Iron-Rich Salt and Antioxidants from Sea Sponge with Added Moringa Leaves as Potential Food Ingredients in Efforts to Alleviate Anemia

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Abstract
Based on the high prevalence of anemia of more than 24% population affected, the natural potential of Indonesia such as Sponge and Moringa leaves to be a salt product high in iron, and interconnection between this theoretical science and Islamic studies related to the utilization of natural materials, we want to explore how potential be a product that can be one of the efforts to alleviate anemia. Initial data collection to explore sponge types and types of plants rich in iron was done using data search portal NCBI (National Center for Biotechnology Information, USA) in the last five years starting from 2016/03/30 to 2021/03/28. Samples were formulated based on the comparison of seawater, sponge (Spongia sp), and moringa leaves in a composition as follows: Sample A (1:1:0.05), sample B (1:1:0.25), sample C (1:1:0.5), and control that did not use the addition of Moringa leaves (1:1). The analysis included testing antioxidant activity using DPPH, moisture content, NaCl, impurities sulfate, magnesium, calcium, and iron levels which were all done in 3 repetitions of each sample or triplicates and using analytical methods from the Association of Official Analytical Chemist (AOAC). This study obtained salt granules as much as 0.92±0.22 kg. There was no difference in the results of salt granules from each sample, and there was no difference in the level of fineness of salt granules, namely 20-35 mesh or equivalent to crude salt. However, there were differences in the level of chemical it contains. Samples B, C, and control correspond to SNI 3556:2016 based on percentage of moisture content, Fe, calcium impurities, and magnesium impurities in all 3 samples. Sample C has the highest iron levels compared to B and control samples, which means that sample C is the best sample and has antioxidant activity that is in line with the purpose of this study, which is an effort to alleviate iron deficiency anemia. This salt is also a salt that is Halal Tayyiban, which means not only halal but also good and healthy if consumed.
1. Introduction

As a large archipelago with wide ocean areas, Indonesia has large natural resources of the sea. Utilization of marine natural resources is not only done through the capture but also needs to be developed with cultivation efforts that lead to sustainable conservation. One of the marine natural resources is the coral reef ecosystem. Coral reef ecosystems are part of marine ecosystems that become a source of life for a wide variety of marine biota. The potential of marine biota as a source of new antioxidant ingredients has been widely studied in recent years, although not as much as on terrestrial biota. A long evolutionary history of marine biota causes marine biota to have a very high molecular diversity. Various types of compounds with various bioactivities have been found from the biota (Fallu, 1991).

Sponge is an invertebrate marine organism belonging to the phylum of Porifera which is characterized by having many pores along its body. Sponge includes animals that are filter feeders (Haedar et al., 2016). Sponge plays a role in carbon cycles, silicon cycles, and nitrogen cycles as well as associations with other organisms where sponge has a role as a primary producer and secondary producer in the provision of microhabitats (Bell, 2008). The diversity of sponge species in a habitat is generally determined by clear water conditions and lack of strong currents. A sponge can also be found in every different depth condition with enough brightness for its growth (Haedar et al., 2016).

The presence of sponges is currently a big concern for researchers because of the content of active compounds in the sponge body. Metabolite extract from sponges is believed to contain bioactive compounds that have cytotoxic, anti-tumor, anti-viral, anti-inflammatory, anti-fungi, anti-leukemia, and enzyme activity inhibitors. In addition to being a source of natural material compounds, sponges also have other benefits, namely used as biological indicators for monitoring marine pollution, indicators in community interactions, and as economically valuable animals for marine aquarium decoration (Suparno, 2005).

Iron-deficiency anemia (IDA) is one of the hematological diseases commonly found in infants, children, and women of reproductive age. Children with IDA will experience developmental disorders, behavioral changes, and motor impairments, thus reducing learning ability and decreasing learning achievement in school. This situation can certainly hinder the development of human resource quality. The main factors causing iron deficiency anemia in women of reproductive age are menstruation and pregnancy. Given the magnitude of the adverse effects of iron deficiency anemia, it is necessary to get enough attention. According to data from the World Health Organization (WHO, 2011), the frequency of iron deficiency in developing countries will increase 2-5 times to iron-deficiency anemia caused by several factors such as infection and malnutrition.

Moving on from the various problems that have been presented above and by interconnecting between scripture and science, researchers are very enthusiastic to explore further things related to the natural wealth of Indonesia's sea and Indonesian land plants into food that is more beneficial for health. Therefore, this study intended to utilize sea sponge into high functional salt Fe with the addition of moringa leaf flour (Moringa oleifera) as sustainable product innovation and beneficial in health, especially minimizing the occurrence of iron deficiency anemia. This is in line with the global government programs listed in the SDGs Sustainable Development Goals point three, namely "Good Health and Prosperity".

Seeing the high prevalence of of more than 24% population, and by looking at the natural potential of Indonesia such as sponge and moringa leaves that have the potential to be a salt product high in iron; and interconnect between this theoretical science and Islamic studies related to the utilization of natural materials, researchers want to explore how potential to be a product that can be one of the efforts to alleviate anemia. Therefore, to achieve systematic implementation of this research, there are several questions of problems that are to be further examined as follows:
A. What species of sea sponge in Indonesian waters has the potential to be iron-containing salt?
B. Can moringa leaves increase iron levels in salt products from this sea sponge?
C. What is the content of moisture, NaCl, impurities (Magnesium Sulfate, Calcium), and iron in salt samples?
D. What is the quality of salt based on the results of the analysis formulated in point 1.2.3 and which is the best formulation?
E. How much potential from the salt as an effort to alleviate iron deficiency anemia?

The purpose of the implementation of this research is to explore the potential of natural materials, especially sponge with the addition of moringa leaves as a functional salt rich in iron and its potential as one of the efforts to alleviate anemia. The objectives are more specific as follows:
A. Knowing the type of sea sponge in Indonesian waters that have the potential as salt-containing iron.
B. Knowing the effect of adding moringa leaves (Moringa oleifera) whether it can increase iron levels in salt products from this sea sponge.
C. Knowing the content of Moisture, NaCl, Impurities (Magnesium Sulfate, Calcium), and Iron in salt samples.
D. Know the quality of salt based on the results of the analysis formulated and which formulation is best.
E. Knowing the potential of salt from this study as one of the efforts to alleviate iron deficiency anemia.

2. Materials and Method

2.1 Initial Data Tracing Method

Initial data collection to explore the types of sponges that have the potential to be produced into a salt used data search portal NCBI (National Center for Biotechnology Information USA) with ("marine"[All Fields]) AND ("porifera"[MeSH Terms] OR "porifera"[All Fields] OR "sponge"["porifera"[All Fields]]) AND NaCl [All Fields]) AND ("2016/03/30"[PDat]: "2021/03/28"[PDat]) and with time limits in the last five years published.

Data related to leaves or natural materials of iron-rich plant types through data research portal NCBI (National Center for Biotechnology Information USA) with ("plant leaves"[MeSH Terms] OR "plant"[All Fields] AND "leaves"[All Fields]) OR "plant leaves"[All Fields] OR "leaf"[All Fields] AND ("iron"[MeSH Terms] OR "iron"[All Fields]) AND ("2016/03/30"[PDat]: "2021/03/28"[PDat]) and with a time limit within the span of five years of publication. Then, data related to prevalence and other data were obtained from the official website of the government and also the World Health Organization (WHO).

2.2 Salt Sample Processing Formulation

The type of marine sponge that is easy to find in Indonesian waters according to Abdjul et al., (2017) is Spongia sp. Therefore, Spongia sp is chosen to be sampled in the manufacture of this salt. Data obtained from Mallya et al., (2017), that moringa leaves have a high iron content and abundant availability is used as an addition to enrich iron in salt products to be produced.

Samples are formulated based on the comparison of sea water, sponge (Spongia sp), and moringa leaves in sequence: Sample A (1:1:0.05); Sample B (1:1:0.25); Sample C (1:1:0.5) and control that did not use the addition of moringa leaves (1:1). Variations of the formulation of these samples aim to find out if there is an influence of increasing concentration of moringa leaves on the results of iron contained in the sample salt. Moringa leaves were previously blended for 5 minutes and then done dried using the freeze-drying method or using Freeze Dryer Lyovapor™ L-200 for 12 hours. The seawater used comes from the beach with a
distance of 10 km from the mainland and is filtered using Whatman filter paper number 42 with a diameter of 11 cm 110 mm to avoid particles of dirt.

After filtering, all the ingredients of each sample A, B, C, and control are stirred or mixed (seawater with sponges and moringa leaf flour), then boiled using medium heat and continuously stirred until boiling so that the salt content is not flammable and resulted in a salt that resembles wet sand.

2.3 Content Analysis

According to (Soesilowati et al, 2013) and SNI 3556:2016, consumable salt is salt with a NaCl content of 94.7% based on dry weight with a maximum impurities content of Sulfate, Magnesium, and Calcium 2% and maximum water content of 7%. Therefore, the analysis conducted includes tests of moisture content, NaCl, impurities content of sulfate, magnesium, calcium, and iron levels which are all done in three replays of each sample or Triplo and using analytical methods from the Association of Official Analytical Chemists (AOAC) (Latimer, 2019). Analysis of antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH).

3. Results and Discussion

3.1 Potential Sea Sponge from Indonesian Waters

Natural products have been at the core of the evolution of organic chemistry since several years ago. About 40 molecules have been detected that have revolutionary or potential effects and become indispensable to humans because their activity can provide health effects (Hanif et al., 2019). Moreover, seeing Indonesia is an island nation whose territory mostly waters, very much Indonesia’s marine biodiversity (Lasabuda, 2013). Like the sea sponge, which is based on a search of data in the form of an international journal indexed NCBI database (National Center for Biotechnology Information USA) it was obtained that there are several types of marine sponge or marine natural products identified about 29,000 types (Hanif et al., 2019). The type of marine sponge that is easy to find in Indonesian waters (Abdjul et al., 2017) is Spongia sp and contains many NaCl (Bauvais et al., 2017). Therefore, this Spongia sp is chosen to be sampled in the manufacture of this salt.

Figure 1. Spongia sp.

3.2 Plants Rich in Iron Content

Similar to a sea sponge, local plants since a few years ago explored the level of nutrients to improve health and nutrition problems in many countries including Indonesia. Indonesia itself is a tropical country overgrown by many types of plants (Akbar et al., 2017). One of the potential biodiversity to become a health product is moringa leaves (Moringa oleifera). Moringa oleifera is a large plant that originated in North India. Almost all parts of the tree can be eaten or used as herb for traditional herbal medicines. This is especially true for its leaves and pods, which are commonly eaten in some parts of India and Africa (Stohs and Hartman, 2015; Mallya et al., 2017).
Moringa leaves are an excellent source of vitamins, antioxidants, and minerals. Based on data from (Departamento de Agricultura de Estados Unidos (USDA), 2019), one cup of fresh chopped leaves (21 grams) contains:
- Protein: 2 grams
- Vitamin B6: 19% of RDA
- Vitamin C: 12% of RDA
- Iron: 11% of AKG
- Riboflavin (B2): 11% of RDA
- Vitamin A (from beta-carotene): 9% of RDA
- Magnesium: 8% of AKG

In Western countries, dried leaves are sold as dietary supplements, both in powder and capsule form. In addition to its high iron content, moringa leaves are also easy to find in Indonesia and even the general public has begun to breed in the yard around the house. Therefore, moringa leaves are chosen as an additional ingredient in the salt processing process of this sea sponge. It is expected that with this addition, the level of iron in the salt produced also increases.

### 3.3 Moisture Content, NaCl, Impurities Sulfate, Magnesium, Calcium, and Iron in Salt Samples

All samples produced salt granules as much as 0.92 ± 0.22 kg. There was no difference in the results of salt granules from each sample and there was no difference in the level of fineness of salt granules, which is 20-35 Mesh or equivalent to crude salt. However, there are differences in the level of chemistry it contains. In sample A with a formulation of seawater, sponge (Spongia sp.), and Moringa leaves of 1:1:0.05, produces a percentage (%) each; moisture content of 4.65 ± 0.50; Fe content of 3.56 ± 0.02; NaCl levels 94.5 ± 0.20; Calcium impurities 1.25 ± 0.03; Sulfate impurities 1.88 ± 0.88, and Magnesium impurities 1.55 ± 0.48. In sample B with a formulation of seawater, sponge (Spongia sp.), and Moringa leaves of 1:1:0.25, produces a percentage (%) each; moisture content of 3.98 ± 0.02; Fe content of 3.97 ± 0.80; NaCl levels 95.8 ± 0.45; Calcium impurities 1.49 ± 0.02; Sulfate impurities 1.91 ± 0.90, and Magnesium impurities 1.67 ± 0.50. In sample C with a formulation of seawater, sponge (Spongia sp.), and Moringa leaves of 1:1:0.5, produces a percentage (%) each; moisture content of 4.75 ± 0.01; Fe content of 4.04 ± 0.06; NaCl levels 95.7 ± 0.22; Calcium impurities 1.60 ± 0.50; Sulfate impurities 1.96 ± 0.70, and Magnesium impurities 1.89 ± 0.60. The Control group that did not use the addition of moringa leaves (1:1) produced a percentage (%) each; moisture content of 5.80 ± 0.60; Fe content of 0.92 ± 1.00; NaCl content of 96.0 ± 0.50; Calcium impurities 1.10 ± 0.68; Sulfate impurities 1.52 ± 1.02, and Magnesium impurities 1.93 ± 0.33.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Water Content (%)</th>
<th>Iron Content (%)</th>
<th>NaCl Content (%)</th>
<th>Calcium Impurities (%)</th>
<th>Sulfate Impurities (%)</th>
<th>Magnesium Impurities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.65±0.50</td>
<td>3.56±0.02</td>
<td>94.5±0.20</td>
<td>1.25±0.03</td>
<td>1.88±0.88</td>
<td>1.55±0.48</td>
</tr>
<tr>
<td>B</td>
<td>3.98±0.02</td>
<td>3.97±0.80</td>
<td>95.8±0.45</td>
<td>1.49±0.02</td>
<td>1.91±0.90</td>
<td>1.67±0.50</td>
</tr>
<tr>
<td>C</td>
<td>4.75±0.01</td>
<td>4.04±0.06</td>
<td>95.7±0.22</td>
<td>1.60±0.50</td>
<td>1.96±0.70</td>
<td>1.89±0.60</td>
</tr>
<tr>
<td>Control</td>
<td>5.80±0.60</td>
<td>0.92±1.00</td>
<td>96.0±0.50</td>
<td>1.10±0.68</td>
<td>1.52±1.02</td>
<td>1.93±0.33</td>
</tr>
</tbody>
</table>
3.4 Best Quality Salt Samples Based on Comparison of Test Results of Each Sample

Based on Soesilowati et al, (2013) and SNI 3556:2016, that consumable salt is salt with a minimum content of NaCl 94.7% based on dry weight with a maximum limit of 2% sulfur, magnesium, and calcium impurities and water content not exceeding 7%. It was obtained that the lowest moisture content in sample B, the lowest calcium impurities in the control sample, and the second-lowest were the A samples whose difference was only 0.15%, compared to the higher B and C samples above, the lowest sulfate impurities in the control sample and the second-lowest was sample A, the lowest magnesium impurities in sample A and the second-lowest was sample B. When viewed from the value (%) The moisture content, calcium impurities, sulfate impurities, and magnesium impurities overall the lowest value is sample A and shows that the increase in the level of variation of moringa leaves does not have much effect on those values. But not only until there, basically all produced are included in the range of Indonesian National Standard SNI 3556:2016, so it can be said that all samples are safe to consume. But by looking at the main goal of producing salts containing iron or iron, that sample C that has a high content of Fe of 4.04±0.06 %. This suggests that the addition of concentration of terraced moringa leaves can
increase the level of Fe in this salt. It can be said that the C sample is the best version of high Fe levels but other chemical values remain in accordance with the SNI range.

Impurities are performed as a determinant of the quality and impurity of food or medicinal product, including salt. In general, this test is intended to determine the content of inorganic substances contained in a product as impactors in an organic substance (Wenno, Thenu, and Cristina Lopulalan, 2012). calcium impurities, impurities sulfate, and magnesium impurities present in a product can have the effect of reducing shelf life, altering physical and chemical properties, and can be toxic if consumed beyond safe limits. Such testing affects not only the quality but also the safety of a product (Wenno, Thenu, and Cristina Lopulalan, 2012). It is therefore very important to do this research process to see the quality and safety of Sponge's salt products. It was obtained that all samples did not exceed 2% or 0.02 in the value of calcium impurities, sulfate impurities, and magnesium impurities in accordance with SNI 3556:2016. This suggests that this product is not only potential but also safe to consume.

![NaCl Level Analysis Results](image)

**Figure 4.** NaCl Level Analysis Results (%)

Based on (Soesilowati et al, 2013) salt consumption is divided into 3 types: (1) food or high grade, namely high-quality salt consumption with NaCl content of 97% water content below 0.05%, clean white color, smooth crystal granules. This type of salt is used for table salt, food flavoring industry, high-quality food industry (snack food, chiki, taro, instant noodles, etc.), sausage and cheese industry, and cooking oil industry: (2) medium grade, i.e. middle-class consumption salt with NaCl content of 94.7-97% and water content of 3-7% for kitchen salt and medium industries such as soy sauce, tofu, animal feed; (3) low grade, i.e. low-quality salt consumption with NaCl content of 90-94.7%, the water content of 5-10%, dull-white color, used for salting fish and agriculture.

In this study, high NaCl salts were produced in the control sample of 96.0 ± 0.50% and the second-highest was sample B at 95.8 ± 0.45%. The lowest NaCl content is found in sample A with the amount of NaCl produced at 94.5 ± 0.20, and this does not correspond to SNI 3556:2016 that the NaCl minimum in salt products is 94.7%. But there is little difference between sample A and SNI 3556:2016 limit, which is the difference of 0.20%, this can be further refined through a more modern salting process or sophisticated tools. Seen in all samples if in total and average get a value of 95.5%, and this corresponds to the range on SNI for salt products or in other words that sponge can be used as salt consumption and included in the type of salt middle class or medium grade.
Furthermore, from some of the above exposures, it is known that samples B, C, and Control fall within the range of SNI 3556:2016 based on (%) moisture content, Fe, calcium impurities, sulfate impurities, and magnesium impurities were contained in all three samples. However, it is known that the C sample is the one that has the highest Fe levels compared to the B and Control samples, meaning that the C sample is the best sample that aligns with the purpose of this study which is to eliminate anemia. Because there are still few salt products fortified or with Fe content.

3.5 Antioxidant Levels

Sample with the best formulation, namely sample C is tested for its antioxidant activity. Obtained percent of antioxidant activity was 12.60 ± 0.05%. This suggests that the salt obtained through the C sample formulation is high in antioxidants. Compared to ordinary salts circulating in the community or that are used daily in the kitchen do not have antioxidant levels. According to Yoo's research, et al (2009), antioxidant levels have many functions, especially helping to protect free radicals, non-communicable diseases (cancer, obesity, heart, diabetes, etc.) and potentially minimize red blood cell deficiency. So, salt formulation C is a salt that Tayyib compared to salt that is consumed daily which is less Tayyib because it can cause various diseases such as hypertension if consumed in excess.

3.6 Sponge Salt Potential as Anemia Alleviation Efforts

The latest data of anemia, when viewed by age group or age in 2018, is as follows; age group 15 to 24 years old by 84.6%, age group 25 to 34 years old by 33.7%, age group 35 to 44 years old by 33.6% and age group 45 to 54 years old by 24% (RI), 2019). This indicates that anemia in Indonesia in all age groups has a prevalence of more than 24%. Anemia is a condition, where Hb or hemoglobin levels in the blood are lower than normal values and in Indonesia are mostly caused by a lack of iron intake and the need for daily consumption of food from a high source of iron (Parulian et al., 2016). The national salt balance shows that in 2020, the national salt requirement reached 4,464,670 tons (Central Bureau of Statistics, 2019). Looking at the data, that salt in Indonesia is indeed a basic necessity that is always consumed, therefore efforts are needed for the fortification of iron or iron in salt that is generally circulated in the community without fortification of iron. This opportunity is projected to have a massive impact both in efforts to alleviate iron deficiency anemia. This is in line with what the World Health Organization (WHO) says that salt today needs not only the fortification of iodine but also iron or iron as a countermeasure to anemia that is still high. Looking at the results of this study contained in figures 3, figures 4, and table 1, that salt derived from the formulation of seawater, sponge (Spongia sp), and Moringa leaves have the potential to be commercialized and felt the effect in efforts to alleviate anemia, especially in Indonesia. So, the important role of salt produced by this study can be a bridge of consumption so that Indonesians are free from iron deficiency anemia. The optimism of this researcher departed from the hadith RW Bukhari, that the Prophet (PBUH) conveyed "That all diseases, of course, there is a cure". In addition, this salt becomes a salt that is halal Tayyib, which means not only halal to be consumed but also Tayyib or good and healthy for the body.

4. Conclusion

Sponge sp. is a type of sea sponge that is easy to find in the Indonesian country and can be combined with Moringa leaves whose availability is also abundant in Indonesia, as a potential salt high in iron. This study obtained salt granules as much as 0.92 ± 0.22 kg. There is no difference in the results of salt granules from each sample, and there is no difference in the level of fineness of salt granules, namely 20-35 mesh or equivalent to crude salt. However, there are differences in the level of chemistry it contains. Samples B, C, and Control
are included in SNI 3556:2016 based on (%) moisture content, Fe, calcium impurities, sulfate impurities, and magnesium impurities were contained in all three samples. However, it is known that the C sample is the one that has the highest Fe levels compared to the B and control samples, which means that the C sample is the best sample and Tayyib from the health aspect and in line with the purpose of this study which is to eradicate iron deficiency anemia.

Notes
As for some shortcomings in this study that cannot be said, so researchers suggest further research as a product development effort that is a detailed calculation of its economic value and tests to animals to find out the continued effects of salt consumption results of this study. Moreover, researchers thanked many parties who have helped so that the implementation of this research and produce an appropriate product that is sponge salt.

Data Availability
No data are associated with this article. Data is available in the table in this main paper.

Conflict of Interest
F, Nurkolis., Dian A. Kumalawati, Reza S. Dewi, Maizer S. Nahdi, Defny S. Wewengkang, Hardinsyah, Nurpudji A. Taslim, Nelly Mayulu, stated that there is no conflict of interest from all of authors.

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Author Contributions
The authors’ responsibilities were as follows—all authors: contributed to the writing and revisions contained in the paper; and all authors have read and approved the final manuscript. H.K.P and F.N. collate study ideas, design and experiment, analyze data, and compile manuscripts. N.A.T, N.R, N.S, M.K, S.R, R.R and N.M analyze and interpret data and critically revise the manuscript. The F.N, S.L.N, D.S.W and H.K.P conducted experiments, analyzed biochemistry, and critically revised the manuscript. N.M, H.H, S.C.B, W.B.G and C.D.V, implemented experimental protocols, assisted in statistical analysis, interpreted data, and critically revised manuscripts. All writers read and approve the final manuscript.

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