CHAPTER I

INTRODUCTION

1.1 Background of the Research

Phytoplankton, equally known as microalgae refer to all the aquatic autotrophs living in suspension in the water column and the name encompasses several phyla which are mostly eukaryotes with the inclusion of photosynthetic prokaryotes called cyanobacteria (Widowati *et al*. 2017). Microalgae as components of the aquatic food chain are obviously very important for their role in food production in the marine environment as well as freshwater. Several studies on phytoplankton were initially focused on their use for food by fish and other aquatic organisms both in their natural habitats and in aquaculture (Brown *et al*. 1997). However developments in science which birthed areas like marine biotechnology have drawn additional attention to marine phytoplankton and other organisms due to their potentials to act as secondary metabolites, thereby serving as drug active ingredients in the pharmaceutical industry. Many studies on phytoplankton in recent times have been targeted at assessing their ability to synthesize secondary metabolites (carotenoids, pigments, lipids etc) for the pharmaceutical industry, biofuel production and aquaculture applications (Talero *et al*. 2015).

Spirulina is a “blue-green” photolithoautotroph, which implies it is able to derive energy from sunlight and use carbon dioxide as its carbon source (Perry *et al*. 2002). It obtains minerals from the inorganic sources of the environment and similar to other cyanobacteria, spirulina is gram negative with a cell membrane, cell wall and an outer membrane. Spirulina has been known for its food benefits for numerous years even before its rediscovery three decades ago (Barnett, 2007) as the little biscuits of dried spirulina biomass known as "Dihe" in present-day Chad was one of the first documented reports on the use of spirulina as food (Cliferri, 1983; Perry *et al*. 2002). *Athrospira maximus* obtained from Lake Texcoco (around present-day Mexico) which was added into bread by the Spanish Conquistadors is another old instance of the use of spirulina for food by man (Cliferri, 1983). More recently, spirulina was used for candy production by *Antenna Foundation* to fight malnutrition in children. According to the report of Vonshak *et al*. (1997) the unique composition in terms of fatty acids and vitamins and the high protein content of Spirulina are the basis for justification of the claimed health benefits it has for human. The feed market, mainly aquaculture is yet another growing market for spirulina products (Vonshak *et al*. 1997).

According to Matic *et al*. (2018) and Gopalakrishnan *et al*. (2016) *Moringa oleifera* is a plant with historical origin from India and surrounding countries like Bangladesh, Pakistan and Afganistan where it is believed to have been first described as a medicinal plant in 2000 BC. Moringa is distributed westward (Egypt, the Horn of Africa, areas of the Mediterranean and to the West Indies in America) and eastward (lower areas in China, Southeast Asia as well as the Philippines). The moringa plant is known with names like "Nebedaye" which translates to "never die" in many African languages, "the Miracle tree", "Drumstick tree" or "Horseradish tree". The tree grows mainly in tropical, subtropical and semiarid regions with dry, sandy soil (Matic *et al*. 2018). Moringa is a very resistant plant and has the ability to withstand severe drought as well as mild frost conditions.

Africans and Indians have used *M. oleifera* in herbal medicine for long and it is often regarded as a "panacea" used for treatment of more than 300 conditions such as diabetics, microbial infections, as an antioxidant, anti-inflammatory, anticancer etc. *Moring oleifera* has notable nutritional potentials, as the leaves are high in protein, seeds abundant in lipids like stearic acid, oleic acids and saturated palmitic acid while both the seeds and pods contain high levels of potassium, sodium, calcium and iron (Raimunda *et al*. 2017). The extracts of *M. oleifera* are widely used by doctors, nutritionists, healers, and community leaders for treating under-nutrition and anaemia, mostly in infants and children (Yang *et al*. 2006; Thurber and Fahey 2009). Animals fed with *M. oleifera* leaves have also reportedly gained weight and higher nutritional status (Mendieta-Araica *et al*. 2011).

One special attribute of moringa is that it can be preserved by freezing or drying for long without loss of value (Katayon *et al*. 2006). Beside the medicinal and nutritional applications of *M. oleifera*, it also has other numerous uses, for instance the seeds are used for oil rich in oleic acids, sterols and tocopherols which can be used for cooking as a substitute for olive oil, but equally for non-food applications like cosmetics, a lubricant for machines and in biodiesel (Tsaknis *et al*. 1999; Fahey, 2005). After the extraction of oil from moringa, the by-product (seed cakes) can be used as an organic fertilizer for improvement of agricultural productivity (Emmanuel *et al*. 2011). The seeds also serve as a natural coagulant which contains a cationic protein with the ability to “purify” turbid water by precipitation of organic and mineral particulates (Ndabigengesere and Subba Narasiah, 1998). Furthermore, the extract from moringa seeds has demonstrated the capacity to eliminate heavy metals such as cadmium, copper, lead, arsenic and chromium from water (Ravikumar and Sheeja, 2013) and the seed extracts also have antimicrobial abilities that inhibit the growth of bacteria, with implications to waterborne disease prevention.

Antioxidants refer to all substances that are capable of inhibiting or delaying oxidation of a substrate, even in low concentrations compared to the “oxidisable” substrate (Young and Woodside, 2001). Spirulina has been reported severally to have antioxidant attributes as already demonstrated in both *in vitro* and *in vivo* studies (Demule *et al*. 1996; Bermejo-Bescós *et al*. 2008; Gad *et al*. 2011; Wang *et al*. 2007). Spirulina acts as an antioxidant either by inhibition of the processes which activate the formation of free radical species or by intercepting the action of free radical (scavenging of the radical) or supressing radical damage amplification (by subsequent interception of the attack of secondary-derived radicals on their biological components) or reduction in iron ions known to trigger many processes that lead to the appearance of free radicals (using iron chelating properties) (Bermejo-Bescós *et al*. 2008).

The antibacterial ability of a substance simply implies the ability of that substance to inhibit or slow the growth and proliferation of a particular bacterium. The antibacterial potential of spirulina has been studied by many authors (Mala *et al*. 2009; Ahsan *et al*. 2015; Manigandan and Kolanjinathan, 2017) but the mechanism through which it occurs seem unclear. According to Alves *et al*. (2013) the carboxylic acid (-COOH), two hydroxyl (-OH) groups in *para* and *ortho* positions in benzene ring as well as a methoxyl (-OCH_3) group in the *meta* positions of phenolic compounds seem to play important roles in overcoming antimicrobial resistance. Demule *et al*. (1996), observed that the γ-linolenic fatty acid which is an active antimicrobial fatty acid available in high quantity in spirulina extracts was responsible for the antimicrobial activity of methanolic extract of spirulina.

Similar to spirulina, many previous studies have reported the antioxidant potential of moringa (Fitriana *et al*. 2016; Kwei, 2012; Mahdi *et al*. 2016; Xu *et al*. 2019). Paula *et al.* (2017) observed that the antioxidative effect of moringa is mainly strong in the leaves, howbeit it the pods also have the potential (Gull *et al*. 2016) as the seed have equally indicated a synonymous effect (Randriamboavonjy *et al*. 2017). In addition, the aquaeous leaf extract of moringa have reportedly cause a tangible rise in the activity of catalase, superoxidase dismutase and glutathione S-transferase as well as a decrease in lipid peroxidation in normal and diabetic rats (Jaiswal *et al*. 2013).

Certain substances in moringa such as phenols including flavonoids, vanillin, gallic acid, kaempferol, quercetin, cinnamic acid etc have exhibited antibacterial activity (Abdull *et al*. 2014; Saini *et al*. 2016).

1.2 Problem Formulation

It is unarguably true that both *S. platensis* and *M. oleifera* are high quality natural products in terms of human nutrition and health. While the former is one of the richest aquatic natural products, the latter is one of the richest on land but incidentally, both *S. platensis* and *M. oleifera* are natural commodities dominant in the tropics/sub-tropics and have both been reported by different scientists as good sources of antioxidants and antibacterial.

However, there are some questions about the antioxidant and antibacterial potentials of these two products that are yet to be answered in an individual research and these include: “which among the two products is higher in antioxidant activity”? “Which of the two is higher in antibacterial activity”? “Does synergism exist in terms of antioxidant and antibacterial activity when the extracts of *S. platensis* and *M. oleifera* are combined to form a complex”?

This research has been designed to provide answers to the above questions by comparing the antioxidant and antibacterial activities of *S. platensis* and *M. oleifera* as well as to determine whether or not there exist any synergistic effect in antioxidant and antibacterial activity when a complex is produced from the two since no such comparison and assessment has been published, as far as available literature search is concerned.

1.3 Aims of the Study

This research aims at the following:

i. To compare the antioxidant activity of *S. platensis* and *M. oleifera* (*in vitro)*

ii. To compare the *in vitro* antibacterial activity of *S. platensis* and *M. oleifera*

iii. To determine whether or not there is synergism in various complexes formed from respective extracts and fractions of *S. platensis* and *M. oleifera* in terms of antioxidant activity

iv. To determine whether or not there is synergism in various complexes formed from respective extracts and fractions of *S. platensis* and *M. oleifera* in terms of antibacterial activity

1.4 Benefits of the Study

The research has the following benefits:

i. The study will provide the reading public with information on the natural product higher in antioxidant activity between *S. platensis* and *M. oleifera*

ii. The study will provide the reading public with information on the natural product higher in antibacterial activity between *S. platensis* and *M. oleifera*

iii. The study will ascertain if there is synergism or not in *S. platensis* and *M. oleifera* complex in terms of antioxidant activity

iv. The study will ascertain if there is synergism or not in *S. platensis* and *M. oleifera* complex in terms of antibacterial activity