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Study on Chemical Composition of *Azolla filiculoides* and *Hydrilla verticillata*

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ABSTRACT

The main objectives of this study were to determine and measure the chemical composition as well as the mineral composition of *Azolla filiculoides* and *Hydrilla verticillata*. Both plants are an aquatic plant that has high potential to be introduced as a new alternative to ruminant animal feed. Samples were collected around Tembila area, Besut, Terengganu and were dried and ground before the plants were analyzed by using proximate analysis as well dry ashing method for Induced Coupled Plasma Optical Emission Spectrometry (ICP-OES). The proximate analysis was used to measure the dry matter (DM), ash, crude fibre (CF), crude protein (CP), ether extract (EE) and Nitrogen-Free Extract (NFE). The minerals composition of Copper (Cu), Zinc (Zn), Iron (Fe) and Calcium (Ca) were measured using ICP-OES. The result of this analysis shows that Azolla contains the highest protein and ether extract content compared to Hydrilla. As in mineral composition, Hydrilla and Azolla showed that it has significant differences ($p < 0.05$) in between the two plants. This study revealed that Azolla is more suitable to be used as a source of fodder mixture to ruminant as it contains a more nutritive value in terms of high crude protein and ether extract that are essential for ruminant diet.

Keywords: Aquatic plants, proximate analysis, ICP-OES, ruminant diet

INTRODUCTION

Azolla is an amazing plant which holds the key to provide a sustainable feed for livestock (Pillai et al., 2002). Azolla is an aquatic free-floating fern plant belongs to the family Salviniaceae. The term of Azolla comes from the combination of two Greek words, *azo* (to dry) and *allyo* (to kill) because the fern will die when drought (Lumpkin & Plucknett, 1980). Azolla hosts the symbiotic relationship with blue-green algae, called *Anabaena azollae* that are accountable for assimilation and fixation of atmospheric nitrogen. Through this relationship, Azolla supplies carbon sources and conducive environment for algae's growth and

development. This high protein produced from natural symbiotic relationship of *Azolla - Anabaena azollae* became one of the unique plant in the world (Pillai et al., 2002).
Another type of aquatic plant which that is native in Asia is *Hydrilla verticillata* that is commonly known as Hydrilla, water thyme, Florida elodea or Indian star-vine. It is submerged aquatic plants that are an inhabitant in the freshwater ecosystem of the world, and it is highly a controversial weed (Sumithran & Raj, 2013). This scientific name comes from the Greek word "hydro" meaning "water" and the Latin word "verticillus" that bring meaning "the whorl of a spindle (Bhavsar et al., 2016).

Previous studies shown that both these aquatic plants are the excellent sources of protein that contains almost all essential amino acid needed for animal nutrition (notable lysine) as well as macronutrients like calcium, magnesium, potassium, and vitamins like vitamin A (precursor beta-carotene) and vitamin B12 (Anitha et al., 2016). For example, the Azolla also contains vitamin B-12 and Beta- Carotene, which also act as growth promoter intermediaries (Pillai et al., 2002). On the dry weight basis, Azolla contains about 25 to 35 % of protein, 10 to 15 % of mineral and 7 to 10 % of amino acids (active substances) and biopolymers as well as low of carbohydrate and fat content. This nutrient composition makes Azolla is a highly efficient and effective feed to be given for livestock. It is easier to digest, and owing to its high protein and low lignin content, the animals can quickly grow accustomed to it. Azolla is also easy and economical to be cultivated (Pillai et al., 2002).

Due to less study of the potential these aquatic plants, there is less utilization of Azolla and Hydrilla as a source of feed mixture in the market. Only in a certain country that are utilizing these aquatic plants, for example, in India where the farmers using the aquatic plant to feed their ruminant as a replaceable to expensive ruminant feed in the market. Therefore, by study the chemical composition in *Azolla filiculoides* and *Hydrilla verticillata*, the information regarding all beneficial nutrient composed in these aquatic plants can be used as one of the sources of ruminant feed mixture in the future.

MATERIALS AND METHODS

Sample collection and sample preparation

Fresh samples of *Azolla filiculoides* and *Hydrilla verticillata* were collected from the lake of UniSZA Campus Tembila, Besut area and were put into the plastic zipper bag. The plant samples were washed properly under the tap water in less than 30 seconds to remove any debris and remaining soils and later were dried in an oven at 65 °C for 72 hours.

Nutritive Value Content Analysis

Plant samples were subjected for proximate analysis for dry matter (DM), ash, crude protein (CP), crude fibre (CF), ether extract (EE) and Nitrogen - Free Extract according to the standard procedures of AOAC (2005). To estimate the Nitrogen content, crude protein content was calculated indirectly by multiplying factor of 6.25 (N x 6.25). The Nitrogen-Free Extract (NFE) supposedly represents soluble carbohydrate of feed such as starch and sugar. The fraction may also contain solubilized hemicellulose and lignin. Calculations of NFE were determined by using the formula:

[Percentage of NFE= 100 – (% EE + % CP + % ash + % CF)].

The samples were prepared in triplicates.

Mineral Analysis

The samples were weighed for one gram and were transferred into crucibles that later were transferred to muffle furnace at temperature 500 ºC. The temperature was kept for two hours. The crucibles were then removed with ash from muffle furnace and left for cool. 3 mL of diluted nitric acid (HNO_3) was added. Samples were dried on a hot plate at 100 to 120 ºC until it was dried completely. The crucibles were placed into the muffle furnace at 500 ºC for an hour. The crucibles were then removed and left for cool. 10 mL

of diluted hydrochloric acid (HCL) were added. The samples were transferred to 50 mL volumetric flask, and deionized water was added to the volume and was mixed thoroughly. The solutions were directly analysed by Induced Coupled Plasma Optical Emission Spectrometry (ICP-OES) (AOAC, 2005).

Statistical analysis

Data collected were analysed by using Independent T-Test Analysis and presented by using mean ± SD along with the significance to compare the chemical and mineral composition of *Azolla filiculoides* and *Hydrilla verticillate* in triplicates sample by using SPSS 25.0 Statistical Software. The value of ($p < 0.05$) was considered as a significant difference.

RESULTS AND DISCUSSION

Proximate analysis

Azolla and Hydrilla both show significant difference on the percentage of proximate composition analysis. The highest percentages of moisture, Ether Extract (EE), crude protein (CP) as well as Nitrogen Free Extract (NFE) were showed in the Azolla whereas, in terms of Crude Fibre (CF) and ash, Hydrilla shows the highest percentage compared to Azolla.

The highest moisture content was shown in Azolla with 94.68 %, whereas Hydrilla has only 92.29 % moisture content. There are significant differences for mean values in the moisture content of both species. The percentages of ash were higher in Hydrilla with 20.84 %, whereas Azolla has only 12.51 %. In percentage of ash, both species show that there were significant differences ($p < 0.05$) for mean values.

The highest percentage of crude protein was shown in Azolla with 24.51 %, whereas Hydrilla has only 18.15 %. There are significant differences for mean values in the crude protein of both species with ($p < 0.05$). For the percentage of crude fibre, Hydrilla shows the highest crude fibre content with 15.49 %, whereas Azolla has only 11.77 %. In crude fibre content, it is shown that there was a significant difference ($p < 0.05$) for the mean value in both species. For ether extract percentage, Azolla has the highest percentage with 4.23 % compared to Hydrilla with only 1.24 %.

There are significant differences for mean values in ether extract of both species with ($p < 0.05$). For a percentage of Nitrogen-Free Extract (NFE), Azolla shows the highest of NFE content with 67.26 %, whereas Hydrilla has only 59.55 %. In the NFE content, there are significant differences for mean values of both of the species.

Table 1: Proximate composition of *Azolla filiculoides* and *Hydrilla verticillata.*

Parameters (%)	Samples Mean ± SD	
	Azolla filiculoides	*Hydrilla verticillata*
Moisture	$94.68^{a} \pm 0.13$	$92.29^{b} \pm 0.20$
Dry matter	$5.31^{b} \pm 0.13$	$7.71^{a} \pm 0.10$
Ash	$12.51^{b} \pm 0.44$	$20.84^{a} \pm 0.17$
Crude protein	$24.51^{a} \pm 0.30$	$18.15^{b} \pm 0.10$
Crude fibre	$11.77^{b} \pm 0.30$	$15.49^{a} \pm 0.62$
Ether extract	$4.23^{a} \pm 0.20$	$1.24^{b} \pm 0.35$
NFE	$67.26^{a} \pm 0.32$	$59.55^{b} \pm 0.50$

[ab] Means with common superscript are significantly different ($p < 0.05$)
SD: Standard Deviation

Proximate composition of both Azolla and Hydrilla are presented in Table 1. In terms of moisture, Azolla shows 2.39 times higher moisture content compared to Hydrilla. This is because these different may be attributed to species variation of Azolla, growing media and locations (Fasakin & Balogun, 2001). According to Santanu (2016), Azolla contained high moisture that is more than 93 % of water content. This result also is near to the previous finding by Sreenath et al., (2015) with 91.81 % of moisture content.

The highest dry matter content shows in Hydrilla with 7.71 % compared to Azolla with only 5.31 %. This percentage has near value with previous research by Anitha et al., (2016) with 4.70 % of dry matter and according to her findings, although Azolla has low percentage dry matter content, Azolla may still be used as supplements in livestock feeds. According to Cherryl et al., (2014), low dry matter content of Azolla may act as a hindrance to be used as a fresh basis because Azolla is needed in bulk to satisfy the dry matter requirements of livestock.

In ash content, Hydrilla shows the highest percentage with 20.84 %. This is supported by Tan (1970) that said ash content in aquatic weeds is higher than grasses and plants. According to Boyd (1968), as Hydrilla is a submerged plant, it has a very large quantity of ash content. Ash is defined as the total inorganic and mineral content in a material. Determination of ash content is crucial as it presents the mineral content in materials for nutrition labelling, quality assessment, microbiology stability and food processing (McClements et al., 2009).

In terms of crude protein, Azolla shows the highest crude protein content, with 24.51 % compared to Hydrilla with only 18.15 %. This is because of the characteristic of Azolla that grows symbiotically with blue-green algae called *Anabaena azollae* (Santanu, 2016). The high protein content is fixed by the endosymbiotic nitrogen-fixing bacterium, *Anabaena azollae* and the variation in heterocyst frequency of endosymbiont and maturity of leaf affect the nitrogen-fixing ability of Azolla (Sreenath et al., 2015). According to Santanu (2016), high protein and low lignin content make the livestock to easily digest Azolla. These crude protein results were almost similar to Cherryl et al., (2014) with 23.49 % and much similar with the previous study by Roy et al., (2016) with 24.6 % of crude protein.

The higher percentages in terms of crude fibre are shown on Hydrilla with 15.49 % compared to Azolla with only 11.77 %. Fibre is the main element of the plant cell wall and primarily constitute of carbohydrates. Cellulose, hemicellulose, and lignin are types of primary components of fibre in the plant (Kung, 2014).

In terms of ether extract, the highest percentage shows in Azolla with 4.23 % compared to Hydrilla with only 1.24 %. The ether extract (EE) has a diversity of composition and is formed by lipids made of galactolipids, triglycerides and phospholipids and other non-polar compounds. Solvents such as phosphatides, steroids, pigments, fat-soluble vitamins, and waxes can be used to extract ether. Gravimetrically extracted residue is properly termed as crude fat due to the disparity between the biochemical and analytical concepts of lipids (Prabina & Kumar, 2010). This result is nearly similar to Anitha et al., (2016) with ether extract content 4.50 %. According to Anitha et al., (2016), this variation of results in ether extract might be influenced by the nutrient input while propagating the Azolla.

The highest Nitrogen - Free Extract (NFE) is shown on Azolla with 67.26 % compared to Hydrilla, with only 59.55 %. According to Sunil Dutt et al., (2013), NFE content of Azolla is 48.62 %, and according to previous research Nampoothiri (2017), NFE was recorded lower with only 47 %. NFE is made up of starches, glucose, oligosaccharides, beta-glucans as well as pectin. Glucose and fructose are monosaccharides sugars, whereas sucrose or saccharose, cellobiose and maltose are examples of disaccharides sugar. This compound of sugars is easy to digest by ruminant animals (Antonius, 2015).

Mineral Analysis

The mineral composition of both *Azolla filiculoides* and *Hydrilla verticillata* were shown in Table 2. Based on mineral composition in terms of iron, Hydrilla shows the highest content with 3849.35 mg/kg compared to Azolla with only 2400.43 mg/kg. These results are significant difference ($p < 0.05$) for the mean value of both species. The result of this study has similar with the findings by Das et al., (2015) and Pal & Nimse (2006), which has of 3580 mg/kg of iron content in the Hydrilla. Iron is needed in high amounts compared

to any other micronutrients as it is fundamental to proteins that are involved in the electron transfer like ferredoxin and cytochromes. During electron transfer, it is reversibly oxidised from Fe^{2+} to Fe^{3+} and helps to activate the catalase enzyme. Iron is also important for the formation of chlorophyll in the plant body (Muztar et al., 1978).

Table 2: Mineral composition of *Azolla filiculoides* and *Hydrilla verticillata.*

Parameters (%)	Samples Mean ± SD	
	Azolla filiculoides	*Hydrilla verticillata*
Iron (mg/kg)	2400.43^{b}±6.97	3849.35^{a}±266.51
Manganese (mg/kg)	642.33^{b}±8.38	2112.83^{a}±554.48
Calcium (mg/kg)	50.45^{b}±71.35	269.25^{a}±380.78
Zinc (mg/kg)	27.25^{b}±13.29	62.10^{a}±16.70
Copper (mg/kg)	88.08^{a}±8.31	48.50^{b}±0.14

[ab] Means with common superscript are significantly different ($p<0.05$)
SD = Standard Deviation

In term of manganese, Hydrilla also shows the highest of manganese content with 2112.83 mg/kg compared to Azolla with only 642.33 mg/kg. This result shows that it is significant difference ($p < 0.05$) for the mean value of both species and almost similar findings with the study by Pal & Nimse (2006). In the plant, manganese is active in activating an enzyme, involved in the formation of amino acids, acts as a coenzyme activity and is required in water – splitting mechanism in photosynthesis as well as chlorophyll synthesis (Soetan et al., 2010).

The highest percentage of calcium is showed in Hydrilla with 269.25 mg/kg compared to Azolla with 50.45 mg/kg and it has significant difference ($p < 0.05$). However, this calcium content is lower compared to the findings by Pal & Nimse (2006). Based on Das et al., (2015), Hydrilla contains 15% of elemental calcium on dry weight basis that the function is to bind between carbohydrates, proteins and polyphenols.

In copper composition between both plant samples, Azolla shows the high composition of copper with 88.07mg/kg compared to Hydrilla with only 48.50 mg/kg. These results are significant difference ($p < 0.05$) for the mean value of both species. Previous research by Muztar et al. (1978) cited that total ash in aquatic plant constitutes more than 30 % calcium content and this might be due to deposition of calcium carbonate content on surface aquatic plants.

CONCLUSION

These studies have shown that different types of aquatic plants have a significant difference on the chemical composition for both *Azolla filiculoides* and *Hydrilla verticillata.* Based on the proximate analysis and mineral composition, there were statistically significant difference ($p < 0.05$) in the mean of both *Azolla filiculoides* and *Hydrilla verticillata.* By these results, both *Azolla filiculoides* and *Hydrilla verticillata's* chemical composition were compared. This study revealed that *Azolla filiculoides* are more suitable to be used as a source of fodder mixture to the ruminant because it has more nutritive value in terms of crude protein and ether extract that are essential for ruminant diet compared to *Hydrilla verticillata.* To encourage more growth and proliferation of *Azolla filiculoides*, a small amount of fertilizer may be applied to boost rapid growth.

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