Nutritive Analysis of Five Aquatic Plants at Selected Area in Kelantan, Malaysia

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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(   )

I certified that the report of this final year project entitled "Nutritive Analysis of Five Aquatic Plants at Selected Area in Kelantan, Malaysia" by Siti Maryam Salamah Bt Ab Rhaman, matrix number F15A0210 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agrotechnology), Faculty of Agro Based Industry, Universiti Malaysia Kelantan.

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ACKNOWLEDGEMENTS

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º	Degree
ºC	Degree celcius
%	Percentage

LIST OF ABREVIATIONS

ANOVA	Analysis of variance
CFU/mL	Colony forming unit per milliliter
E	East
Fe	Iron
g	Gram
HCl	Hydrochloric acid
kg	Kilogram
M	Molarity
Mg/L	Milligram per liter
mm	millimeter
Mn	Manganese
N	North
NaOH	Sodium hydroxide

Ni   Nickel

PCA   Plate count agar

TVC   Total viable count

Nutritive Analysis of Five Aquatic Plants at Selected Area in Kelantan, Malaysia

ABSTRACT

In terms of providing a low cost feeds for animal consumption, the nutrient component for different species of aquatic plants were evaluated. This study emphasize on the nutrition comparative between 5 species of aquatic plants such as *Hydrilla verticillata* (order: Hydrocharitales)*, Pistia stratiotes* (order: Arales), *Chara corallina* (order: Charales)*, Myriophyllum spicatum* (order: Haloragidales) and *Azolla pinnata* (order: Salviniales) were collected at the selected area in Kelantan (6.1254º N, 102.2381ºE). The objectives of this study is to differentiate the nutrient component for five different species of aquatic plants. The study shows that the crude fiber values for *H. verticillata, P. stratiotes, C. corallina, M. spicatum,* and *A. pinnata* were 15.35±0.35%, 14.10±0.10%, 20.05±0.55%, 25.60±5.10% and 42.95±2.25% respectively. While the percentage of ash content recorded for *H. verticillata, P. stratiotes, C. corallina, M. spicatum,* and *A. pinnata* were 18.8%, 31.8%, 20.6%, 28.9% and 12.0% respectively. The organic matter content for *H. verticillata, P. stratiotes, C. corallina, M. spicatum,* and *A. pinnata* were 81.20±0.10%, 68.20±0.10%, 79.40±0.20%, 80.80±0.10% and 88.05±1.45% respectively. The crude protein value for *H. verticillata, P. stratiotes, C. corallina, M. spicatum,* and *A. pinnata* were 23.18±0.13%, 16.89±0.08%, 22.30±0.13%, 22.78±0.76% and 21.70±0.09% respectively. While the microbial analysis based on total bacterial count for *H. verticillata, P. stratiotes, C. corallina, M. spicatum,* and *A. pinnata* were 2.71CFU/mL, 2.88CFU/mL, 2.16CFU/mL, 0.49CFU/mL and 1.68CFU/mL respectively. In conclusion, the nutrient component for five different species of aquatic plants can are differs from each other. So, the nutrient for five different species of aquatic plants are suitable for animal feed.

Keywords: Aquatic plant, nutrition, animal feed.

Analisis Nutritif Terhadap Lima Jenis Tumbuhan Akuatik di Kawasan yang Terpilih di Kelantan, Malaysia

ABSTRAK

Dalam istilah penyediaan makanan haiwan yang berkos rendah, komponen nutrien untuk spesis tumbuhan akuatik yang berbeza telah dapat dinilai. Kajian ini memberi penekanan terhadap perbandingan nutrien diantara 5 spesis tumbuhan akuatik iaitu *Hydrilla verticillata* (order: Hydrocharitales), *Pistia stratiotes* (order: Arales), *Chara corallina* (order: Charales), *Myriophyllum spicatum* (order: Haloragidales) dan *Azolla pinnata* (order: Salviniales) telah dikumpulkan dari kawasan yang terpilih di Kelantan (6.1254º N, 102.2381ºE). Objektif kajian ini adalah untuk membandingkan komponen nutrient untuk lima tumbuhan akuatik yang berlainan spesis. Kajian ini menunjukkan nilai serat mentah bagi *H. verticillata, P. stratiotes, C. corallina, M. spicatum,* dan *A. pinnata* masing-masing adalah 15.35±0.35%, 14.10±0.10%, 20.05±0.55%, 25.60±5.10% dan 42.95±2.25%. Sementara itu kandungan abu direkodkan untuk *H. verticillata, P. stratiotes, C. corallina, M. spicatum,* dan *A. pinnata* masing-masing adalah 18.8%, 31.8%, 20.6%, 28.9% dan 12.0%. Manakala nilai bahan organik untuk *H. verticillata, P. stratiotes, C. corallina, M. spicatum,* dan *A. pinnata* masing-masing adalah 81.20±0.10%, 68.20±0.10%, 79.40±0.20%, 80.80±0.10% dan 88.05±1.45%. Nilai protein mentah bagi *H. verticillata, P. stratiotes, C. corallina, M. spicatum,* dan *A. pinnata* masing-masing adalah 23.18±0.13%, 16.89±0.08%, 22.30±0.13%, 22.78±0.76% dan 21.70±0.09%. Manakala nilai bagi analisis microbial berdasarkan jumlah kiraan bakteria bagi *H. verticillata, P. stratiotes, C. corallina, M. spicatum,* dan *A. pinnata* masing-masing adalah 2.71CFU/mL, 2.88CFU/mL, 2.16CFU/mL, 0.49CFU/mL and 1.68CFU/mL. Secara konklusinya, komponen nutrient untuk lima tumbuhan akuatik yang berlainan spesis berbeza antara satu sama lain. Jadi, nutrient untuk lima tumbuhan akuatik yang berainan sepsis sesuai untuk dijadikan makanan haiwan.

Kata kunci: Tumbuhan akuatik, nutrisi, makanan haiwan.

CHAPTER 1

INTRODUCTION

Aquatic plant is the plant that lives in the water bodies that have ecological roles towards the aquatic environments and have substantial contribution towards the organization, function and the service of the aquatic ecosystem (O'Hare et al., 2018). Most of the aquatic plants can adapt with the aquatic environment either it is saltwater or freshwater. There are different kinds of aquatic plants that survive in the aquatic environment. The aquatic plants can be classified based on their adaptation in the water. Most of the aquatic plants can grow in different ways such as emergent, submerged and floating.

According to Schlesinger (2013), emergent aquatic plants can be defined as the majority vegetation in all types of wetlands. Emergent plants usually have root that grabbed by the soil and have firm stems that makes them can stand above the water surface but they also can be found living in the water bodies because of the water level at the area are rising (Texas A & M Forest Service, n.d). Aquatic plants also can grow as submerged plants where it is the plants that totally grow in the water bodies while for floating plant is the plants that survive on the water surface where the root and the body part of the plant floating on the water. The aquatic plants especially for the *Hydrilla verticillata, Chara corallina* and *Myriophyllum spicatum* can rapidly grow in the water

and form mat-like structure that invaded deep in the water body. While *Pistia stratiotes* and *Azolla pinnata* are floating aquatic plants that usually block the entrance of the sunlight to *penetrate* deeper into the water

The aquatic plants like *H. verticillata, P. stratiotes*, *C. corallina, M. spicatum* and *A. pinnata* can be classified as the one of the invasive species that give problem to the water bodies especially in Malaysia. It is because of the distribution and their aggressive growth rate is fast as they can grow into mat dense in the water only an inch for a day. For *H. verticillata*, they can rapidly grow in the water and form mat-like structure that invaded deep in water body. The thick mat-like structure can block the sunlight from strike towards lower part of the water body and cause the ground plant in the water cannot receive enough amount of sunlight. The aquatic plants disturb the human water activities such as swimming, fishing and water driving because of their growth. They usually stuck to the motorboat engine and cause failure operation towards the boats.

1.1   Problem statement

Important aspect for animal feeding is the nutrition provided in the food itself. In India, Bishnoi *et al.,* (2011) reported that the nutrition is the most crucial issue on animal productions. It is because of the costing for the feeds itself is high. Bishnoi *et al.,* (2011) stimulate few research in searching the non-conventional feeds for the animals. Malaysia recorded about 70% of the cost of the livestock feeds where it is the highest cost in livestock production (Wan and Wong, 2009). The problem of animal production is because of the costly animal feeds. They need to reduce the feed cost by using non-conventional feeds to the animals. However, there are extensive of aquatic plant grows

especially in India and the aquatic plant been chosen as the non-conventional feeds to animal.

1.2   Hypothesis

H null: The nutrient component of aquatic plants are the same among the species

H alternate: The nutrient component of aquatic plants differs from each species.

1.3   Objectives

i.   To differentiate the nutrient component of *Hydrilla verticillata, Pistia stratiotes*, *Chara corallina, Myriophyllum spicatum* and *Azolla pinnata*

1.4   Scope of Study

The study is focusing on the determination of the nutrient component of aquatic plants with different species of aquatic plants (*H. verticillata, P. stratiotes*, *C. corallina, My. spicatum* and *A. pinnata)*. The nutrient of *H. verticillata, P. stratiotes*, *C. corallina, M. spicatum* and *A. pinnata* will be determine by proximate analysis method. The nutrition component of *H. verticillata, P. stratiotes*, *C. corallina, M. spicatum* and *A. pinnata* being analysed.

1.5   Significance of Study

These invasive species were treated as weeds. But they have potential as animal feeds. These invasive species which is a nuisance in water bodies can be utilized and reuse to feed the livestock. From this study, the disturbance caused by the aquatic plant as invasive species in irrigation canals and artificial lakes that cause trouble to the water transport and other deep aquatic creature will be decreased and it may not only produced a low cost with high nutrition animal feeds in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1   Essential Nutrients

Every living organisms need to uptake enough amount of nutrient for themselves to lead a healthy and proper growth development. Nutrients refer to the molecules in food or substance that needed by all organisms for producing and providing energy, growth development and reproduction (Amanda, n.d). Nutrients that been intake by particular organism will be broken down into particular parts that will be used by the organism for future function. There are two types of nutrients which are macronutrients and micronutrients. The essential elements for plant growth development is the macronutrients (Kulcheski, Correa, Gomes, Lima and Margis, 2015). Macronutrients represent the nutrients that needed by the particular organisms in a large amounts where it consist of carbohydrate, protein and lipids. While for micronutrients, they represent the nutrients that needed by the particular organisms only in small quantity where they consist of minerals and vitamins. Lacking of the nutrient consumption by the human, animal or plant can caused them to have a nutrient deficiency. In human terms, lacking of nutrients can caused malnutrition that can lead to the disease where they can affects human function (Lindsay, 1995).

2.2   Aquatic Plants

According to International Plant Protection Convention (2012), aquatic plant is the plant that live in water and have their habitat in the water ecosystems. Aquatic plants are include the flowering plants, mosses, macrophytes, charophytes, algae and any other plant that can be found standing or floating on the water. There are different adaptation of aquatic plants which are submerged, emergent and floating on the water surface. For the submerged aquatic plants, it is the plant that grow entirely under the water. Each part of the plants include the root, stem and leaves mostly can adapt in the water area. However, submerged plants can be divided into two which are underwater plants and emergent water plants. The different between underwater plants and emergent water plants is based on their foliage. If the aquatic plants is underwater plants, their foliage grow totally under the water while for the emergent water plant, their foliage grow under the water and also above the water surface.

However, aquatic plants sometimes can add the aesthetics values towards the pond or lake but they also difficult to be identified either they are desired aquatic plants or not (Dunns Fish Farm, 2014). The aquatic plants especially that live in the lake in a park can add beautiful element to the area. For example the water hyacinth that grows in the water can increase the landscape at the area because of the flower produced. According to the research reported by Omotayo, Adelakun, Kehinde, Amali and Ogundiwin (2016), the excessive growth of the aquatic plants in the lakes and waterways cause the lakes and ponds becomes weedy where they become worldwide issues.

2.2.1 Issues Related to Aquatic Plants

There is issues regarding the invasive species of aquatic plants. The excessive breeding of the aquatic plants can cause trouble especially for the water activities such as fishing, riding boat and for the living of the aquatic organism such as fishes. Aquatic Ecosystem Restoration Foundation (2014) reported that other than can disturb the human activities in water and change the habitat quality for fish, invasive species of aquatic plants also can suppress the growth of the native aquatic plants that live their habitat at the area. This issues can cause the localized extinction towards the native aquatic plant species. The excessive growth of the invasive aquatic plants species, they also reduce the water quality where they increase the biomass by increasing the dense of the forming canopies in the water (Ecosystem Restoration Foundation, 2014). The invasive species also create big obstacle towards the boat traffic, clog the hydropower generation turbines (Madsen, 1991a; 1997b).

2.2.2 Advantages of Aquatic Plants

Despite of having disadvantages, aquatic plants also have their own positive side. The aquatic plants can encourage the fish growth especially for the fish that live at the ecosystem (Ling, 1960). Ling (1960) also reported that the existence of small amount of water hyacinth in the water bodies in China can promote the fish in the area because the fish usually find food among the roots of the aquatic plants. Other than being the food source for the fish that live in the area, the aquatic plants also act as the sources of the food for the animal especially for the poultry and ruminant (Ali et al., 2006). Ali et al., (2006) reported that the aquatic plants also can be processed as the fish feed formulation

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(Little ECS, 1979). Apart from that, the aquatic plants also can act as phytoremediation agent where the aquatic plants can absorb the toxic heavy metals in the water (Malik, 2007). Besides being a phytoremediation agents, aquatic plants also can act as compost and medicinal plants where most of the people in China and India use to cure minor disease (Deepa et al., 2009).

2.3   Macrophytes

According to the National Aquatic Resource Survey (n.d), the terms of macrophytes can be define as the aquatic plant that have distribution and complete their life cycle in or near the water source such as lake, pond or river. Most of the macrophytes can grow in different ways such as emergent, submerged and floating. Macrophytes also can be claimed as a large plants that dominate the wetlands in the world. However, most of the macrophytes which are claimed as aquatic plants, they are angiosperm where they can produce flowers (Bornette and Puijalon, 2009).

Macrophytes usually give benefits towards the wetland itself where they gives shelter for the fish and other aquatic invertebrates to lives under it for a protection purpose. Other than that, National Aquatic Resource Survey (n.d) also stated that the macrophytes also can provide oxygen to the aquatic organism and at the same time they also produce food for the aquatic life such as fish and other wildlife. The existence of the macrophytes in the water body such as water hyacinth, *H. verticillata* and duckweed can provide the indicator for the health of the waterbodies. However, the indicators are based on the depth, density and diversity of the macrophytes itself. A studies stated that if there is absence of the macropytes around the water body, they may highlight the water quality

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matter that occurs at the area. The macrophytes usually indicate the turbidity, salinization or acidity of the water where they courage the growth of the plants.

2.4   Charophyte

Charophytes are from the six distinct groups where they are mostly the freshwater green algae. Charophytes can be related to the modern land plants. Charophytes actually been derived from the unicells to the branching form such as *C. corallina sp.* The species actually is an algae where they have specialized into a complete form of the aquatic plants (Richard, Charles and Kenneth, 2004). The charophytes also can be known as streptophyte algae where they are the group of green algae that ancestral to plants (Domozych, Serensen and Popper, 2017). The taxanomy of the charophytes itself give the largest number of characteristic that can be easily found in modern plants.

Back in 15th century where Wood and Imahori (1965) reported that the charophytes was firstly recorded were called *Equisetum*. However, in 17th century, the charophytes were called as horsetail where in Latin they called as "smelly horsetail creeping underwater" (Schneider, Garcia, Closas and Chivas, 2014). In 18th century, they finally assigned the new genus which is Chara. Charophytes usually have a morphological that can adapt with high light intensity (Schneider, Garcia, Closas and Chivas, 2014).

Charophytes also can be classified as providers towards the ecosystem services where they fully contribute towards the multitude of the ecosystem services and get fully attention from the scientists. Charanphytes are stated to be economic value because they are responsible towards the purification of the water bodies. From the observation in the research made by Schmeider (2004), the charophytes that were harvested in the Lake

Constance were used as the fertilizers in the vegetables field. The charophytes also reported can be used in homeopathic medicine (Brand and Groeger, 2012; Schneider, Garcia, Closas and Chivas, 2014).

2.5   *Hydrilla verticillata*

Hydrilla verticillata (L.f.) Royle is the aquatic plant that can rapidly grow in the freshwater ponds, lakes and rivers. *Hydrilla verticillata* (L.f.) Royle also known as water thyme, Florida elodea or Indian star-vine is the macrophytes that lives in aquatic ecosystems. This water thyme is from Hydrocharitaceae family (Verkleij, Pieterse, Horneman & Torenbeek, 1983). *Hydrilla verticillata* (L.f.) Royle sometimes can be confused with *Elodea verticillata* (L.f.) F. Muell because of their appearance. *Hydrilla verticillata* have two types of biotypes which are dioecious and monoecious. Both biotypes of *H. verticillata* which are dioecious and monoecious were found in lake Gaston, North Carolina, Virginia and it was the first coexistence of the biotypes reported (Ryan, Coley and Kay, 1995). *Hydrilla verticillata* is differ from other aquatic macrophytes because of their morphology. *Hydrilla verticillata* have long stem that can grow up to 25ft height where they also can produce branches. However, *H. verticillata* is a polymorphic aquatic plants where they can grow into various shape of plants. They do not have specific shape within the species. Apart from that, *H. verticillata* also differ from Elodea and Brazilian Elodea. The difference between them can be vividly seen by the number of the whorls attached to the stem where *H. verticillata* have 3 to 10 whorls while *Elodea* only have 3 whorls attached to the stem (CABI, n.d). For *Brazilian Elodea*, they have 4-6 whorls. From the number of whorls, the *H. verticillata* can easily recognized. From that, *H. verticillata* can reproduce from seeds or tuber and also from the turions

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where the axillary buds produced. However, the *H. verticillata* can be monoecious and dioecious based on the area they grow. In Southern population, the *H. verticillata* is predominantly dioecious female while in the population North of South Carolina include New York, the *H. verticillata* is monoecious (New York Invasive Species Information, 2012).

This aquatic plants is unisexual plants where they have translucent white female flower and three broadly ovate petals while they have similar shape and size for the male flower (Olson, 2004). *Hydrilla verticillata* have two types of biotypes which are dioecious and monoecious. Both biotypes of *H. verticillata* which are dioecious and monoecious were found in lake Gaston, North Carolina, Virginia and it was the first coexistence of the biotypes reported (Ryan, Coley and Kay, 1995). *Hydrilla verticillata* can be listed in the group of rooted floating weeds (Shashikant, Arvjen, Elisabeta, Bashkim and Ismail, 2014). Langeland (1996) reported that in year 1960 this aquatic plants was first discovered in Florida. However, *H. verticillata* is stated to be occurred on all continents except for Antartica (Jacono, Richerson, Morgan, Baker and Li, 2018).

Study by Langeland (1996) showed that *H. verticillata* is one of the most studied aquatic plants because of their attribution in surviving in many type of condition. This kind of adaptation makes the *H. verticillata* become a perfect aggressive aquatic plants. However, *H. verticillata* can be considered as a polymorphic aquatic plant where they can grow in different form depends on their environmental condition (Verkleij *et al.,* 1983). "A sizeable number of introduced, naturalized and native plant species in Malaysia have established and spread as invasive species and some are classified as scheduled pests under the Plant Quarantine Act 1976 and Plant Quarantine Regulations 1981" (Bakar, 2004). *Hydrilla verticillata* is the one of the invasive species that give problem to the water bodies in Malaysia. It is because of the distribution and their aggressive growth rate

is fast as they can grow into mat dense in the water only an inch for a day. *Hydrilla verticillata* can rapidly grow in the water and form mat-like structure that invaded deep in water body. The thick mat-like block the sunlight from strike towards lower part of the water body and cause the ground plant in the water cannot receive enough amount of sunlight. *Hydrilla verticillata* is highly distributed macrophyte where the origin of *H. verticillata* is from the dam of South African (Paul *et al.,* 2007).

2.5.1 Taxonomy of *Hydrilla verticillata*

Kingdom: Plantae

Class: Monocotyledonae

Order: Hydrocharitales

Family: Hydrocharitaceae

Genus: Hydrilla

Species: *Hydrilla verticillata*

Sources: CABI (n.d)

2.6 *Chara carollina*

Chara carollina also known as muskgrass is from the family of Characeae where they are from the genus of charophyte green algae. *Chara carollina* have stem-like and leaf-like structures where they normally can be found in the muddy bottom area. *Chara carollina* cannot easily found in the mosquito larvae breeding area. They usually can be found clustered form in the bottom of the ponds, lakes, slow moving rivers and ditches. They look like other underwater plants where their size usually 5cm to 1m length (Department of Ecology State of Washington, n.d). They have stem that connected with

whorls of branchlets and clustered at regular spaced joints. *Chara carollina* usually have unpleasant smell when crushed. They have strong garlic odor (Department of Ecology State of Washington, n.d). They usually propagates by spores that carried away by the water and the waterfowl. That is why they can be classified as invasive species where they can rapidly grow and their growth population usually scattered because the spores being transported to another area by water.

According to the research made by Environment Agency (2009), *Chara carollina* have important role in aquatic ecosystems where they can enhance the water quality by reducing the flow rates and aid sediment deposition. *Chara carollina* is reported to be a substance for phytoremediation selenium contaminated agriculture drainage water (Lin, Souza, Pickering and Terry, 2002). They can accumulates mostly high concentrations of heavy metal in their tissues. In the research made by Lin, Souza, Pickering and Terry (2002), it is stated that the *Chara carollina* can be proved that they can absorb iron (Fe), manganese (Mn) and nickel (Ni) better than in cattail (*Typha latifolia L.*).

2.6.1   Taxonomy of *Chara corallina*

Kingdom: Plantae

   Class: Charophyceae

   Order: Charales

      Family: Characeae

      Genus: Chara

         Species: *Chara corallina*

Sources: Olson (2004)

2.7   *Pistia stratiotes*

Pistia stratiotes also known as water lettuce is from the family of Araceae where it is classified as monocotyledon plant. Linnaeus (1753) classify the genus of water lettuce which is *Pistia* as a monospecific with the one and only species which is *Pistia stratiotes*. The name of water lettuce is because the shape of the plant resembles the head of a lettuce. This plant is a free floating aquatic plants that have pale green leaves. The size of the leaves can grow up to 20cm long and 10cm wide. The texture for the leaves for this water lettuce usually same like cactus which is succulent where they tend to have high water content and fleshy. *Pistia stratiotes* is the aquatic plants that have no branches. They also have fibrous long roots. For the reproduction system, *Pistia stratiotes* can reproduce vegetatively throughout the growth season. They can spreads by vegetative and by seeds. Water lettuce can survive longer even in mud banks. However, they are so sensitive towards frost. However, the Department of Agriculture and Fisheries (2016) reported that this free floating aquatic plants grow in water that have high nutrient concentrations. *Pistia stratiotes* also can be classified as invasive aquatic plants. It is a restricted invasive plant that been placed under Biosecurity Act 2014 (Department of Agriculture and Fisheries, 2016). It is because of their habitat where they can rapidly grow on the water and block all of the water activity.

2.7.1   Taxonomy of *Pistia stratiotes*

Kingdom: Plantae

Class: Monocotyledonae

Order: Arales

Family: Araceae

Genus: *Pistia*

Species: *Pistia stratiotes*

Sources: Olson (2004)

2.8   *Myriophyllum spicatum*

Another invasive submerged aquatic weed is *Myriophyllum spicatum*. This weed also known as water-milfoil. There are many kind of species of *Myriophyllum* which are *M. alterniflorum, M. verticillatum*, *M. sibiricum, M. hippuroides, M. mattogrossense* and *M. spicatum* (Hutchinson, 1970). The most common species that exist in Malaysia is *M. spicatum*. This invasive species is stated to be originated from the North Africa and native to Europe, Asia and North Africa.

2.8.1   Taxonomy of *Myriophyllum spicatum*

Kingdom: Plantae

Class: Dicotyledonae

Order: Haloragidales

Family: Haloragidaceae

Genus: Myriophyllum

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Species: Myriophyllum spicatum

Sources: Olson (2004)

2.8.2 Morphology of *Myriophyllum spicatum*

Other than being called as water milfoil, *M. spicatum* also known as Eurasian water milfoil (Aiken, Newroth and Wile, 1978). The chromosome numer of *M. spicatum* is 2n = 24. This aquatic plant have 1.8-6m long. However, in the research that made by Aiken, Newroth and Wile (1978), this aquatic plant have 0.5-7m long with leafy shoot branching. They usually grow with green, reddish-brown or whitish pink stem. This aquatic plants usually have olive green leaves where they are feather-like texture. They have whorled leaves with 1.5-4.0cm long where each of the whorls contains 4 leaves with 14-24 pair filiform divisions. However, *M. spicatum* can be easily distinguish from *M. sibiricum* where the leaflets of *M. spicatum* at the uppermost leaves are taper to a squarish tipped leaf while M. sibirium have long leaflets for less than 11 pairs and extend almost to the tip of the leaf (Aquatic Nonindigenous Species, n.d). *M. spicatum* usually grows up to the surface of the water. *M. spicatum* usually vegetatively propagates and through seeds. The vegetative propagation is the main reproductive system. They can reproduce through roots where the roots will breaks and autofragmentate. The nodes will developed. Autofragmentation usually occurs after flowering process done. While for the seeds, the plants can produce over 100 seeds. However, the germination of the seeds of *M. spicatum* usually rare to be happened (Aquatic Invasive Species, n.d).

2.9   *Azolla pinnata*

Azolla pinnata is from the family of *Azollaceae*. They have symbiotic relationship with *Cyanobacteria Anabaena azollae* for nitrogen fixation process. In the researched reported by Pfingsten, Thayer and Howard (2018), *A. pinnata* also known as feathered mosquito fern. *A. pinnata* have free floating body that clustered in large mats (Bodle, 2008). The diameter of the plant is 1-2.5cm with triangular shape. *Azolla pinnata* have 1-2mm overlap leaves (Periera et al., 2011). *A. pinnata* have unicellular rhizome papillae and elliptical dorsal lobe shape (Periera et al., 2011). *A. pinnata* is reported to be native in Australia (Madiera et al., 2013). *A. pinnata* also can be found in the ponds, ditches and wetlands of tropical region around the world (Ferentinos et al., 2002). This species grow in water or wet mud but dies within few hours in dry condition. They can survive in water that have pH 3.5-10 but the optimum growth of *A. pinnata* is between range pH 4.5 and 7. However, the growth of *A. pinnata* will decreases if the salinity of the water increase (Ferentinos et al., 2002).

2.9.1   Issues regarding *Azolla pinnata*

Ferentinos et al., (2002) reported that *A. pinnata* becomes the noxious weeds in the waterbodies of Europe, Africa and New Zealand. So, to overcome the issue, people use *A. pinnata* as substitutes of animal feed. However, Tamizhkumaran and Rao (2012) reported that *Azolla pinnata* cultivation as cattle feed is not sustainable. The practices is stated to be unsuitable because the adoption for the *A. pinnata* was built around the subsidies instead of demand among of the farmers. In the coastal area of Villupuram district in Tamil Nadu, the NGO promotes the cultivation of *A. pinnata* to the dairy

farmers but only half of the farmers adopt the *A. pinnata* cultivation practices because of the loans and subsidies provided by the NGOs. Most of the farmers stop grows the *A. pinnata* because of poor growth of *A. pinnata* because of the heat excess, pest attack and lack of attention (Tamizhkumaran and Rao, 2012).

2.9.2   Taxonomy of *Azolla pinnata*

Class: Filicopsida

Order: Salviniales

Family: Azollaceae

Genus: Azolla

Species: *Azolla pinnata*

Sources: Olson (2004)

2.10   Super food

Super food refers to the high level of essential nutrition and other nutrient component contained in the food that can give thousands of benefits to the consumers especially for human and animals (Bethany, 2011). In non-medical terms, super food also can be defined as food that can have properties that can help to improve the health such as decreasing the disease risk or maintaining the health of mental or physical. However, Holly (2016) reported that there is no such terms regarding the super food because the super food terms only for increasing the awareness on how the food can give effect to the health.

Apart from that, to increase the health level, most of the people also include the super food in their daily diet. A researcher reported that this super food is good for heart health when the super food being included into the balanced diet that low in protein, contains lots of fruits and vegetable (American Heart Association, 2017). For animal, when super food being added in the diet of the animal, their production will increase. For example, if the super food such as micro algal been added in the diet of a chicken, the quality of the eggs will increase where the micro algal itself will increase the omega 3 level in the eggs (Zoey, 2016).

In terms of nutrition, *H. verticillata* can be considered as super food because this plant is so rich in nutrients (Dilip and Satish, 2005). It is because every single part of the *H. verticillata* such as the stems, leaves, turion and tubers are suitable for medicinal and food purpose and can be produced in any form. Dilip and Satish (2005) reported that the calcium content in the *H. verticillata* is more than any food source on earth. When *H. verticillata* been introduced in the ruminants and poultry daily diets, their milk, meat and eggs production will increase. The research stated that when the cows and chickens consumed the *H. verticillata*, the production of milk and eggs will increase by 20% and 14% (Dilip and Satish, 2005).

Other than that, there are also lots of enzymes contained in the *H. verticillata* (Pal and Nimse, 2006). The enzymes in the *H. verticillata* makes them to be unique among the other macrophytes. Studies also stated that *H. verticillata* also contain chlorophyll that can help in healing remedies and act as antiseptic agent. Moreover, *H. verticillata* also recorded to have lot of protein compared to *Eichhornia crassipus* and *Ceratophyllum demersum* where the protein recorded was 21% (Bishnoi, Sharma and Nag, 2011)

CHAPTER 3

METHODOLOGY

3.1   Experimental Design

In this experiment, the aquatic plants were collected in the freshwater ecosystem at selected Kelantan area (6.1254º N, 102.2381ºE) which are in UMK Jeli drain (5°44'56''N 101º52'), two of fish pond in Agropark UMK Jeli (5º44'56''N 101º52'9''E), in the abandoned pond near the residential area at Politech Jeli (5º42'43''N 101º51'6''E) and in the river at Kampung Nara, Pasir Puteh (5º49'58''N 102º24'19''E). From the different location area, 5 different species (*H.verticillata, A. pinnata, P. stratiotes, M. spicatum* and *C. corallina*) were collected and were undergoes the proximate analysis. The *H.verticillata* species were collected in UMK Jeli drain (5°44'56''N 101º52') while for *A. pinnata* and *P. stratiotes* species were collected in two of fish pond in Agropark UMK Jeli (5º44'56''N 101º52'9''E). For *M. spicatum* species were collected in the river at Kampung Nara, Pasir Puteh (5º49'58''N 102º24'19''E) and *C. corallina* species were collected in the abandoned pond near the residential area at Politech Jeli (5º42'43''N 101º51'6''E). However, the water sample collected from the selected area of the aquatic plant species then was analyzed using serial tenfold dilution and plate count agar for the colonies counting by using 2 times replication.

3.2   Plant Sampling

After identify the sampling location, the whole body for different species of aquatic plants such as water thyme (*Hydrilla verticillata*), water lettuce *(Pistia stratiotes)* muskgrass (*Chara corallina*), water milfoil *(Myriophyllum spicatum)* and mosquito fern (*Azolla pinnata*) were collected about 1kg fresh samples for each of the different aquatic plants. The sample for each species were collected at different location. For *H. verticillata* sample, it was collected in UMK Jeli drain ($5^O44'56''$N $101^o52'$). While for *P. stratiotes* and *A. pinnata* were collected in the fish pond of Agropark UMK Jeli ($5^o44'56''$N $101^o52'9''$E). For *C. corallina*, it was collected in the abandoned pond near the residential area at Politech Jeli ($5^o42'43''$N $101^o51'6''$E) and *M. spicatum* in the river at Kampung Nara, Pasir Puteh ($5^o49'58''$N $102^o24'19''$E). The plant sample were immediately placed in the zipper bags and were transported in an ice box to UMK Jeli (5.7461^oN, 101.8654^oE) within 4-6 hours to avoid the changing of the nutrition in the plant sample.

The sampling process were based on the identification of the aquatic plants through Texas A&M Forest Service (n.d). *H. verticillata* is based on the physical characteristics which is the leaves of the *H. verticillata* is strap-like and pointed leaves. The leaves grow in 4-8 whorls. The *H. verticillata* have stem that grow up to 762cm long. While for *P. stratiotes*, it can be identified by the physical characteristic where from the first looking the *P. stratiotes* float on the water and the leaves of *P. stratiotes* have clustered leaves on short branches. The shape of the leaves are in wedges or oval that point upwards. For the *A. pinnata*, this aquatic plant have feathery physical characteristic. The shape of *A. pinnata* usually in triangle shape. The leaves of *A. pinnata* usually in 0.039-0.078cm long. For *M. spicatum*, the usually identified by their physical

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characteristic. The leaves *of M. spicatum* grow in 3 to 5 whorls. They have horizontal stem and the roots usually branches from the stem. Finally for *C. corallina*, it is been distinguished by their habitat where they usually found near the submerged community of plant (Marine Volunteer Lake Monitoring Program, 2009). The physical characteristic of *C. carollina* is usually have stem that comprised of chains of single tube shape cell. They also have simple rhizoid structure. They also have stem that arranged with whorls.

3.3 Sample Preparation

After collecting the samples, the plant samples were separated and washed in distilled water to remove the debris that stick on the plants. The plants were wet weighed and heated for 24 hours at 70ºC and reweighed. The dried sample were crushed using electric high speed mill grinder until powder form. Then, the sample were sieved using 1.0mm sieve. The 10g of powdered sample were placed into sanitized sample bottle and labelled for future analysis.

3.4 Crude Protein Analysis

The crude protein analysis conducted by following the standard methods outlined by AOAC (2005). Approximately 1.0g of the powdered samples were digested with 1g Kjeldahl tablet in the 12mL concentrated sulfuric acid at 400ºC temperature in fume hood. After that, the sample was cooled and diluted with 250ml of distilled water. Then, the mixture were distilled in 4% boric acid and will be titrated against 0.1M HCl. The 2.5mL bromoscresol green and 1.75mL methy red were used as indicators that produce light pink

for final color as the end point for the titration. The percentage of the total nitrogen and crude protein were obtained by using the formula:

$$\% \text{ Total Nitrogen} = \frac{(14.01 \times M \times 100)}{\text{mg sample}} \times (\text{mL titrant} - \text{mL blank}) \times 100$$

(3.1)

*where 14.01 is the atomic weight for Nitrogen, M is the molarity of the acid (mol/L)

$$\% \text{ Crude Protein} = \text{Total N} \times 6.25$$

(3.2)

3.5 Crude Fiber Analysis

The experimental aquatic plant that were analysed for crude fiber analysis standard methods outlined by AOAC (2005).

3.5.1 Preparation of Fiber Bags for Crude Fiber

The fiber bags were dried for 1 hour at 105°C. After that the fiber bags were placed in the desiccator for 30 minutes. The fiber bags were weighed and value for M_1 were recorded. Approximately 1g from the sample were weighed and the value for M_2 been recorded. The fiber bags with glass spacers been inserted into the carousel.

3.5.2 De-Fatting of The Sample, especially if the fat content is > 10%

The samples were washed in petroleum ether 40/60 for three times. Then, the samples were dried for 2 minutes. After that, the samples were digested into 360ml boiling H_2SO_4 for 30 minutes after start to boil. After 30 minutes, the samples were

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washed three times in hot water. Next, the sample were digested into 300ml NaOH for 30 minutes after boiling. Then, the samples were washed again with hot water for three times. The fiber bags were removed from the carousel. After that the fiber bags been put in oven for 4 hours oven drying at $105^{\circ}C$ then were placed into desiccator for 30 minutes. After 30 minutes for the oven dry, the sample were weighed for M_3. Then, the samples in the crucible were placed in furnace for $600^{\circ}C$ for 4 hours. Then, the sample were weighed with the crucible for M_4. The percentage of crude fiber were calculated using formula:

$$\% \text{ crude fiber} = [(M_3 - M_1 - M_4) - (B_3 - B_1 - B_4) \text{ x } 100] / M_2$$

(3.3)

Denomination:

M_1 = Fiber bag (g)

M_2 = Initial sample weight (g)

M_3 = incinerating crucible and dried fiber bag after digestion (g)

M_4 = incinerating crucible and ash (g)

B_1 = blank value of the empty fiber bag (g)

B_3 = Incinerating crucible and dried fiber bag blank value after digestion (g)

B_4 = incinerating crucible and ash blank value (g)

3.6   Ash analysis

The ash analysis standard methods outlined by AOAC (2005). Approximately 1.5g of the samples were placed into the crucible. Then, they were incinerated in the

furnace for 500ºC overnight. The ash produced were cooled down in desiccator and reweighed. The percentage for ash content were calculated using formula:

Ash % = ash weight (g) / weight of dry sample (g) x 100

$$(3.4)$$

3.6.1 Organic matter content

The organic matter content of the samples were obtained from the results of ash content by using the formula below:

100 - % Ash = Organic matter content

$$(3.5)$$

3.7 Microbial Analysis

3.7.1 Water Sampling

The water samples were taken in the 15ml falcon tube that been wrapped with aluminium foil from the sampling location for each 5 different sampling locations. The water sample were placed in the polystyrene box and were transported to the lab for microbial analysis.

3.7.2 Plate Count Agar Preparation

Approximately weighed 4.5g of agar, 2.5g of Tryptone, 1.25g of yeast extract and 0.5g of glucose were placed into the beaker that contains 500ml of distilled water. The

agar mixture were mixed well on the hot plates and were transferred into the 500ml Duran sample bottle. The agar mixture were incubated for 2hrs and were transfer into the agar plates. The plates were sealed with parafilm and store in the chiller for future used.

3.7.3 Total Viable Bacterial Count

The plate count agar (PCA) medium were used for estimation of the heterotrophic bacteria by using spread plate method for the water samples in 48h at 35 degree celcius on the basis of culturing in non-selective nutrient agar medium. The water samples were shacked well and the serial tenfold dilution were prepared with one drop (0.01ml) of each dilution were spreaded with sterile hockey stick on the agar plates. The PCA were incubated at 35 degree celcius for 48h and the colonies formed on the agar were counted. The plate with the colonies that have more than 30 colonies but less than 300 colonies were chosen for colony forming calculation in CFU/ml because the range is considered statistically significant.

Colonies or plagues ÷ amount dilution plated (m/s) x dilution = CFU/ml

(3.6)

3.8 Statistical Analysis

All the data were tested by using one way ANOVA and Turkey to determine the significance of nutrient component of aquatic plants with different species of aquatic plants (*H. verticillata, P. stratiotes, C. corallina, M. spicatum* and *A. pinnata)*. Statistical analysis done using SPSS software (IBM SPSS Statistics Version 22). All the data were recorded and then calculated to gain the value for crude protein, crude fiber, ash content, organic matter content and the microbe in the water.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Overview

In this chapter, the nutrition components for five of the aquatic plants were analyzed using proximate analysis which contained of crude fiber content, ash, organic matter and crude protein. For the water sample, they were analyzed using plate count agar (PCA) for total bacterial count (TBC).

4.2 Crude Fiber Content

Table 4.1: The mean of the percentage crude fiber content for different aquatic plant.

Species	Percentage of crude fiber content (%)
Azolla pinnata	42.95±2.25
Hydrilla verticillata	15.35±0.35
Pistia stratiotes	14.10±0.10
Myriophyllum spicatum	25.60±5.10
Chara corallina	20.05±0.55

Table 4.1 showing the percentage of crude fiber content of the samples where 42.95±2.25 for *A. pinnata*, 15.35±0.35 in *H. verticillata*, 14.10±0.10 in *P. stratiotes*, 25.60±5.10 for *M. spicatum* and 20.05±0.55 for *C. corallina*. The crude fiber content refers to the residue of acid hydrolysis that is insoluble. The crude fiber content is done to divide the carbohydrates into two which are digestible and indigestible fractions (AAFCO, 2017). If the content of the crude fiber is high, the amount of the energy for the feed is low because the crude fiber content can be considered as indigestible substances. Crude fiber is part of the carbohydrate that is insoluble. However, in this study the results obtained shows that *A. pinnata* contains the highest amount of the crude fiber compared to *H. verticillata, P. stratiotes, M. spicatum and C. corallina*. Prior to this study, there is contra with the value of the crude fiber content for *A. pinnata* reported by Anitha, Rajeshwari, Prasanna and Shilpa (2016) where they reported less value which is 14.70%.

Another research made by Alalade and Iyayi (2006) also reported that the *A. pinnata* has lower value of crude fiber which is 12.7%. All of the values reported by the researcher shows that the crude fiber content for *A. pinnata* does not exceed 20% of the nutrition of the *A. pinnata*. There is difference between the result that obtained from this study and the research that have been done by Alalade and Iyayi (2006). It is because of the different method that have been applied to obtain the crude fiber for *A. pinnata*. The method that used by Alalade and Iyayi was the method of Goering and Van Soest (1970) while this study use the method from AOAC (2005). From the method, there are difference between the fiber analyses.

Gilles, Jean and Gerald (1991) reported that the method of Goering and Van Soest (1970) use the acid detergent to wash the sample after extraction while in this study use hot water to wash the fiber residues after digestion process. The washing process is a

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crucial methods for crude fiber analysis because the function of washing the residue is to remove the fiber solution and the soluble feed components. The voids in the sample trap the solution and components in it and when the sample been washed 3 times using hot water (95-100ºC), the solution and component from the digestion process being removed because the uses of hot water and the multiple times of rinsing process. The hot water was used to enlarge the pores of the voids so that the solution and components in the void easily removed (AAFCO, 2017). So, that is the reason the crude fiber for this study recorded to be the highest.

A research by Joysowal et al., (2018) stated that the broilers can digest the crude fiber contained by *A. pinnata*. The digestibility of the crude fiber in the *A. pinnata* is not a limiting factor when using *A. pinnata* as animal feeds. When the comparison have been made with the research that have been done by Anitha, Rajeshwari, Prasanna and Shilpa (2016) and Alalade and Iyayi (2006), there are big difference in the range value for the crude fiber. It is might due to the changes towards the method that used for crude fiber analysis.

Apart from that, *P. stratiotes* recorded the lowest value for the crude fiber among the other species. However, the crude fiber content for *P. stratiotes* was closed to the agreement of the values that obtained by Omotayo et al., (2016) which is 14.13±0.88% but slightly differs with the crude fiber value in the research that have been done by Wasagu et al., (2013) which in range of 17.50-20.50%. The values of the crude fibers in the species indicates the cellulose content of the samples (Wolverton and McDonald, 1976).

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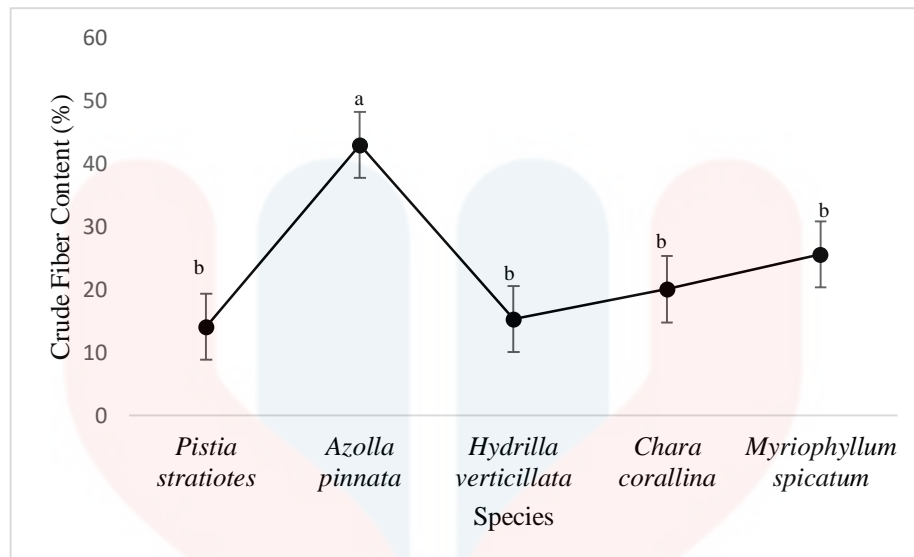
Figure 4.1: Comparison of the mean of fiber content between each species based on homogeneity test
Note: Different letter represents different significant level which 'b' represent not significant while 'a' represent the most significant.

Figure 4.1 shows the fiber content for each of the species of aquatic plants shows the difference of the fiber among of them. From the comparison, in terms of fiber content richness, we can see that *A. pinnata* is significant among *P. stratiotes, H. verticillata, M. spicatum* and *C. corallina.* The suitable crude fiber value for animal feed requirement especially for the lactating cows is around 19 to 21% for minimum dietary ADF where *P. stratiotes, H. verticillata* and *C. corallina* can fulfill the requirement (National Research Council, 1989). However, in terms of fiber in animal feeds, *P. stratiotes* is more suitable to be in the animal feeds. Physically ruminant animals require less of fiber in their diet consumptions so that they can allow their rumen function normally (DPP, 2004). Plants that have high crude fiber content is not suitable to use for animal feeds. Crude fiber is the division of the carbohydrates and cellulose where the feeds that have high fiber content is less digestible. From the study, the aquatic plants species that suitable to be chosen as animal feeds in terms of crude fiber content is *P. stratiotes.*

4.3   Ash Content

In the study, the ash content for *P. startiotes* was reported to have highest value for the ash content. The percentage of ash content recorded for *P. stratiotes* is 31.8% while for *A. pinnata* is 12.0%. For the percentage of ash content recorded by *H. verticillata* was 18.8%, *M. spicatum* is 28.9% and for *C. corallina* is 20.6% as shown in Table 4.2.

Table 4.2: The mean value for the percentage of ash content

Species	Percentage of ash content (%)
Azolla pinnata	11.95± 1.45
Hydrilla verticillata	18.80± 0.10
Pistia stratiotes	31.80± 0.10
Myriophyllum spicatum	19.2± 0.10
Chara corallina	20.60± 0.20

The value of total ash content for *P. stratiotes* recorded the highest value compared to the other species. From the study, it shows that *P. stratiotes* contain more minerals compared to the other species. It is because of the water bodies where the *P. statiotes* were sampled which is at the agriculture area in the fish pond of Agropark UMK. In the pond, there is agriculture waste leaching into the pond as the pond located near the planting field. The fertilizer used for planting deposited in the pond and contribute to the increasing of the minerals content of the *P. stratiotes* that live in the pond. Apart from that, the increasing the ash values in *P. stratiotes* also because of the fish feeds that been applied to the fish in the pond. The fish in the pond consumed the fish feeds and the fish

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stool produced contributes to the increasing of the organic matter in the water and resulting numerous of nutrient uptakes in the *P. stratiotes*.

Besides, the value for ash content of *A. pinnata* recorded the lowest. However the total ash of *A. pinnata* in this study was close to the value obtained by Anitha, Rajeshwari, Prasanna and Shilpa (2016). Compared to other species, *A. pinnata* recorded the lowest ash content because the habitat for the *A. pinnata* is at the undisturbed fish pond in Agropark UMK. The infiltration in the pond is less compared to the fish pond that has habitat of *P. stratiotes*. It is because the pond located slightly abandoned from the fish pond. When the ash content of aquatic plants recorded to be lower, the mineral content of the aquatic plants also will decrease.

Apart from that, the total ash for *C. corallina* in this study (20.60± 0.20) were contradict with the ash value reported by Muztar, Jabbar, Slinger and Burton (1978) which is 76.5%. In this study, the total ash for *C. corallina* is higher than *M. spicatum, H. verticillata* and *A. pinnata*. It is due to mineral inputs in the cultivation of *C. corallina*. The value for ash content for *M. spicatum* in this study (19.2± 0.10) is lower than the value of ash content for *M. spicatum* repoted by Boyd (1968) which is (40.6%). The value of ash is high because the sample of *M. spicatum* lives in the brackish water. It is because the waste from the residential area were dumped into the pond and increase the mineral content in the water.

However, the value of ash content for *H. verticillata* recorded is 18.80± 0.10. The value of the total ash for *H. verticillata* in this study was close towards the agreement with the values obtained by Tan (1970) which is 17.64%. From the result obtained in the study, the ash content vividly shows that the content of ash depends on the water of the habitat of the species live. The leachate that contains water soluble inorganic compound

from the surroundings into the water can cause the potash to form and contributes to ash

contents (Babayemi, Dauda, Nwuda and Kayode, 2010).
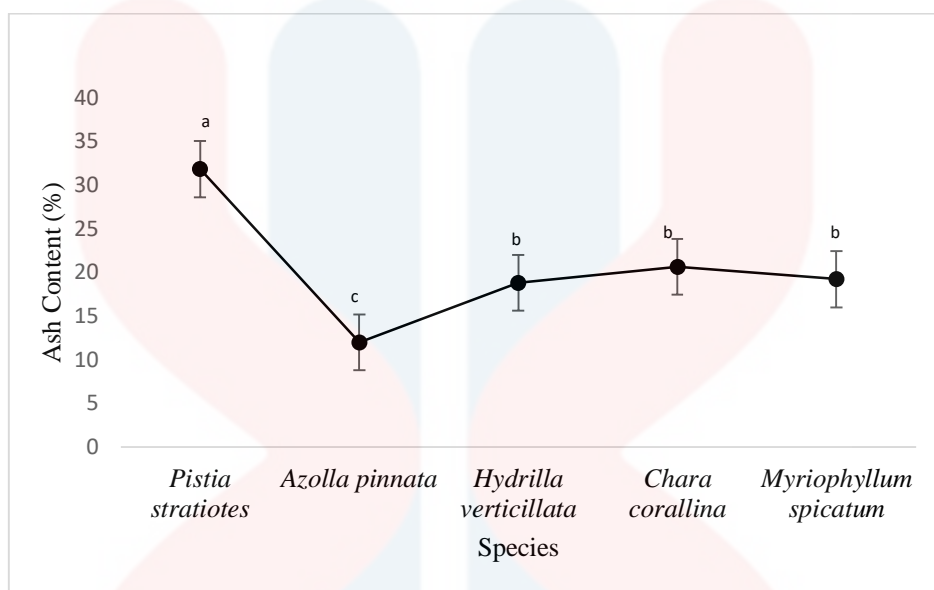
Figure 4.2: The mean comparison for the mean of ash content between each species based on homogeneity test.
Note: Different letter represents different significant level which 'a' represent most significant while b and c represent not significant.

The Figure 4.2 shows that the mean ash content for *P. stratiotes* is significant

among *A. pinnata*, *H. verticillata*, *M. spicatum* and *C. corallina.* The ash content in P.

stratiots is high due to the contamination of soil during the harvesting process of the plant

material (Michael, n.d).

4.4   Organic Matter Content

The organic matter content for the aquatic plant were presented in Table 4.3

showed that the organic matter content for *A. pinnata* is 88.05±1.45%, *H. verticillata* is

81.20±0.10%, *P. stratiotes* is 68.20±0.10%, *M. spicatum* is 80.80±0.10% and *C. corallina*

is 79.40±0.20%. The highest percentage of organic matter content of the aquatic plant is *A. pinnata* while the lowest value for organic matter content is *P. stratiotes*.

Table 4.3: The mean value for percentage of organic matter content in different species of aquatic plants

Species	Percentage of organic matter content (%)
Azolla pinnata	$88.05 \pm 1.45_a$
Hydrilla verticillata	$81.20 \pm 0.10_b$
Pistia stratiotes	$68.20 \pm 0.10_c$
Myriophyllum spicatum	$80.80 \pm 0.10_b$
Chara corallina	$79.40 \pm 0.20_b$

Note: Different letter represents different significant level which 'a' represent most significant while b and c represent not significant.

The increasing the number of ash content will affect the organic matter values where they tend to decrease the organic matter content in the aquatic plant species (Banerjee & Matai, 1990). The organic matter in the species shows the amount of nutrition value of the aquatic plants (Anam Al-Arif et al., 2017). The organic matter values for *A. pinnata* in this study was exceed the agreement with the values obtained from the research that have been made by Anitha, Rajeshwari, Prasanna and Shilpa (2016) where the values was 82.66% of organic matter. The finding of this study for organic matter content shows that the higher results than the research that have been made by other researchers.

The organic matter for *H. verticillata* in this study recorded 81.20±0.10% compared to the research that have made by Chand and Mali (2015) where they obtained 88.05% for shoot part and leaf part is 82.25%. While for *P. stratiotes*, they recorded was

68.20±0.10% compared to the study that have been done by Omotayo et al., (2016) which is 83.8%. The study have a large difference between the organic matters. The difference may connected with the variations in the geographical location of the species growth and the development of maturity stage (Ogbe and John, 2011).

Apart from that, the organic matter value recorded for *M. spicatum* in this study was 80.80±0.10% compared with the study that have been done by Muztar, Jabbar, Slinger and Burton (1978), the value was 49.8% where it was recorded the lowest value of organic matter among of all of the researcher. The reasons for the big gap between the two comparison values are due to the handling of the samples. The sample might exposed to the environments exceeding their time which is supposed in rage of 4-6 hours.

The organic matter might change due to the contamination towards the species. Apart from that, the organic matter values recorded by *C. corallina* was 79.40±0.20%. While for the research that have been done by Muztar, Jabbar, Slinger and Burton (1978) recorded 23.5% for the value of the organic matter. However, the organic matter in the aquatic plants depends on the temperature and the heating times of the samples (Muztar, Jabbar, Slinger and Burton, 1978).

4.**5**   **Crude protein content**

In this study *H. verticillata* recorded the highest value for crude protein content compared with other species (*A. pinnata, P. stratiotes, M. spicatum* and *C. corallina*). The crude protein content for each species of the aquatic plant which are *A. pinnata, H. verticillata, P. stratiotes, M. spicatum* and *C. corallina* are presented in Table 4.4

Table 4.4: The mean value for percentage of crude protein content in different species of aquatic plants

Species	Percentage of crude protein content (%)
Azolla pinnata	21.70±0.09
Hydrilla verticillata	23.18±0.13
Pistia stratiotes	16.89±0.08
Myriophyllum spicatum	22.78±0.76
Chara corallina	22.30±0.13

The mean value for percentage of crude protein content for *A. pinnata is* 21.70±0.09, *H. verticillata* is 23.18±0.13, *P. stratiotes* is 16.89±0.08, *M. spicatum* is 22.78±0.76 and *C. corallina* recorded 22.30±0.13. From this study, the protein content of *H. verticillata* recorded the highest value compared with the value of protein for *M. spicatum, C. corallina, A. pinnata* and *P. stratiotes.* On the contrary, Parsons and Cuthbertson (2001) were reported the crude protein value of *H. verticillata* obtained was 12.9%. While the percentage of the crude protein recorded by Tan (1970) was 17.64%. The crude protein of *H. verticillata* obtained by Rini, Happy and Anik (2016) was 44.13% and it was the highest value recorded among the researcher.

Apart from that, the value of crude protein content for *A. pinnata* for this study was recorded 21.70±0.09. The value of the crude protein for *A. pinnata* almost close with the value obtained by Anitha, Rajeshwari, Prasanna and Shilpa (2016) which was 22.48%. However, the value of crude protein for *P. stratiotes* in this study is 16.89±0.08. The research that have been made by Rodriguez, Julio and Palma (2000) reported that the value for crude protein of *P. stratiotes* was 8.62%. Previous research indicates the percentage of crude protein for *P. stratiotes* was below than 1.0% which was 0.78% (Boyd, 1969).

The crude protein value recorded in this study for *M. spicatum* is 22.78±0.76 compared to the crude protein value recorded by Boyd and Paul (2016) which were in ranged between 13.8 to 18.0 percent. The crude fiber content was close agreement with the value recorded by Lin, Goodrich, Meiske and John (1973) which was 25.83%. Besides, the crude protein content for *C. corallina* recorded in this study is 22.30±0.13. The crude protein recorded by Boyd (1968) was 17.5%.
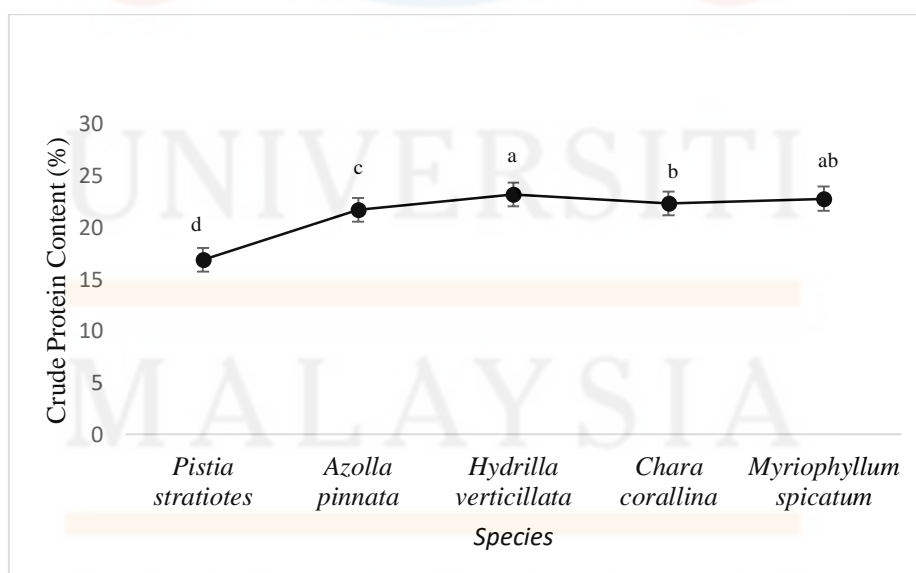

Figure 4.3: The mean comparison value for mean of crude protein between each species based on homogeneity test
Note: Different letter represents different significant level which 'a' represent most significant while 'b, c, d and ab' represent not significant

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From Figure 4.3, the *H. verticillata* species recorded the highest crude protein values where they tend to have high nitrogen content (Baydan et al., 2016). The value of crude protein content in the aquatic plants are comparable with the crude protein value in the conventional dry forages that most of the people used as animal feeds (Boyd and Paul, 2016). The crude protein in the *H. verticillata* can enhance the growth of the animal who consume it in their diet. Jeerasak, Kabuan and Auraiwan (2009) reported that the increasing of the crude protein in the total mixed rations (TMR) can enhance the digestibility of the crude protein and can help to increase the growth rate of the animal. The mean protein content for *H. verticillata* is significant among *A. pinnata, P. stratiotes, M. spicatum* and *C. corallina*. The *H. verticillata* is suitable for animal feed because they contain high protein where they can help to increase the flesh of the fish (David, Vernon and Jeffrey, 1986). Rini, Happy and Anik (2016) reported that the substitution of 10% fish feed with *H. verticillata* can increase the productivity of Tilapia. According to Cooperative Extension (2011), the suitable protein requirements for animal especially for lactating cows require 9.6% of crude protein. So, according to this study, all of the aquatic plants exceed the protein requirement for the lactating cow. While the suitable protein requirement for fish that reared for human consumption require 36% to 42% of protein (Frank and Richard, 2009).

4.6   Microbial Analysis on Each Species

In this study *P. stratiotes* recorded the highest value for total bacterial count compared with other species (*A. pinnata, H. verticillata, M. spicatum* and *C. corallina*).

The total bacterial count for each species of the aquatic plant which are *A. pinnata, H. verticillata, P. stratiotes, M. spicatum* and *C. corallina* are presented in Table 4.5

Table 4.5: The mean value for total bacterial count (CFU/mL) for each species of aquatic plant

Species	Total Bacterial Count (1ml x 10^6)		
	Mean	**± S.D**	**Log Mean**
Hydrilla verticillata	2.71	0.36	6.43
Pistia stratiotes	2.88	0.16	6.46
Azolla pinnata	1.68	0.80	6.23
Chara corallina	2.16	0.47	6.33
Myriophyllum spicatum	0.49	0.14	5.69

From the result, the highest bacterial count shows the pollution level for the water at the habitat area of the aquatic plants. The bacteria presence at the water bodies at the habitat of *P. stratiotes* is high. The mean of total bacterial count for *P. stratiotes* was 2.88×10^6 CFU/mL. Mohammed Rawway, Momen Salah and Usama (2015) reported that the uses of the total bacterial count usually for evaluate the total amount of bacteria and the microbial status of the water bodies of the sampling area. In this study, the location of *P. stratiotes* is recorded to have high microbial status because the water bodies is located at the agriculture area. The location also near to the cow grazing area where the water might contaminate with the cow dung and resulting in increasing the bacterial count. Research by Amira et al, (2014) stated that high manure application to soil can increase the contamination and pathogen in the water bodies. The mean of total bacterial count for *H. verticillata* was 2.71×10^6 CFU/mL while the mean of total bacterial count for *A. pinnata* was 1.68×10^6 CFU/mL. The bacterial counting for *C. corallina* was

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2.16x10^6 CFU/mL and the bacterial counting for *M. spicatum* recorded was 0.49x10^6

CFU/mL where it was the least number recorded.
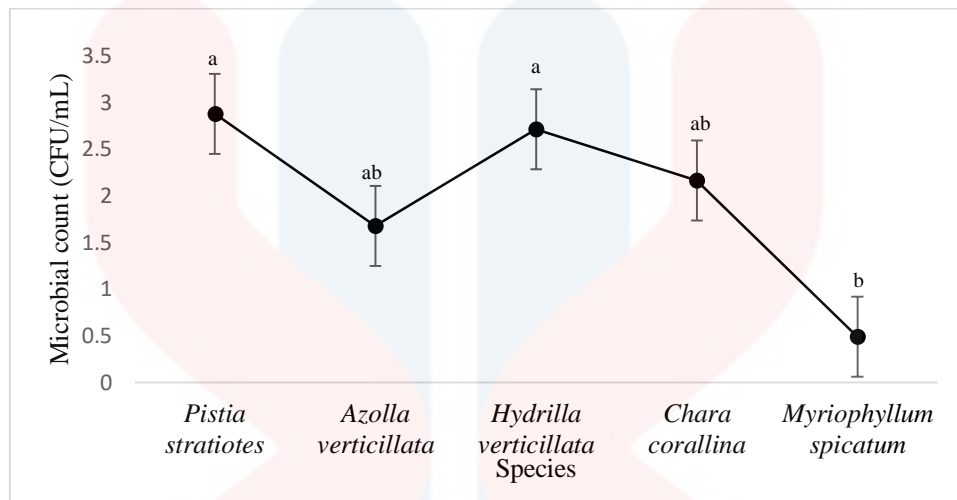
Figure 4.4: The mean comparison for the microbial count for each species crobial count for each water for each species

Note: Different letter represents different significant level which 'a' represent most significance while 'b' represent not significant

Figure 4.4 shows the water bodies at the habitat of *P. stratiotes* and *H. verticillata*

are significance than other water bodies at the habitat of the aquatic species *(A. pinnata,*

M. spcatum and C. corallina). It shows that *P. strtaiotes* reach the microbial limit for

animal feed suitability where the limits prescribed by the Regulation ranging from 10-

800 in 1 g (Elzbieta, Krzysztof and Magdalena, 2005).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

Based on the result of the study, the nutrient component of *Hydrilla verticillata, Pistia stratiotes*, *Chara corallina, Myriophyllum spicatum* and *Azolla pinnata* can be differentiate from each other. Each of the species are nutritious and the nutrition component between the five species of the aquatic are different from each other. However, the aquatic plants can be replaced as unconventional feed resources that has high potential for animal feeds especially for ruminant and fish based on the nutrition component and the microbial requirement for the animal.

For recommendation, the study should have done for 3 times of replication so that the result will be more accurate. Other than that, the recommendation for plant sampling, the sampling for aquatic plants should not exceed December because in Kelantan there will be monsoon on that month and the sampling for aquatic plant will be difficult because the water level of the river arise.

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APPENDIX A

Table A.1: The Percentage of Ash Content

Replicate	Species				
	Pistia stratiotes	*Azolla pinnata*	*Hydrilla verticillata*	*Chara corallina*	*Myriophyllum spicatum*
1	31.9	10.5	18.7	20.4	19.3
2	31.7	13.4	18.9	20.8	19.1

Table A.2: The Percentage of Organic Matter Content

Replicate	Species				
	Pistia stratiotes	*Azolla pinnata*	*Hydrilla verticillata*	*Chara corallina*	*Myriophyllum spicatum*
1	68.1	89.5	81.3	79.6	80.7
2	68.3	86.6	81.1	79.2	80.9

Table A.3: The Percentage of Crude Fiber Content

Replicate	Species				
	Azolla pinnata	***Hydrilla verticillata***	*Pistia stratiotes*	*Myriophyllum spicatum*	***Chara corallina***
1	40.7	15.0	14.2	20.5	19.5
2	45.2	15.7	14.0	30.7	20.6

Table A.4: The Percentage of Crude Protein Content

Replicate	Species				
	Azolla pinnata	***Hydrilla verticillata***	*Pistia stratiotes*	*Myriophyllum spicatum*	***Chara corallina***
1	21.79	23.32	16.97	22.69	22.21
2	21.61	23.05	16.81	22.87	22.40

Table A.5: Print screen of SPSS Descriptive on Each Analysis

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
ash_content	Pistia stratiotes	2	31.8000	.14142	.10000	30.5294	33.0706	31.70	31.90
	Azolla pinnata	2	11.9500	2.05061	1.45000	-6.4740	30.3740	10.50	13.40
	Hydrilla verticillata	2	18.8000	.14142	.10000	17.5294	20.0706	18.70	18.90
	Chara corallina	2	20.6000	.28284	.20000	18.0588	23.1412	20.40	20.80
	Myriophyllum spicatum	2	19.2000	.14142	.10000	17.9294	20.4706	19.10	19.30
	Total	10	20.4700	6.79134	2.14761	15.6118	25.3282	10.50	31.90
fiber_content	Pistia stratiotes	2	14.1000	.14142	.10000	12.8294	15.3706	14.00	14.20
	Azolla pinnata	2	42.9500	3.18198	2.25000	14.3610	71.5390	40.70	45.20
	Hydrilla verticillata	2	15.3500	.49497	.35000	10.9028	19.7972	15.00	15.70
	Chara corallina	2	20.0500	.77782	.55000	13.0616	27.0384	19.50	20.60
	Myriophyllum spicatum	2	25.6000	7.21249	5.10000	-39.2016	90.4016	20.50	30.70
	Total	10	23.6100	11.36119	3.59272	15.4827	31.7373	14.00	45.20
organic_matter_content	Pistia stratiotes	2	68.2000	.14142	.10000	66.9294	69.4706	68.10	68.30
	Azolla pinnata	2	88.0500	2.05061	1.45000	69.6260	106.4740	86.60	89.50
	Hydrilla verticillata	2	81.2000	.14142	.10000	79.9294	82.4706	81.10	81.30
	Chara corallina	2	79.4000	.28284	.20000	76.8588	81.9412	79.20	79.60
	Myriophyllum spicatum	2	80.8000	.14142	.10000	79.5294	82.0706	80.70	80.90
	Total	10	79.5300	6.79134	2.14761	74.6718	84.3882	68.10	89.50
protein_content	Pistia stratiotes	2	16.8900	.11314	.08000	15.8735	17.9065	16.81	16.97
	Azolla pinnata	2	21.7000	.12728	.09000	20.5564	22.8436	21.61	21.79
	Hydrilla verticillata	2	23.1850	.19092	.13500	21.4697	24.9003	23.05	23.32
	Chara corallina	2	22.3050	.13435	.09500	21.0979	23.5121	22.21	22.40
	Myriophyllum spicatum	2	22.7800	.12728	.09000	21.6364	23.9236	22.69	22.87
	Total	10	21.3720	2.42146	.76573	19.6398	23.1042	16.81	23.32

Table A.6: ANOVA Test for Significance Value

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ash_content	Between Groups	410.756	4	102.689	118.169	.000
	Within Groups	4.345	5	.869		
	Total	415.101	9			
fiber_content	Between Groups	1098.674	4	274.669	21.794	.002
	Within Groups	63.015	5	12.603		
	Total	1161.689	9			
organic_matter_content	Between Groups	410.756	4	102.689	118.169	.000
	Within Groups	4.345	5	.869		
	Total	415.101	9			
protein_content	Between Groups	52.672	4	13.168	660.377	.000
	Within Groups	.100	5	.020		
	Total	52.771	9			

Table A.7: The Homogeneous Subsets

ash_content

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Azolla pinnata	2	11.9500		
Hydrilla verticillata	2		18.8000	
Myriophyllum spicatum	2		19.2000	
Chara corallina	2		20.6000	
Pistia stratiotes	2			31.8000
Sig.		1.000	.407	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

fiber_content

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
Pistia stratiotes	2	14.1000	
Hydrilla verticillata	2	15.3500	
Chara corallina	2	20.0500	
Myriophyllum spicatum	2	25.6000	
Azolla pinnata	2		42.9500

| | | | Sig. | | .106 | 1.000 | |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

organic_matter_content

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Pistia stratiotes	2	68.2000		
Chara corallina	2		79.4000	
Myriophyllum spicatum	2		80.8000	
Hydrilla verticillata	2		81.2000	
Azolla pinnata	2			88.0500
Sig.		1.000	.407	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

protein_content

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
Pistia stratiotes	2	16.8900			
Azolla pinnata	2		21.7000		
Chara corallina	2			22.3050	
Myriophyllum spicatum	2			22.7800	22.7800
Hydrilla verticillata	2				23.1850
Sig.		1.000	1.000	.094	.155

Means for groups in homogeneous subsets are displayed.

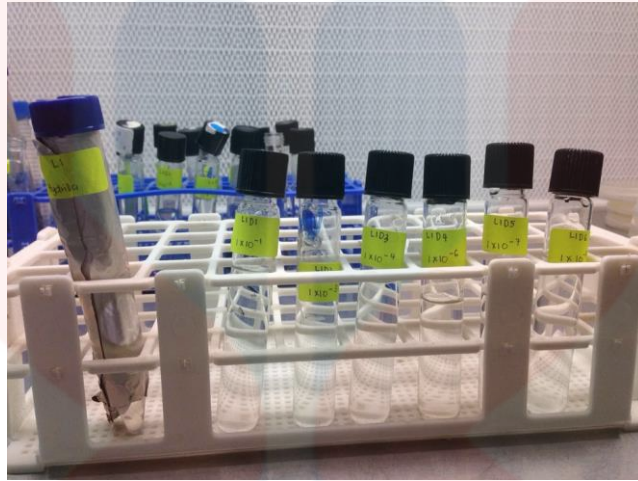a. Uses Harmonic Mean Sample Size = 2.000.

APPENDIX B

Figure B.1: Serial tenfold dilution for microbial analysis

Figure B.2: After overnight ashing for fiber content analysis

Figure B.3: Light pink after titration process for crude protein

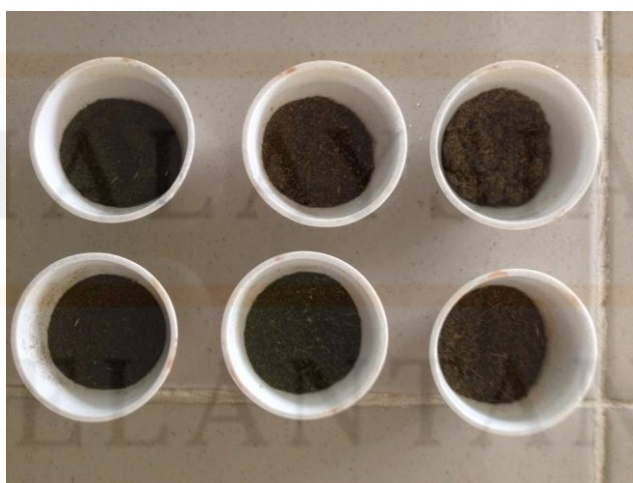Figure B.4: Titration result after titrate with NaOH
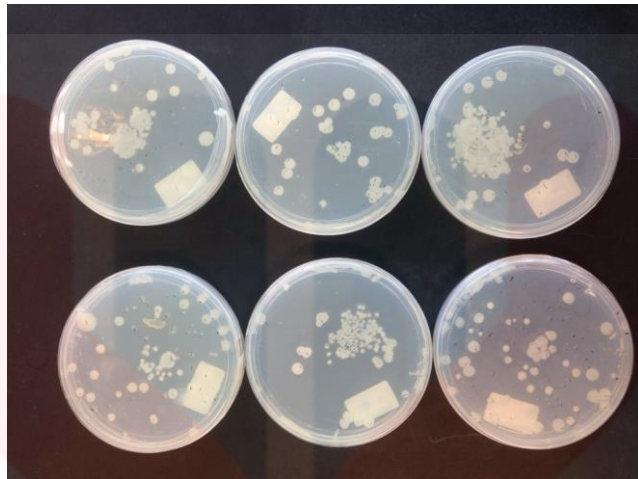

Figure B.5: Ash in the crucible for ash content

Figure B.6: Bacteria colony for Total Bacterial Counting

Figure B.7: Upper view of *Pistia stratiotes*

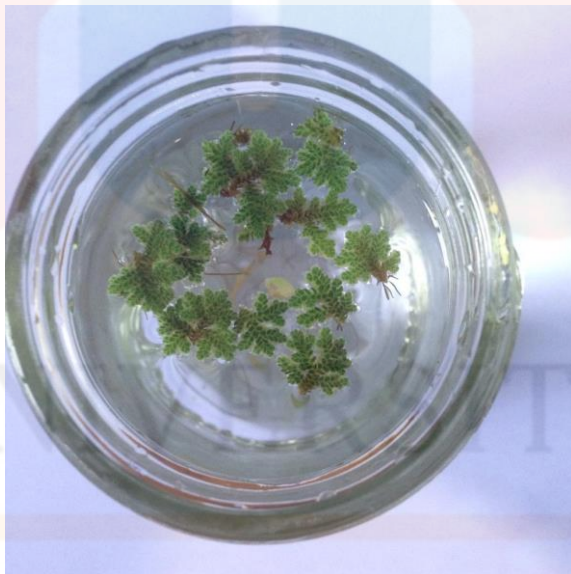Figure B.8: Side view of *Myriophyllum spicatum*

Figure B.9: Upper view of *Azolla pinnata*

Figure B.10: Side view of *Hydrilla verticillata*

Figure B.11: Fresh *Chara corallina*