

Original article

Species identification by DNA barcoding and bioprospecting of Indonesian seahorses

Mala Nurilmala^{1,*}, Evi Maya Sari¹, Asadatun Abdullah¹, Agus Oman Sudrajat¹ and Yoshihiro Ochiai²¹ Faculty of Fisheries and Marine Science, Bogor Agricultural University, Indonesia² Graduate School of Agricultural Science, Tohoku University, Aramaki, Aoba, Sendai, Miyagi 980-0845, Japan

* Correspondence: mnurilmala@ipb.ac.id

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Introduction

Seahorse (*Hippocampus* spp.) is one of the marine organisms belonging to the family Syngnathidae. The habitat of seahorses is in shallow tropical and temperate waters around the world [1]. They are unique organisms exhibiting male pregnancy. The trading of seahorse involves live and dead conditions. It has been known that seahorse is utilized as a traditional medicine, particularly traditional Chinese medicine (TCM) and its derivatives. This is mainly traded in dead specimen, while the live seahorse is for ornamental display [2,3]. Although seahorse is easy to be recognized, many species are similar in appearance. Thus, the method for accurate species identification is needed. Recently, DNA barcoding has become a popular and useful method for this purpose by using short nucleotides.

Indonesia is one of the tropical countries, having high biodiversity including seahorse in the vast coastal area. There are actually nine species so far reported in Indonesian waters [2]. Seahorse is being harvested from the wild throughout the year in Indonesia, then the dried products are traded internationally. However, there have been a few reports on Indonesian seahorse and thus the available information is quite limited. Therefore, the exploration of seahorse was carried out in this study with molecular approach using DNA barcoding for identification of seahorse species accurately. The proximate composition and free amino acid profiles were also determined, and the secondary metabolites were further investigated.

Materials and methods

The specimens of seahorse were collected from the coasts of Sumatera and Seribu Islands, Indonesia. The proximate analysis of water, protein, lipid, and ash was carried out based on AOAC methods [3]. The ethanol extract from the whole body of seahorse was subjected to phytochemical analysis to determine its secondary metabolites.

The species identification was carried out by a DNA barcoding method using mitochondrial *cytochrome oxidase subunit 1 (COI)* as a molecular marker. The DNA of the specimens was isolated using DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The purity of the isolated DNA was assessed by horizontal electrophoresis. The amplification of isolated DNA was carried out by PCR at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 45 sec, annealing at 58°C for 1 min 45 sec, and extension at 72°C for 1 min, followed by final extension at 72°C for 6 min. The PCR products were assessed by electrophoresis in 1.25% (w/v) agarose gel in 1 × TBE buffer at 100 V for 25 min. The sequences were subjected to further bioinformatical analysis.

Results and discussion

The DNA was isolated and amplified successfully using the primers as designed from *COI* gene sequences. BLAST analysis showed that the species of seahorse examined in this study were *H. kuda* and *H. comes*. The results of this study showed that molecular identification was reliable and effective to identify the species of sea horse. This reminds us the report that a molecular forensic method revealed the authentication of dried seahorse in Taiwan [3].

The results of chemical composition analysis showed that the seahorse (*H. comes*) consisted of 66.16 ± 0.33% moisture; 22.73 ± 0.17% protein; 1.18 ± 0.23% lipid; and 9.55 ± 0.15 ash (n=3). Hydrolysates of seahorse proteins were found to contain the amino acids such as glutamic acid, aspartic acid, arginine, and alanine as the major components. In addition, the results of the qualitative phytochemical analysis showed that the ethanol extract contained flavonoids, triterpenoids, steroids, saponins, and phenol hydroquinone. These bioactive compounds could be the promising targets for further effective utilization of natural products from the seahorse.

Conclusions

DNA barcoding revealed that the specimens of seahorse in this study. The species of sea horses were *H. kuda* and *H. comes*. The secondary metabolites of seahorse are considered to be the potential bioactive compounds beneficial for human health.

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