01$^{bc}$ | 0.33±0.01$^{abc}$ | 0.39±0.01$^{abc}$ | 0.054±0.01$^{a}$ | 0.070±0.01$^{a}$ |
| Sandy seeds | 7.66±0.16$^{d}$ | 4.02±0.15$^{abcd}$ | 3.71±0.01$^{bcd}$ | 0.21±0.01$^{ad}$ | 0.30±0.04$^{ad}$ | 0.042±0.01$^{a}$ | 0.061±0.03$^{a}$ |
| Canopy seeds | 5.50±0.60$^{bce}$ | 4.33±0.04$^{abce}$ | 4.15±0.01$^{bde}$ | 0.29±0.02$^{abcde}$ | 0.24±0.03$^{de}$ | 0.045±0.01$^{a}$ | 0.034±0.02$^{a}$ |

*V. marina* seeds (5.10±1.44 mg $g^{-1}$) grown on sandy are a and creeping on the tree canopy (4.98±0.16 mg $g^{-1}$) were not significantly different.

The fat content of *V. marina* leaves (8.54±1.33 mg $g^{-1}$) creeping in the tree canopy was significantly higher than the fat content in the leaves grown on sandy area (6.22±0.61 mg $g^{-1}$) and under canopy (4.94±0.13 mg $g^{-1}$) (F = 30.34; p = 0,000; Tukey Test). Furthermore, the fat content of seeds grown on sandy area was significantly higher (p<0.05) than that in canopy leaves as well as shaded leaves, while it was not significantly different from fat content in sandy leaves (p = 0.36). Nevertheless, almost the fat content in leaves of *V. marina* was not significantly different among habitat except the fat content in leaves of *V. marina* that climbed on the canopy of trees and on sandy area which had significantly different content (p = 0.03). Nonetheless, statistical analyses with one-way ANOVA showed that the carotenoid content in leaves and seeds of *V. marina* was also significantly different among habitat (F = 72.77; $p = 2.36\times10^{-7}$). Advanced Tukey Test showed that carotenoid content in the canopy seeds (18.14±2.29 mg/100 g) and in sandy seeds (25.06±0.91 mg/100 g) of *V. marina* was significantly lower (p< 0.01) than that in sandy leaves (240.46±19.28 mg/100 g), leaves under the canopy (233.32±27.27 mg/100 g) as well as leaves on the canopy (287.35±4.45 mg/100 g). Meanwhile, the carotenoid content was not significantly different between sandy seeds and canopy seeds. Similarly, the carotenoid content in leaves of *V. marina* was not significantly different among habitat types. Moreover, the content of ascorbic acid in leaves and in seeds of *V. marina* was significantly different among habitat types (F = 157.78; $p = 6.4\times10^{-9}$). Advanced Tukey Test showed that ascorbic acid content in leaves of *V. marina* that climbed on sandy (6.27±0.25 ppm), on the canopy (5.84±0.10 ppm) and climbed under the canopy of trees (5.56±0.17 ppm) was significantly different (p<0.01). Although, the ascorbic acid content in seeds of *V. marina* that climbed in sandy (5.17±0.04 ppm) and on the canopy of trees was not significantly different (p = 0.05) but the ascorbic acid content in seeds of *V. marina* was significantly lower as compared to that in its leaves for all habitat types (p<0.01).

**Micronutrients:** The micronutrients content in leaves and seeds of marine bean (*V. marina*) grown in Toronipa beach Southeast Sulawesi is presented in Table 2. The Na content in sandy seeds of *V. marina* (7.66±0.16 mg $g^{-1}$) was significantly higher than that in sandy leaves (5.52±0.35 mg $g^{-1}$), canopy seeds and both leaves under the canopy (4.79±0.07) as well as leaves on the canopy (3.77±0.21) of trees (F = 19.14; p = 0.000 and Tukey Test). Meanwhile, the lowest of Na content was found in the canopy leaves (3.77 mg $g^{-1}$). On the contrary, the K content in leaves and seeds of *V. marina* ranges from 4.02±0.15-4.33±0.04 mg $g^{-1}$ and seemed to show less significantly different (F = 3.69; p = 0.043), while the content of K in seeds only showed significantly different (p = 0.034) but it was not significantly different in others (p>0.05). Meanwhile, the Ca content in leaves of *V. marina* ranges from 3.33±0.01-3.83±0.68 mg $g^{-1}$ and ranges from 3.71±0.01-4.15±0.01 mg $g^{-1}$ in its seeds. Statistical analyses by one-way ANOVA of Ca content in leaves and seeds of *V. marina* was significantly different (F =16.51; $p = 2.1\times10^{-4}$). Advanced Tukey Test showed that the content of Ca in sandy leaves (3.83±0.68) of *V. marina* was significantly higher (p<0.01) than that in leaves under the canopy (3.33±0.01), leaves on the canopy (3.50±0.01) and sandy seeds (3.71±0.01), though the content of Ca in canopy seeds

(4.15±0.01) was significantly lower than that in sandy leaves (p<0.05). Moreover, the higher Fe content was found in canopy leaves (0.33±0.01) of *V. marina*, while it was the lowest in sandy seeds (0.21±0.01 mg $g^{-1}$) and was significantly different (F = 4.084; p = 0.0324). Advanced Tukey Test showed the only Fe content in sandy seeds were significantly lower (p = 0.0363) than that in leaves on the canopy (0.32±0.01) and under the canopy of trees, while the Fe content among others were not significantly different. In addition, the higher Zn content was found in leaves of *V. marina* that climbed under the canopy (0.39±0.01 mg $g^{-1}$) and on the canopy (0.37±0.01 mg $g^{-1}$) of trees, while the lowest was found in seeds (0.24±0.03 mg $g^{-1}$) of *V. marina* that climbed on the canopy of trees. One-way ANOVA showed that the content of Zn in leaves and seeds of *V. marina* was significantly different (F = 20.07; p = $5.98\times10^{-5}$). Advanced Tukey Test exhibited that the Zn content in leaves of *V. marina* that climbed on various habitat types was significantly higher (p<0.01) than that in seeds of *V. marina*. Similarly, the Zn content of *V. marina* was significantly different (p<0.01) between sundy seeds and canopy seeds. However, the content of Zn in leaves of *V. marina* was not significantly different among habitat types. Moreover, the content of Mn leaves and seeds of *V. marina* ranges from 0.042±0.01-0.054±0.01 mg $g^{-1}$. Meanwhile, the content of Cu in leaves and seeds of *V. marina* ranges from 0.034±0.02-0.070±0.01 mg $g^{-1}$ and they were not significantly different.

**Anti-nutrition:** Table 3 shows the anti-nutrient content of hydrogen-cyanide (HCN) in young leaves and seeds of marine bean that grown in several habitat types at the Toronipa beach, Southeast Sulawesi. The HCN content ranges from 0.083±0.061-0.095±0.027 mg $kg^{-1}$ but it was not significantly different (F = 1.16; p = 0.38) among organ from different habitat types. However, the HCN content in leaves and seeds of marine bean was very low (<0.1 mg $kg^{-1}$) indicating that the leaves and seeds of marine bean are safe to use as food and antioxidants.

Table 3: The antinutritional of hydrogen cyanide (HCN) content in leaves and seeds of marine bean (*Vigna marina*) grown in Southeast Sulawesi, Indonesia. Similar letters in the same column indicate no significantly different at 5% by one-way ANOVA and advanced Tukey test

| Marine bean organs | HCN content (mg $kg^{-1}$) |
|---|---|
| Sandy leaves | 0.087±0.011[a] |
| Leaves on the canopy | 0.095±0.027[a] |
| Leaves under the canopy | 0.095±0.026[a] |
| Sandy seeds | 0.083±0.061[a] |
| Canopy seeds | 0.084±0.010[a] |

## DISCUSSION

The present results clearly showed that the nutrient contents of protein and fat of marine bean were significantly higher in its seeds than that in its leaves, while antioxidant contents of ascorbic acid and carotenoid were significantly higher in its leaves than that in its seeds. Although the fat content in leaves and seeds of marine bean (0.49-0.85%) is lower than that reported by Rusydi *et al*.[15] for Mung bean (5.69%), soy bean (20.07%) and non-germinated of white rice (1.42%), black rice (1.29%), red rice (1.5%) as well as brown rice (1.89) but it was higher than that in germinated of white rice (0.18%) and Baririo (0.07-0.34%). Meanwhile, protein content in seeds of marine bean (0.5%) was lower than that in non-germinated of white rice (6.45%), black rice (8.6%), red rice (6.06%) and brown rice (6.35) as reported by Rusydi *et al*.[15]. However, the Ca content in leaves and seeds of *V. marina* ranged from 3.33-4.15 mg $g^{-1}$, which were higher than that of the Ca content in seeds of Cowpea (0.77 mg $g^{-1}$), Gude (1.25 mg $g^{-1}$), Green bean (1.25 mg $g^{-1}$) and soybean (2.27 mg $g^{-1}$) as reported by Utomo and Antalita[16]. In addition, the Fe content in leaves and seeds of marine bean (0.21-0.33 mg $g^{-1}$) were much higher than the Fe content in seeds of Cowpea (0.065 mg $g^{-1}$), Gude (0.04 mg $g^{-1}$), Green bean (0.067 mg $g^{-1}$) and soybean (0.08 mg $g^{-1}$) as reported by Utomo and Antalita[16]. Thus, higher micronutrients contents of Ca and Fe in the seeds and leaves of marine bean provide important information for coastal people as this legume is widely distributed in the coastal areas of the tropical and subtropical regions. Many people in West Africa, Tanzania, Mozambique and South Africa have been used this legume as new food source in traditional methods to substitute coffee[17], while in Maladewa, the seeds are consumed asfood[18]. In Tonga, the leaves of marine bean is applied to clean the eyes, nose and mouth and is rubbed on the body to treat diseases, while Samoans use a leaf infusion to treat a certain type of fever in children[19].

The higher ascorbic acid content in the leaves and seeds of marine bean is also used for medicinal purposes, such as the powder of leaves and seeds which has been used to treat skin burns of babies in the Marshall Islands[20]. Furthermore, the leaves of marine bean have also been used to treat urinary tract infection, blood vomiting and puerperal infection[20]. Therefore, the results of this study exposed the potential uses of marine bean as renewable food, antioxidant sources and substitution in food industry. Thus, the marine bean as a renewable food source for the coastal community is very important to support the health of society especially living in coastal areas, Southeast Sulawesi, Indonesia.

The micronutrient values in leaves and seeds of marine bean indicated the importance of this plant as source of trace elements. This is because micronutrients play critical role to maintain the human body functioning[21-23]. Moreover, Iron (Fe) is part of hemoglobin proteins and myoglobin[24,25], while the Caisis necessary for protein metabolism[26]. Thus, the micronutrient deficiency may cause serious health problems in humans including anemia, decreased immune function[21,23], etc. Meanwhile, the HCN content of marine bean is very low and is safe to use as food and antioxidants for local people in this region.

## CONCLUSION

The leaves and seeds of *V. marina* contained higher nutrient and antioxidant contents. Meanwhile, the micronutrient contents in leaves and seeds of *V. marina* were also higher. Moreover, the HCN contents in leaves and seeds of this marine legume was very low indicating its safe use as food and antioxidant sources. The finding of this study realized the high potentiality of marine bean, a tropical legume as a food source for coastal communities in Southeast Sulawesi, Indonesia.

## SIGNIFICANCE STATEMENT

This study discovers the nutritional and antioxidant capacity of marine bean that can be used as renewable food source for local communities as well as food industry. This study will help the researcher to uncover the critical areas of renewable food nutrition that many researchers were not able to explore. Thus, new theory on nutritional and antioxidant capacity of wild legumes may be arrived at.

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