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BIOPROSPECTING MANGROVES: ANTIOXIDANT SOURCE AND HABITAT FOR THE ENDEMIC *Bubalus* sp. IN RAWA AOPA WATUMOHAI NATIONAL PARK, INDONESIA

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ABTRACT

This study determines the antioxidant contents in mangroves leaves, and elucidates strategies feasible for the management of mangroves forest and conservation of the endemic *Bubalus* sp. in RAWN Park, Indonesia. The antioxidant contents including anthocyanin, alkaloid, tannin and vitamin C were determined in leaf samples of *Lumnitzera racemosa, Ceriops tagal* and *Ceriops decandra* mangrove trees grown in RAWN Park. The leaves of *C. tagal* contained the highest anthocyanin (0.068%) in comparison to *C. decandra* (0.047%) and *L. racemosa* (0.042%). On the contrary, alkaloid content of *L. racemosa* leaves (0.067%) was significantly higher than that of *C. tagal* (0.046%) and of *C. decandra* (0.048%). Similarly, the tannin content of *L. racemosa* leaves (29.66%) was significantly higher than *C. tagal* (23.53%) and *C. decandra* (7.11%). In addition, the vitamin C content of the *L. racemosa* leaves (283,11 mg/100g) was significantly higher than *C. decandra* (231,35 mg/100g) and *C. tagal* (216,82 mg/100g). The footprints of areas found in these mangroves forest, which indicated that they might be used mangrove as feeding and resting areas. The results of study imply the potentiality of mangroves in RAWN Park as antioxidant and food source of *Bubalus* sp. The findings of this study realized the important of mangroves as antioxidant and food sources, as well as habitat of lowland anoas. Therefore, sustainable management mangrove forests must be given priority as an important habitat for endemic animal.

Key words: Anthocyanin, alkaloid, tannin, vitamin C, *Bubalus* sp., mangroves, RAWN Park

INTRODUCTION

Coastal plants grow and thrive in extreme environments because of their ability to produce secondary metabolites such as antioxidant compounds, required to adapt under saline conditions (Dat *et al.*, 2000; Sairam *et al.*, 2004). Various types of antioxidants which are also bioactive compounds include the phenolics, tannins, carotenoids, glycosides, fatty acids, organic acids, vitamins, pectin and others (Jawad *et al.*, 2013; Djilas *et al*., 2009).

Mangroves have unique biochemical characteristics and produce secondary metabolites for their survival at extreme environmental conditions. Several studies have elucidated the antioxidants production in mangroves as their biochemical adaptation and source of bioactive compound (Asha *et al.*, 2012; Ravindran *et al.*, 2012; Agoramoorthy *et al.*, 2008; Deepanjan *et al.*, 2008; Rahim *et al.*, 2008; Jithesh *et al.*, 2006). Mangroves are known to have various metabolites possessing antibacterial and antifungal (Abeysinghe *et al.*, 2006), anti-feedant (Wu *et al.*, 2008) and antiplasmodial (Ravikumar *et al.*, 2010) properties. The antioxidants produced by mangroves are important compounds for human and may also benefit the animal health. Some mangroves can be used as ruminant feedstock (Mouafi *et al.*, 2013). The mangrove *Sonneratia alba* is one of the preferred ruminants (Agustina *et al.*, 2014). Meanwhile, the lowland anoa (*Bubalus depressicornis*), found in Sulawesi is inhabited on mangrove, beach, reverie, lowland, and lower mountain forests (Mustari, 1995), and they are known to feed on grasses, ferns, saplings, palm,

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ginger, herbs, fruit, and marsh and aquatic plants (Popenoe, 1981; Nowak, 1999). However, little information is known regarding foods and preference habitat of lowland anoas in mangrove forest.

Mangrove forest at RAWN Park is one the most fascinating protected forests in Southeast Sulawesi, Indonesia due to its high mangrove diversity and presence of threatened and endemic animal of anoas as of their footprints are found in several mangroves stands of *Lumnitzera racemosa* and *Ceriops* sp. (Analuddin *et al.*, 2013). The mangroves of *Lumnitzera racemosa* and *Ceriops* spp are dominant near the land site (Analuddin *et al.*, 2013) and might become habitat and potential food source for endemic animal of *Bubalus* sp. There is a lack information regarding the supporting ability of mangrove forest as habitat of endemic animal also commonly known as Anoa (*Bubalus* sp.) till now.

Population of anoas is on threat status due to habitat destruction and land conversion to agriculture as well hunting, and therefore, the prevention of habitat loss at key sites, and determination of the status of remaining populations are needed (Burton *et al.*, 2005). Meanwhile, Sutherland (1996) suggested the important data of population size as a function of environmental change and habitat disturbance to predict the longterm persistence of animal populations. Previous study of anoas in South East Sulawesi showed that population densities were approximated about 0.9 and 1.1 anoas/km² in Tanjung Peropa and Tanjung Amolengo Wildlife Reserves (Mustari 2003). Nevertheless, estimation of anoas abundance is difficult although previous studies about estimation population sizes of anoas were done by using line transects and camera trapping (Mustari 2003, Riley *et al.*, 2001a, b). However, Dierenfeld (1997) argued that for zoo management and species conservation that it is important to know what kind of diet of anoas should receive in captivity because their nutrition contributes to their health status and reproductive success. Thus, scientific information concerning the bioprospect of mangroves as habitat and food sources of anoas are essential for conservation and management strategies of mangroves and habitat of endemic lowland anoas in Rawa Aopa Watumohai National Park and its surrounding areas. Therefore, the objectives of this study were to elucidate the antioxidants and nutrition content including anthocyanin, alkaloid, tannin and vitamin C on mangroves leaves, and to improve the strategy for management of mangroves forest and endemic animal of lowland anoas in RAWN Park, South East Sulawesi.

MATERIALS AND METHODS

Study site

This study was carried out in Rawa Aopa Watumohai National Park (RAWN Park), Southeast Sulawesi, Indonesia (03°50'S, 122°30'E, Fig. 1, RAWN Park, 2011). The mangroves in RAWN Park provided excellent site for studying mangroves antioxidant production and mangroves function as habitat for lowland anoas where *Lumnitzera racemosa*, *Ceriops tagal* and *Ceriops decandra* showed good growing condition. The mangrove *L. racemosa* is monospecific dominant on the border of the land site, and the tree canopy is closed due to crowded stands, and shows different growth stages and stand performance (Analuddin *et al.*, 2016). The growth performances of these mangroves are mostly seedlings and saplings, though they showed as poles in some stands. These mangroves showed clearly zonation, where mangrove *L. racemosa* stands is located near the inland area, while *C. decandra* and *C. tagal* stands are located in the middle area (Analuddin *et al.*, 2013). These mangroves became a habitat of endemic animal *Bubalus* sp. as of their footprints found in mangroves habitat as of footprints of anoas in sampling plot of mangrove forest (Fig. 2). Three mangroves stands with same growth performance of seedlings and saplings were selected.

Sampling methods

We did survey in the mangroves stands to make sure the presence of lowland anoas (Fig. 2). The leaves samples of mangroves were taken from different canopy layers of *Ceriops tagal*, *Ceriops decandra* and *Lumnitzera racemosa* grown at the RAWN Park. All selected samples were mature leaves. The leaves samples were 3 replications from each canopy layer of these mangroves. The samples were placed in the plastic and labelled, then kept in box with dry ice. The samples then brought and placed in cold freezer at Laboratory Forensic at Halu Oleo University. The leaves samples then crushed and extract was obtained for determination of anthocyanin, alkaloid and tannin as well as vitamin C.

Anthocyanin extraction begins by weighing 2 g of leaves from each mangrove species and mixed with a half portion of 25% acetic acid solution (100 mL) and then transferred to a test tube. The extraction was centrifuged for 10 minute and filtered by using Whatman 41 paper. The filtrate samples were analyzed using spectofotometry on wave length of 510 nm. The total content of anthocyanin was calculated by formula used by Amelia *et al*.

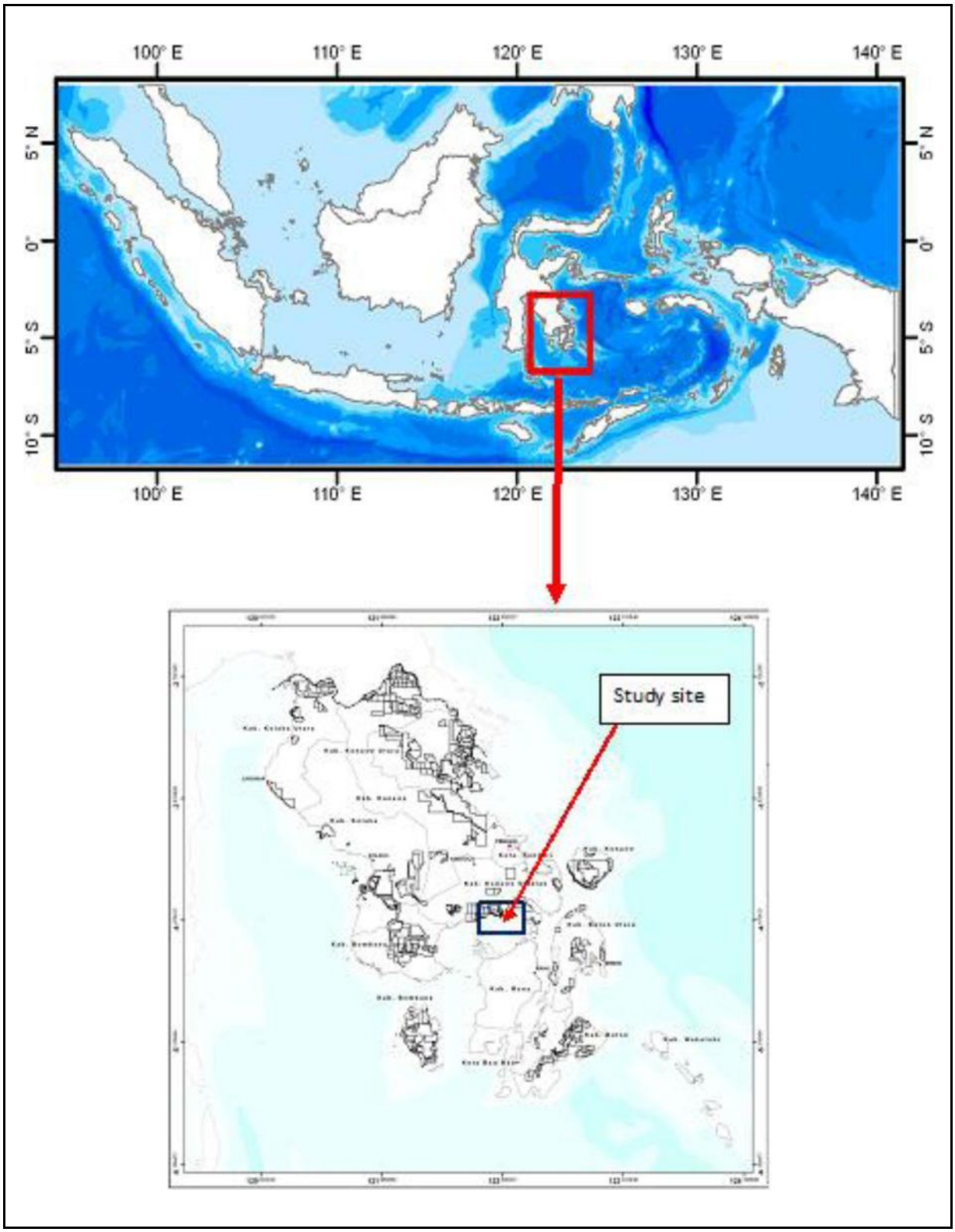

Fig. 1. Maps of Indonesia (upper) and of Southeast Sulawesi (below). Small box is study site of Rawa Aopa Watumohai National Park.

(2013) and by Wrolstad and Giusti (2001), i.e. Each sample was dissolved in potassium chloride-hydrochloride acid buffer solution pH 1.0 and sodium acetate trihydrate (CH3COONa.3H2O) buffer solution pH 4.5. The absorbance was determined at 510 nm in a UV-Visible spectrophotometer. The dilution factor for each sample was firstly determined by dissolving the sample in KCl buffer

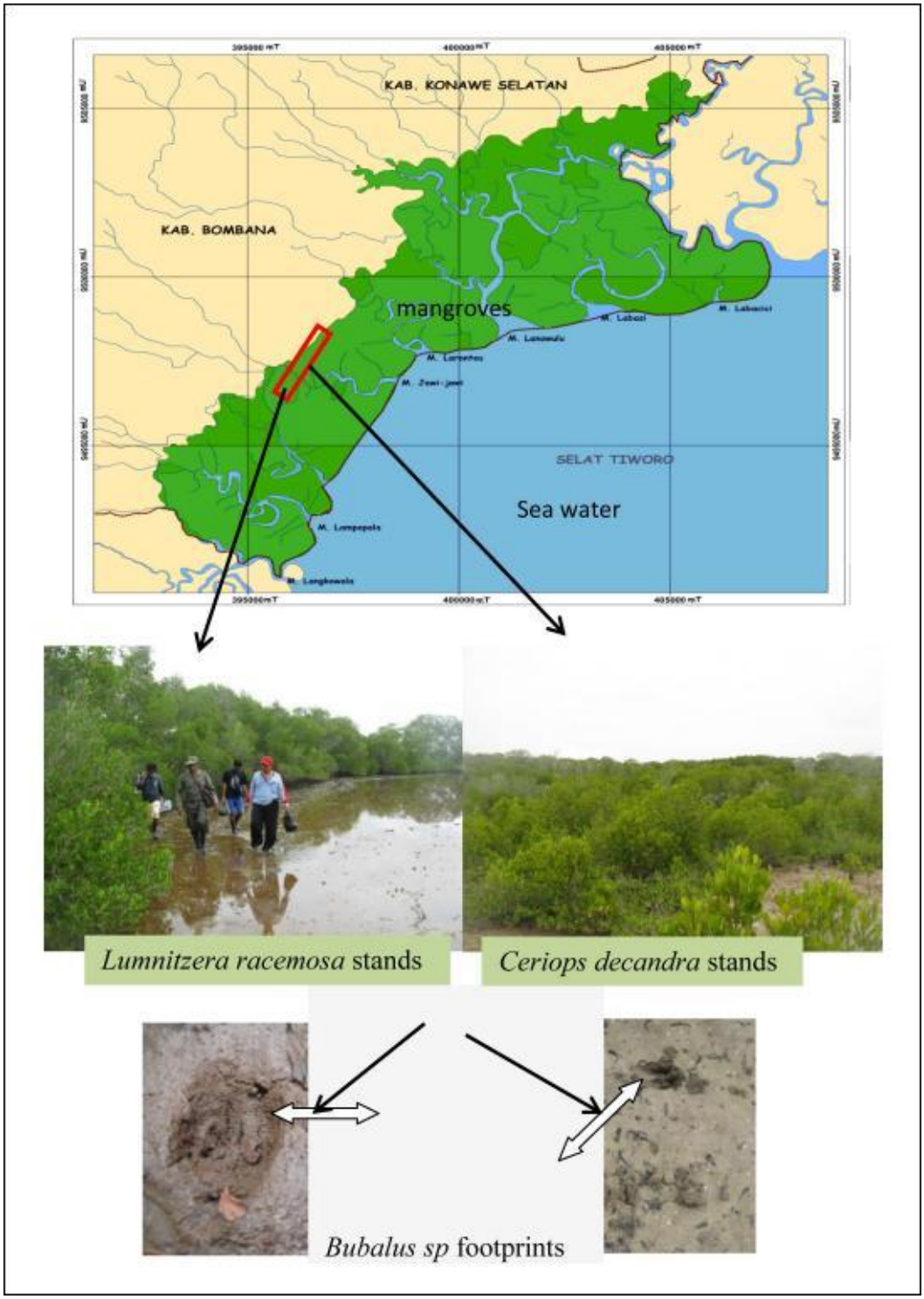

Fig. 2. Upper, Map of RAWN Park (small box is sampling plot); Middle, the mangroves forest as home range lowland anoas in *Lumnitzera racemosa* (Left) and *Ceriops tagal* (right) stands; Bottom, footprints of lowland *Bubalus* sp. inside mangroves of RAWN Park.

pH 1.0 until its absorbance at 510 nm obtained less than 1.2 versus the KCl buffer pH 1.0 as blank. Sample was then dissolved in KCl buffer pH 1.0 (allowed to stand for another 15 min.) and CH3COONa.3H2O buffer pH 4.5 (allowed to stand for another 5 min.) based on the dilution factor. The absorbance for each sample was then read versus the buffer solution pH 1.0 and buffer solution pH 4.5 as the blank at λ = 510 nm (for the cyanidin 3-glucoside) and λ = 700 nm (for correction factor). The final absorbance (A) was calculated by using the formula as follows:

$$A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$$

Total anthocyanin concentration was calculated by using the following formula:

$$TAC = \frac{A}{\varepsilon L} \times MW \times DF \times \frac{V}{Wt} \times 100\%$$

where TAC is total anthocyanins content (mg. 100 g^{-1} of sample); [ε is molar absorption coefficient of cyanidin 3- glucoside (226,900 L $(mol.cm)^{-1}$); L is width of cuvette (1 cm); MW is molecular weight of cyanidin 3-glucoside (449.2 g. mol-1); DF is dilution factor; V represents final volume or sample volume after dilution (L); and Wt is weight of original extract (g).

Alkaloid content of mangrove leaves was analyzed according to the method of Harborne (1973). Alkaloid extraction was done by crushing 5 g of leaves from each mangrove and then added with 200 mL of 10% acetic acid in ethanol and covered for 4 hours. After the solution was filtered, then the extract was concentrated on a water bath to 1/4 of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitation was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Tannin content of mangrove leaves were determined by the spectrophotometric method developed by Rangana (1979). Standard curves were made by dissolving 0.5 mg tanat acid into 50 ml distilled water. The concentrations standard solution raised from 0.02 to 0.1 mg / ml, and added with 0.5 Folin Denis reagent and 1 ml of saturated sodium carbonate solution and made up to 10 ml with distilled water. The absorbance measured at a wavelength of 725 nm using the blank solution that showed absorbance of 0 (zero).

Vitamin C content was determined by destruction of mangroves leaves, then extracted with water-citrate acid solute (9:1) for 30 minute, then centifuged for 10 minute and filtered using Whatman paper. About 10 ml of filtrate was taken and observed its absorbation at 269 nm according to method by Devlin (1975) and Qasim *et al.* (2009). To the filtrated sample solution a few drops of bromine water were added until the solution became colored (to confirm the completion of the oxidation of ascorbic acid to dehydroascorbic acid). Then a few drops of thiourea solution were added to it to remove the excess bromine and thus the clear solution was obtained. Then 2, 4-Dinitrophenyl hydrazine solution was added thoroughly with all standards and also with the oxidized ascorbic acid. Total vitamin C employing coupling reaction of 2, 4-Dinitrophenyl hydrazine dye with vitamin C and followed by spectrophotometric determination.

Data Analysis

Statistical analysis by *t-test* at significant level of 5% was done for chemical content in leaves of among canopy layer for each mangrove species. Statistical analysis was also done for chemical content in leaves among mangrove species.

RESULTS

Anthocyanin content

The anthocyanin content in mangroves leaves of *Lumnitzera racemosa*, *Ceriops decandra* and *Ceriops tagal* represented by Fig. 3. The anthocyanin content in leaves of *L. racemosa* (Fig. 3A) was significantly higher (P = 0,03) in top canopy leaves (0,049%) than bottom canopy leaves (0,032%) and middle canopy leaves (0,034%), respectively. These trend indicated that top leaves of *L. racemosa* produced high anthocyanin as compared other leaves because top leaves might receive higher light intensity than middle and bottom canopy leaves. On the contrary, the anthocyanin content in leaves of *C. decandra* (Fig. 3B) was not significantly different (P > 0,05) between top leaves (0,057%) and middle leaves (0,058%), but it was significantly different (P = 0,01) between top leaves and bottom leaves (0,028%), as well as between middle leaves and bottom leaves (P = 0,03). Similarly, the anthocyanin content in leaves of *C. tagal* (Fig. 3C) was estimated significantly higher in middle leaves (0,103%) than in the top leaves (0,057%) (P = 0,006) and bottom canopy leaves (0,049%) (P = 0,001). However, the average of anthocyanin content in leaves of *C. tagal* (0,068%) was significantly higher (Fig. 3D) than *C. decandra* (0,047%) (P = 0,02) and *L. racemosa* (0,042%) (P = 8,02 x 10^{-5}). In addition, the anthocyanin content in leaves of *C. decandra* and *L. racemosa* was significant different (P = 0,01).

Alkaloid content

The alkaloid content in leaves of *Lumnitzera racemosa*, *Ceriops decandra* and *Ceriops tagal* was described by Fig. 4. The alkaloid content in leaves of *L. racemosa* (Fig. 4A) was not significantly different among leaves (P > 0,05), i.e. it was estimated as 0.071% for top leaves, 0.063% for middle leaves and 0.066% for bottom leaves. Similarly, the alkaloid content in leaves of *Ceriops decandra* (Fig. 4B) was not significantly different (P > 0,05) between than top leaves (0,043%) and middle leaves (0,071%), as well as between top

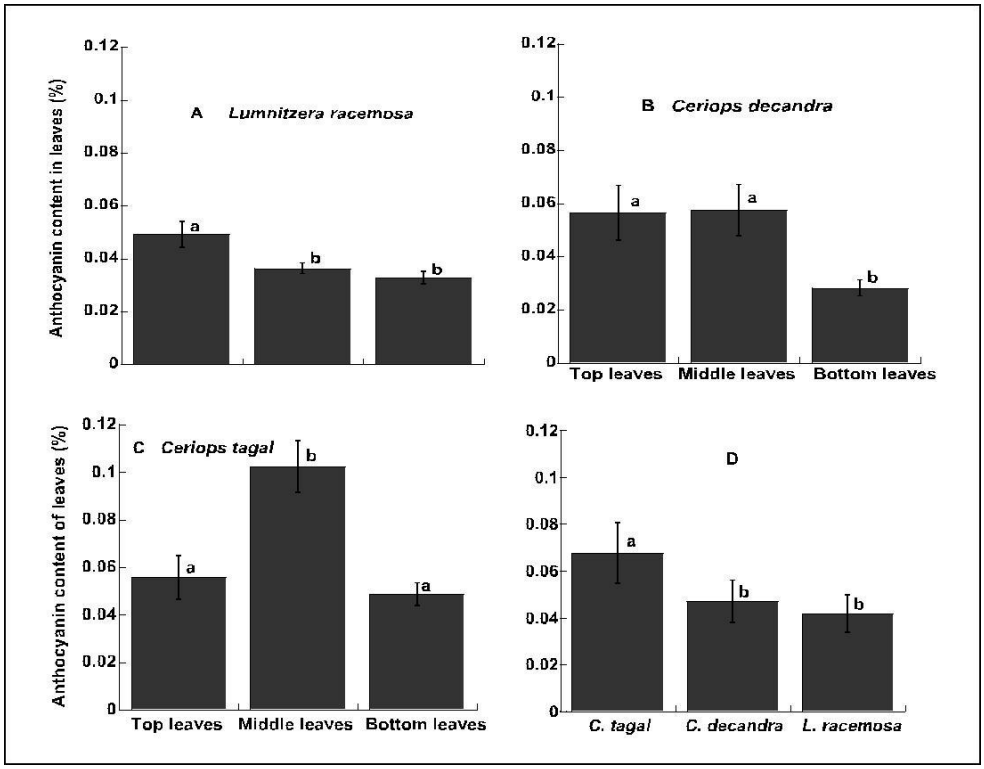

Fig. 3. The anthocyanin content in leaves (Mean ± SE) of three mangroves grown at TNRAW Park. Vertical lines are Standard Error SE of data. The same letters of *a* and *b* are not significantly different at significant level of 5%.

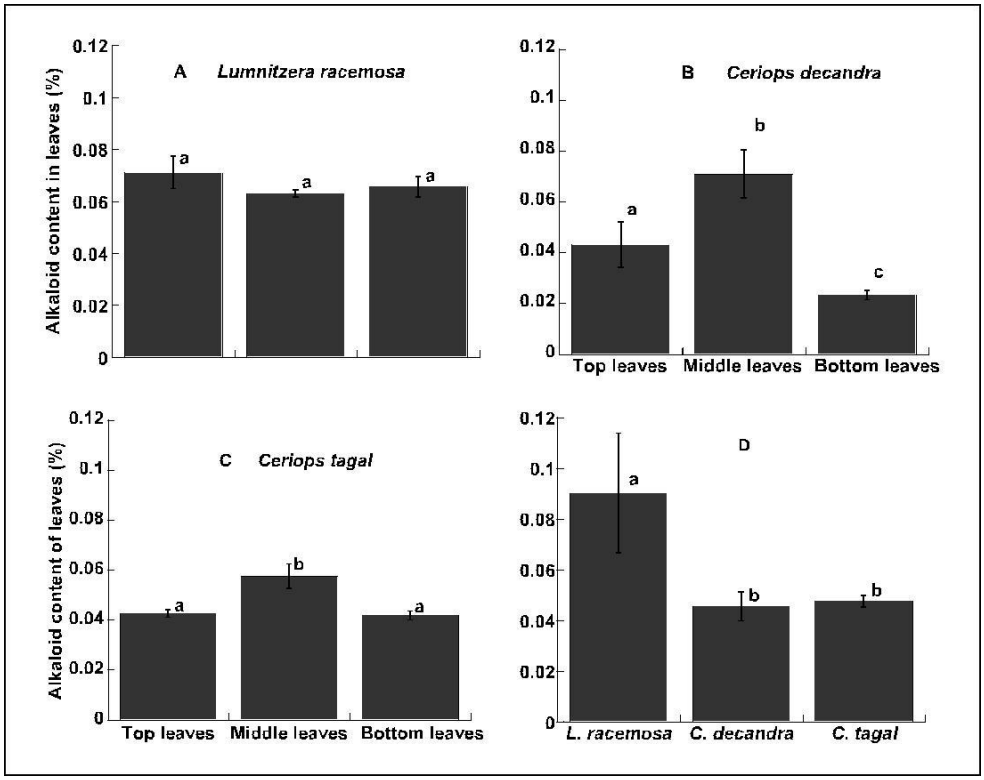

Fig. 4. The alkaloid content in leaves (Mean ± SE) of three mangroves species grown at TNRAW Park. Vertical lines are Standard Error SE of data. The same letters of *a*, *b* and *c* are not significantly different at significant level of 5%.

leaves and bottom leaves (0,023%), excepting the alkaloid content of midldle leaves and bottom leaves was significantly different (P = 5,1 x 10^{-4}). In addition, the alkaloid content in leaves of *C. tagal* (Fig. 4C) was not significantly different among leaves (P > 0.05). It was estimated to be 0,043% for top leaves, 0,058% for middle leaves and 0,042% for bottom leaves. However, the average alkaloid content (Fig. 4D) in leaves of *L. racemosa* (0,067%) was significantly higher than *C. tagal* (0,046%) (P = 3,6 x 10^{-2}) as well as for *C. decandra* (0,048%) (P = 5,4 x 10^{-2}). Meanwhile, the alkaloid content in leaves of *C. decandra* was not significantly different to *C. tagal* leaves (P = 0,79).

Tannin content

Fig. 5 represents the leaves tannin content of *Lumnitzera racemosa*, *Ceriops decandra* and *Ceriops tagal* mangroves. The leaves tannin content of *L. racemosa* was significantly different (P = 0,03) both among the top canopy and bottom canopy leaves, as well as among the middle and bottom canopy leaves (Fig. 5A), which was estimated as 39.11% at the top leaves, 34.09% for middle leaves and 8.83% for bottom leaves. On the contrary, the leaves tannin content of *C. decandra* was not significantly different (P > 0,05) among canopy layers (Fig. 5B), which was estimated as 10.56% for top leaves, 7.67% for middle leaves and 3.11% for bottom leaves. Similarly, the tannin content in leaves of *C. tagal* was not significantly different (P > 0,05) among canopy leaves (Fig. 5C), which was estimated to be 24.19% for top leaves, 26.61% for middle leaves and 19.79% for bottom leaves. However, the average tannin content in leaves of *C. decandra* (7.11%) (Fig, 5D) was significantly lower than in leaves of *L. racemosa* (29.66%) (P = 4,1 x 10^{-4}) and in leaves of and in leaves of *C. tagal* (23.53%) (P = 1,9 x 10^{-4}), but it was not significantly different between tannin in leaves of *L. racemosa* and *C. tagal* (P = 0,20). Therefore, these mangroves can be considered as high potentiality of antioxidants source and food source for endemic animal *Bubalus* sp. due to high tannin contents in leaves.

Vitamin C content

Fig. 6 describes vitamin C content in leaves for *Lumnitzera racemosa*, *Ceriops decandra* and *Ceriops tagal*. The vitamin C content in leaves for *L. racemosa* (Fig. 6A) was estimated to be 291.51 mg/100g for top leaves, 284.10 mg/100g for middle leaves and 273.72 mg/100g for bottom leaves, and was not significantly different (P > 0.05) among canopy leaves. Similarly, vitamin C content in leaves of *Ceriops decandra* (Fig. 6B) was estimated

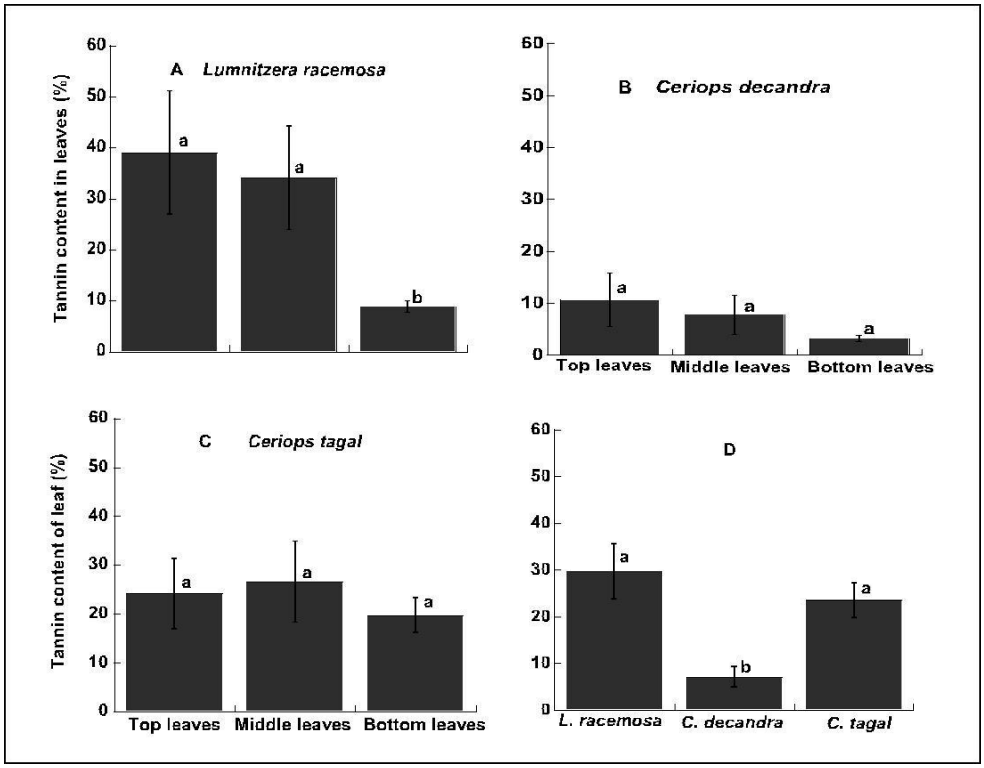

Fig. 5. The tannin content in leaves (Mean ± SE) of three mangroves species grown at TNRAW Park. Vertical lines are Standard Error SE of data. The same letters of *a* and *b* are not significantly different at significant level of 5%.

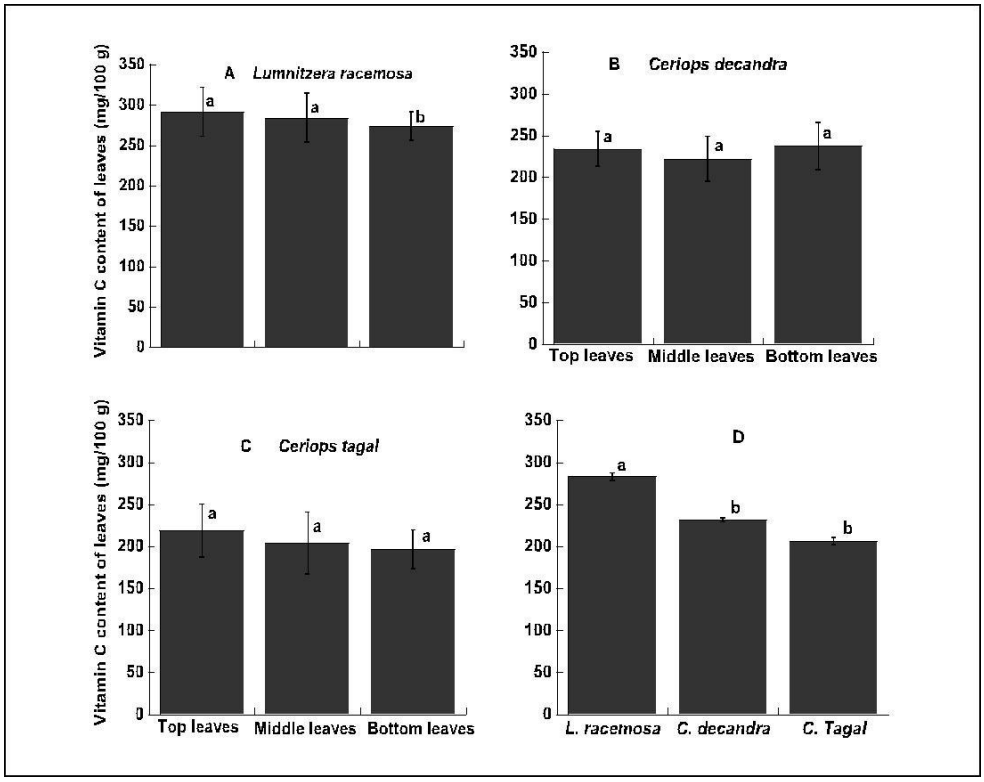

Fig. 6. The vitamin C content in leaves (Mean ± SE) of three mangroves species grown at the TNRAW Park. Vertical lines are Standard Error SE of data. The same letters of *a* and *b* are not significantly different at significant level of 5%.

to be 234.13 mg/100g for top leaves, 222.24 mg/ 100g for middle leaves and 237.69 mg/100g for bottom leaves, and it was not significantly different ($P > 0.05$) among canopy layers. In addition, vitamin C content in leaves of *Ceriops tagal* (Fig. 6C) was not significantly different ($P > 0.05$) among canopy layer. It was estimated to be 218.85 mg/100g for the top leaves, 203.99 mg/100g for middle leaves and 196.22 mg/100g for bottom leaves. However, Fig. 6D showed that the vitamin C content in leaves of three mangroves was significantly higher in *L. racemosa* (283.11 mg/100g) than in *C. decandra* (231.35 mg/100g) ($P = 7{,}4 \times 10^{-3}$) and in *C. tagal* (216.82 mg/100g) ($P = 5{,}4 \times 10^{-3}$). The higher vitamin C content in these mangroves forest can be considered as potential food source and suitable habitat for endemic animal *Bubalus* sp.

DISCUSSION

The present result clearly showed the variety of antioxidants production on mangroves leaves of *L. racemosa*, *C. decandra* and *C. tagal* that are grown in the RAWN Park. This study realized that anthocyanin production on leaves of these mangroves is affected by leaves position on the canopy layers (Figs. 3A, 3B and 3C). Similarly, alkaloid production on *C. decandra* and *C. tagal* is affected also by leaves position at the canopy (Figs. 4B and 4C). The same trend showed for tannin and vitamin C production on leaves of *L. racemosa* (Figs. 5A and 6A). The tannins content on mangrove leaves in this study are on the ranges of 20-40% in leaves tissue of mangroves as reported by other studies (Kraus *et al.*, 2003; Lin *et al.*, 2006, 2007). On the other hand, vitamin C production on the leaves of *C. decandra* and *C. tagal* was not depend on the leaves position at the canopy layers (Figs. 6B and C). However, the top canopy leaves of *L. racemosa* (Fig. 3A) and *C. decandra* (Fig. 3B) produce much higher anthocyanin, while higher alkaloid content was found in the middle leaves of *C. decandra* (Fig. 4B) and *C. tagal* (Fig. 4C). Meanwhile, bottom leaves these three mangroves tended to produce lower antioxidant.

Antioxidants production in mangroves varied among species. The *C. tagal* produces much higher anthocyanin than *C. decandra* and *L. racemosa* (Fig. 3D), while *L. racemosa* produces much higher alkaloid, tannin and vitamin C compounds on its leaves than *C. decandra* and *C. tagal* (Figs. 4D, 5D and 6D). These differences might be due to different biochemical adaptation of mangroves on the extreme environmental condition. Anthocyanins are produced and accumulated in plants as a form of

adaptation to salinity stress (Meneguzzo *et al.,* 1999). Anthocyanin plays an important roles for protection of plants on salt and light stresses (Smith *et al*., 2000; Selmar, 2008). Stresses of high salt concentration and light intensity might induce the plants to produce freeradical compounds, which become toxic if high concentration in the plants tissues. Selmar (2008) pointed out that synthesis of anthocyanin controled by enzimatic activity and environmental condition. Smith *et al.* (2000) mentioned that the plants produce anthocyanin to scavenge the freeradical compounds. Thus this study result realized that anthocyanin compounds might protect mangroves against environmental stresses of both biotic and abiotic by preventing the formation of oxygen species that are harmful to mangroves. Doke *et al.* (1994) verified that many environmental factors that cause plant stress and induce formation of anthocyanin compounds such as temperature, salinity, heavy metals, and UV light radiation.

High tannins content on mangroves leaves might act as an inhibitor compounds for micro-organisms activity, which inhibited the growth of plants. Tannin inhibits the growth of micro-organisms by inhibiting the enzyme activity of the enzymes needed for the growth of microorganisms. Previous studies showed that *Excoecaria agallocha* mangrove produced antioxidant with potential toxicity in its habitat (Kathiresan and Thangam, 1987, 1990). The tannins are known to have many beneficial roles on animal nutrition and health (Crozier *et al.,* 2009), influences on the cell signaling pathways (Achike *et al.,* 2003), anti-oxidative effects (Koleckar *et al.,* 2008), anti-helmintic (Lisonbee *et al.,* 2009) and anti-microbial (Buzzini *et al.,* 2008) activities. In ruminants, tannins have a particularly important positive effect on dietary protein protection from ruminal microflora attack (McNabb *et al.,* 1996), and improve the utilization of nitrogen by ruminants, as well as reduction in ruminal gas production (Makkar *et al.,* 1995). Thus, tannins can be associated with improvements in animal growth and productivity and consequentially minimization of effects to the environment (Lamy *et al.,* 2011).

From the point of view conservation of RAWN Park as home range of *Bubalus* sp., high content of tannin and vitamin C on these three mangroves are important as food source and habitat of endemic animal *Bubalus* sp. Saifullah (1984) mentioned that mangrove swamps are rich in food materials which are readily consumed by estuarine animals. Qadri and Jamil (1993) reported high energy values of the *C. tagal* fruit and hypocotyl, which are potential food source of nutrients and hence may be supplemented for animal feed. Therefore, the lowland anoas might be eaten mangrove leaves to support their nutrition. Saenger and Hutching (1987) pointed out that the animals consume the leaves of mangroves due to high tannin content. Melisch (1995) pointed out that the lowland anoas seem to favour swampy areas within forests along the coast, including mangroves. Our study realized that when the leaves of *L. racemosa, Ceriops decandra* and *C. tagal* were given directly to the goats at nursery, they were eaten firstly the leaves of *L. racemosa* than leaves of *C. decandra* and *C. tagal*. Therefore, the lowland anoas might be eaten mangrove leaves to support their nutrition and health. Meanwhile, knowing on the antioxidant properties of mangroves could be helpfull for conservation strategy and uses of mangroves for various purposes. Thus, *L. racemosa* might become preferable food and to be habitat for *Buballous* sp. due to leaves contain of high tannin and vitamin C. However, this mangrove is known to remove almost its leaves in dry season. Thus, the *Bubalus* sp. might use intensively *L. racemosa* in rainy season, while mangroves *C. tagal* and *C. decandra* might use as main habitat and food source in dry season. The *Bubalus* sp. might be eaten on mangroves leaves not only for their growth and development, but it might be eaten for their body maintenance. Hassapour *et al.* (2011) pointed out that tannins have positive effects on animals by antimicrobial, anthelmintic, protein by passed effects in ruminants. Flores-Miyamoto *et al.* (2005) described that the anoa shows a digestive physiology typical for an intermediate feeder/grazer that is evident from the selective particle retention in the fore stomach, digestibility coefficients, and faecal water content. Osuga *et al.* (2008) stated that chemical composition of the foliage species plays a crucial role in the extent to which they are utilized by ruminant animals of goats and sheep. Therefore, managing the mangroves is important for ruminants including endemic animal of *Bubalus* sp. food source and their habitat.

The mangroves at surrounding areas of RAWN Park have been degraded, and reduced the home range of *Bubalus* spin this region. Thus, several efforts for improving the mangroves at RAWN Park and its surrounding areas for ensuring sustainable of mangroves and *Bubalus* sp. :

1) Managing existed natural mangroves regeneration by moving debrish from the site, and providing fertilizer for better growth of seedlings of *L. racemosa, C. tagal* and *C. decandra.*

2) Growing new seedlings of these three mangroves at difficult site where natural regeneration is not possible.

3) Maintaining the existed crowded young stands of these three mangroves by cutting some branches, which allow sunlight to reach at the ground, and support new seedlings growth and develop.

However, management of mangroves ecosystem must be addressed to ensure the sustainability of mangroves and their supporting habitat for various animals at the RAWN Park and its surrounding areas.

CONCLUSION

Our study provide important information on the production of antioxidants by mangroves that are grown at the Rawa Aopa Watumohai National Park. The following mangroves; *L. racemosa, C. decandra* and *C. tagal* contained high content of antioxidants. Therefore, in conserving the habitat of *Bubalus* sp., an endemic species, it is necessary to conserve these mangroves which contain high tannin and vitamin C in their leaves. The study provides important message for the conservation and management of mangrove forest at RAWN Park, which is also the habitat anoa (*Bubalus* sp.), at present enlisted as most threatened species under the 2002 IUCN Red List of threatened species (World Conservation Union 2002). Thus, the finding of this study can be helpful for taking some management plan during *insitu* conservation of endemic lowland anoas in mangrove forest. The proper management of existed mangroves can be a potential habitat for *Bubalus* sp., which can improves capacity of RAWN Park as nature reserve in near future.

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