Phytochemical Composition And Antibacterial Activity Of Eichhornia Crassipes In Lake Victoria, Kisumu

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Abstract: Water hyacinth (Eichhornia crassipes) is an aquatic weed infesting rivers, dams, lakes and irrigation channels. The plant has affected the marine environment with billions of shillings being lost yearly in controlling it and also in economic losses. The plant is causing severe hindrances to the individual nation's developmental activities. It clogs waterways making boating, fishing and all other water activities impossible. The plant spreads via the waves from the bay to bay blocking waterways and affecting aquatic life as it takes up oxygen from the water. Owing to its tremendous growth, it has threatened the diversity of local native plants alongside the physical and chemical composition of the aquatic environment. It grows very fast and spreads widely across the water body. However, despite this problem the plant has the potential to be used as a medicinal plant. The primary objective of the study was to determine the phytochemical composition and the antibacterial properties of the plant against selected strains of bacteria and determine whether it can be exploited for therapeutic purposes. The plant material for use in the study was obtained from Lake Victoria and classified taxonomically at Botany Department, Egerton University. The crude extract of Eichhornia crassipes was analyzed for phytochemical composition. The crude extract was then subjected to antibacterial assay against bacterial isolates such as Bacillus subtilis, Salmonella typhimurium, Staphylococcus aureus, and Escherichia coli. Phytochemical analysis of Eichhornia crassipes depicted the presence of flavonoids, alkaloids and the terpenoids. Additionally, the crude extract of the plant portrayed potential antibacterial activities against some bacterial isolates. Bacillus subtilis and Staphylococcus aureus showed some level of sensitivity to the crude extract of Eichhornia crassipes. However, there was no activity against Escherichia coli and Salmonella typhimurium. The diameter of the zone of inhibition was measured for the bacterial isolates that showed sensitivity towards the aqueous extract.

Index Terms: Eichhornia crassipes, antibacterial, phytochemicals

1 INTRODUCTION

water hyacinth, an aquatic plant belonging to the family Pontideraceae, is listed as one of the most productive plants on earth and is considered the world's worst aquatic weed (Grodowitz, 1998).It originated in the state of Amazon, Brazil, spread to other regions of South America and was carried by humans to tropical and subtropical regions (Gao-Lei and Bo, 2004). The plant invaded Africa and has since caused severe ecological, economic and social problems in the region. Its dense mat covers lakes and rivers, blocking waterways and eventually interfering with the water transport of agriculture products, tourism activities and irrigation of the agricultural fields. The plant can grow fast consuming vast quantities of nutrients that promote its growth over other aquatic species. It has also been uncovered that when the plant dies and decomposes, it releases nutrients to water bodies leading to deterioration of water quality, becoming a threat to human health. It has been shown by the high prevalence of waterborne diseases in regions that have been affected by the plant especially Lake Victoria and its environs.[i] It is this challenge that has led to an increased interest in determining whether the plant can be used for economic gains. Little is known about how the South American water hyacinth invaded Africa's largest fresh water lake but there's no doubt as to the damages it has caused. In 1989, the weed was spotted in the lake and seven years later, it had clogged 80% of Uganda's shoreline. In the areas bordering Lake Victoria, the weed was first recorded in Lake Kyoga (Uganda) in May 1988(Twongo, 1991) Within Lake Victoria, it was observed in the Ugandan sector in 1989(Ondongkara and Twongo,2000). A reduced catch and lowered income threatened to trigger widespread famine. The weed was reported in Tanzania in 1989 and Kenya in 1990 (Bwathondi and Mahika, 1994). In ancient times, plants were vastly used in treatment of ailments ranging from stomach to wound infections. Plant secondary metabolites have beneficial medicinal effects on humans due to their interaction with potential target sites (Pandey et al, 2011). This is due to the fact that they constitute a wide range of novel chemical compounds which are of potential use in medicine. They contain a variety of active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids that are deposited in specific parts such as leaves, flowers, bark, seeds, fruits, root, etc. (Gadekar et al, 2010). The beneficial medicinal effects of plant materials typically result from the combination of these secondary products (Wink, 1999) That the medicinal actions of plants are unique to particular plant species or groups is consistent with this concept as the combinations of secondary products in a particular plant are often taxonomically distinct (Wink, 1999). The chemical components present in the plant have a positive effect on the body in the event of an infection. In this study, the antibacterial activity of Eichhornia crassipes obtained from Lake Victoria was determined. The aqueous was tested against the selected bacterial strains and the zones of inhibition determined in relation to the effect of the extracts on the bacteria. The zone of inhibition was determined as stipulated by the Clinical Laboratory and Standards Institute's procedure (CLSI document no. M100-S15, 2002). Since the potential of water hyacinth as a medicinal plant especially from Lake Victoria has not been extensively studied, the attempt made in the study was to help provide information on its medicinal activity.

1.1 Problem Statement

Water hyacinth, a pervasive aquatic plant, is known to cause ecological and socioeconomic challenges. Kenyan communities depending entirely on the lake for income have been greatly affected by the presence of the weed which is choking up the lake. This is due to the death of fish and other aquatic organisms in the lake. It is attributed to the deprivation of aquatic oxygen. Kisumu town's economic growth is at stake since the lake is threatened by the weed which is covering more than half of the lake. This is because of a reduction in income derived from export of fish and its products. In addition the weed has brought with it health associated risk factors with regard to the prevalent waterborne diseases for instance bilharzia. This is due to a good breeding ground provided for by the weed. The study was aimed at identifying the potency of the plant as an antibacterial agent by use of different plant extracts. In addition, the phytochemical studies done were meant to check for the presence of the different medicinal components. The plant could be harvested for large scale production of the compounds of medicinal value.

1.2 Objectives

1.2.1 General Objective
To determine the phytochemical composition and antibacterial properties of Eichhornia crassipes obtained from Lake Victoria in Kisumu, Kenya.

1.2.2 Specific objective
1. Determine the phytochemical composition of Eichhornia crassipes obtained from Lake Victoria.
2. Determine the antibacterial activity of Eichhornia crassipes against selected strains of bacteria.

1.3 Hypothesis
1. Eichhornia crassipes obtained from Lake Victoria has no phytochemicals.
2. Eichhornia crassipes obtained from Lake Victoria has no antimicrobial activity.

1.4 Justification.
Water hyacinth is a highly invasive plant that is currently the main weed eating up water bodies with streams, rivers, dams and lakes being seriously affected. Fishing activities have been hampered owing to the fact that the weed uses up the aquatic oxygen at the expense of the fish hence they are deprived of the same. In addition, getting to the fishing grounds has become a struggle leading to a reduced catch and diminished income which might trigger widespread economic challenges and increase the poverty levels in communities that are dependent on fishing activities for livelihood. Rotting vegetation, under the suffocating blanket of weeds fouls drinking water resulting to an offensive smell in the environment. In areas where the weed is prolific, there is a general increase in several diseases such as bilharzia, cholera and malaria as the weed creates an excellent breeding area for mosquitoes and other insects. In addition, there are increased incidences of skin rash, cough, malaria, encephalitis, bilharzias, gastro-intestinal disorders, and schistosomiasis (Opande and Wagai, 2004). The spread of invasive alien species is neither easy to manage nor reverse, threatening not only biodiversity but also economic development and human wellbeing. Native to the Amazon Basin in South America, water hyacinth has emerged as a major weed in more than 50 countries in the tropical and subtropical regions of the world with profuse and permanent impacts. Worryingly, climate change may allow the spread of water hyacinth to higher latitudes. However, the use of plants in the management of diseases is on the increase and thus the need to explore the medicinal potential of water hyacinth. The study, therefore, aims at determining the potential use of water hyacinth as an antibacterial agent. It is an economic hazard to the country but its use as a medicinal plant could serve as a remedy to particular infections. In addition, it investigates the phytochemical composition of the plant with a goal of exploiting those properties for therapeutic use against selected infections.

2.0 LITERATURE REVIEW

2.1 Origin of Eichhornia crassipes
Eichhornia crassipes is believed to have originated from South America in the Amazon basin. Eichhornia crassipes is an aquatic plant and is considered the world's most invasive plant. The beautiful, large purple and violet flowers of the South American water hyacinth make it a very popular ornamental plant for ponds (Zhang, 2012). It is widely reported that water hyacinth is indigenous to Brazil having first been described from wild plants collected from Francisco River in 1824. In Africa, it was first reported in Egypt between 1879 and 1893. By the early 1990s it had spread to virtually every country in the continent (Makhanu, 1997). It was first reported in the Ugandan portion of Lake Victoria in 1990 (Thompson, 1997). It is found in lakes, estuaries, wetlands, marshes, ponds, slow flowing rivers, streams, and waterways in the lower latitudes where growth is stimulated by the inflow of nutrient rich water from urban and agricultural runoff, deforestation, products of industrial waste and insufficient wastewater treatment. Lake Victoria in Africa is the second largest freshwater lake in the world and currently supports approximately 30 million people. Infestation of water hyacinth in the lake has been a serious nuisance; generating public outcry (World Agroforestry Centre, 2006). The plant has a very high reproductive capacity and has spread in major water bodies across the world. It is not exactly clear how the plant got to Lake Victoria in Kenya but several theories are in place as to how the weed spread to Africa's largest fresh water lake. It was introduced in Africa around 1879, and 110 years later, established itself on the continent's largest lake, Lake Victoria. Spreading along the shorelines, the plant formed thick mats that covered an estimated 20,000 hectares (about 77 square miles) of the lake by 1998 (Global Invasive Species Database, 2007). The weed covered a substantial portion of Uganda by 1990 and has since spread to the rest of the three East African countries which share the lake. It is also thought that the plant might have come through River Kagera and found itself in the lake. The Kenyan coastline and town Kisumu which harbor the lake is currently experiencing a major problem due to the menace brought about by the weed. The plant has since spread covering a larger percentage of the lake which is approximately 27000 square miles. It has such a high growth rate that, according to (Ntiba et al, 2001), it can double its area in only five days. It is still rapidly spreading throughout Africa, where new infestations are creating life-threatening situations as well as environmental and cultural upheaval (Adams et al, 2012).

2.2 Effects of Eichhornia crassipes in water bodies
Water hyacinth has had a very negative impact on Lake Victoria as a result of its spread. This has indirectly or directly brought about a number of socio-economic challenges. Economic impacts being currently felt are the effects on transportation in the lake. Boating has been made difficult as the weed is creating havoc by blocking water ways. Fishermen are unable to access the fishing grounds as a result of this hurdle. This has seriously impaired fishing activities and the people who derive their livelihood are now experiencing a

number of economic challenges as a result of loss of income. The weed also interrupts local subsistence fishing, blocking access to the beaches (World Bank 1996). Studies show that although fish stocks have fallen since 1990, this decline appears to have been at least temporarily halted by the declining catchability of fish because of the growing abundance of water hyacinth. The impact of the hyacinth on the availability of fish was greatest in the Kenyan section of Lake Victoria. Although hyacinth has many negative effects, it effectively hinders fishing and thereby paradoxically stops or at least postpones serious overfishing (Eseza et al 2009). It has also greatly destroyed the water quality as it decomposes. This has resulted in extra costs in water treatment systems. Where the weed is prolific, there is a general increase in several diseases, as the weed creates excellent breeding areas for mosquitoes and other insects. There are increased incidents of skin rash, cough, malaria, encephalitis, bilharzias, gastro-intestinal disorders, and schistosomiasis. Water hyacinth also interferes with water treatment, irrigation, and water supply (Opande et al, 2004). Electricity production through hydroelectric technology has also been affected due to a reduction the water quantity and blockage and this affects electricity distribution across the country. Changes to water hyacinth density have the potential to affect other ecological and human communities in areas where it is established. The changes may be perceived as positive or negative depending on the designated or beneficial uses of the water body (Gibbons et al, 1994). Lake Victoria is also a source of food, drinking and irrigation water and the presence of this weed has greatly impaired access to these features. Interviews conducted amongst communities living in the lake region have cited a number of challenges ranging from a rise in certain diseases, challenges in transportation and a very low catch during fishing. In Kenya, Kisumu town, Homabay and Kendu bay are amongst those that have been greatly affected by the spread of the weed.

2.3 Control Measures in managing the spread of Eichhornia crassipes in Lake Victoria.

Water hyacinth has emerged as one of the most aggressive aquatic weeds in the world and many efforts have been put into place to try and reduce its spread to manageable levels. Biological and chemical methods are in trial to see if the weed can be eradicated. Debate has arisen as to the best method of control against the invasive weed. Governments have tried the use of mechanical means and herbicides to eliminate the weed but that too has attracted a lot of criticism from the local environmental authorities. It relates to the effects the methods will have on both the environment and aquatic life. Scientists have come up with biological means of controlling the weed. Dr. Mic Julien, the CSIRO biologist who was leading the assault on the Sepik River, took before-and-after photographs from PNG to show African authorities what could be achieved. He also argued that bio-control offered the only long-term, sustainable answer. He came up with weevils which could help eradicate the aquatic weed. Ugandan and Kenyan scientists travelled to Australia for training in biological control techniques while Dr. Julien and a colleague, Dr. Tony Wright ran a course in Kenya to teach local communities how to raise the Neochetina weevils in drums and tanks. The first weevils were released onto water hyacinth on Lake Victoria off Uganda and Kenya in 1997 while official attention was still fixed on the continuing debate over herbicides and harvesters. The weevils kill the plant by feeding on the leaves. When the population is high this alone can destroy the leaves. But more importantly, larvae tunnel in the petioles (leaf stalks) and into the crown of the plant, destroying the growing points. When severe, the damage allows water to enter the plant and secondary rotting occurs. The combined damage reduces the plant's ability to flower, set seed, send out off-shoots and replace damaged leaves. Under heavy attack the plants rot and become water logged and eventually sink. Members of the genus Neochetina are semiaquatic weevils that feed only on species of Pontideraceae. The Kenya Agricultural Research Institute (KARI) claims that the more than 200,000 weevils had been released into the lake to feed on the weed and had succeeded in reducing its spread, but the residents as well as a visit to the lake by Africa news confirm that little has been achieved. (Africa news, 2009).

2.4 Potential medicinal value of Eichhornia crassipes

Research on the phytochemical and antibacterial properties of Eichhornia crassipes in Lake Victoria has not been fully exploited although its ecological effects have been studied in detail. The effects of the plant have been so profound that scientists have diverted many efforts in evaluating possible beneficial uses of the plant which can be commercially utilized and this has been directed specifically to identifying the potency of the plant for medicinal use. This plant is known to cause major ecological and socio economic changes (Murphy and Villamagna, 2009). Although it is a most obnoxious weed, it possesses nutritionally important compounds like phenolics, flavonoids, glutathione and many metabolites (Malik, 2007). Several phenalenones have been isolated from the ethyl acetate extract of the plant (Tamokou et al, 2012). Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins. Alkaloids and flavonoids have been used as antiviral, antibacterial and anticancer agents (Pandey & Karanwal, 2011). The uses of plant derived products as disease control agents have been studied, since they tend to have low toxicity to mammals, less environmental effects and wide public acceptance (Lee et al, 2007) Studies done by Aboul-Enein et al proved that the plant has antibacterial effects especially against the gram positive and gram negative bacteria. The study showed that it was about 50% as potent as tetracycline. They were also able to determine antifungal activities especially by the crude extracts especially against Candida albicans. Chandran et al., 2013 were able to determine the antibacterial activity of different extracts in accordance with the disc diffusion method. Kumar et al., 2014 in a study done were able to identify antimicrobial activity of plant extracts against gram positive Bacillus cereus, Streptococcus mutants and gram negative Proteus vulgaris and Salmonella typhi ,Bordetella bronchiseptica all determined by Kirby-Bauer disc diffusion method. They were able to identify considerable antibacterial activity against the gram negative bacteria some gram positive bacteria exhibited resistance towards the plant extracts. Research has further been directed at determining the phytochemical composition of the plant which could serve as potent therapeutic agents. These are due to the secondary metabolites that the plants exhibit. The medicinally useful bioactive constituents belonged to alkaloids, flavonoids, phenolic, essential oils and polyphenols (Huv et al, 2000). Alkaloids play an important metabolic role and control development in living systems

(Aniszewsk, 2007). They were also involved in protective function in animals and used as medicine, especially the steroidal alkaloids (Hendriks et al ,1997).Water hyacinth has been studied before and it has been determined that it contains a substantial amount of phytochemical compounds. This plant is endowed with many potent phytochemicals like flavonoids, tannins, terpenoids, sapiens, cardiac glycosides, quinolones and many others. Therefore, we have to exploit the potential use of this plant which possesses high therapeutic value in management of common infections, especially bacterial infections.

3.0 MATERIALS AND METHODS

3.1 Sample collection, authentication and preparation.

Fresh water hyacinths were collected from Lake Victoria with the help of local people and the identification done at the Department of Biological Sciences at Egerton University. The root portion was removed and the plant washed thoroughly to free any debris, washed several times with tap, distilled and sterilized water then air dried. The rinsed leaves were dried in an oven at a temperature of 160°C for 1 hour and shade dried. The dried leaves of each plant were ground, using a motor and pestle, to obtain a powered form. The powdered form of these plants were stored in airtight glass containers and protected from sunlight until required for analysis.

3.2 Preparation of aqueous extracts of plant samples

The aqueous extract of each plant sample was prepared by soaking 10g of the powdered samples in 100ml of distilled water for 24 hours. The extract was then filtered using filter paper or Whatman filter paper. The powdered samples was soaked in dilute water with a ratio of 1:10 w/v (plant extract to solvent) and extracted for 24 hours at room temperature. The extract was stored for future use at -20◦C.

3.3 Qualitative analysis of the phytochemical constituents of the plant extracts.

3.3.1 Test for phlobatannins

10ml of the aqueous plant was boiled in 1% aqueous hydrochloric acid in a conical flask or test tube. Formation of red colored precipitate was to confirm a positive result.

3.3.2 Test for terpenoids

An amount of 0.8 g of selected plant sample was taken in a test tube, then 10 ml of methanol poured into it, shaken well and filtered to obtain 5 ml extract of plant sample. Then 2 ml of chloroform was mixed in the extract of selected plant sample and 3 ml of sulphuric acid was added in the selected sample extract. Formation of reddish brown color was to indicate the presence of terpenoids in the selected plants.

3.3.3 Test for flavonoids

For the confirmation of flavonoid in the selected plants, 0.5 g of each selected plant extract was added in a test tube with 10 ml of distill water, 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of 1ml concentrated H_2S0_4. Indication of yellow color showed the presence of flavonoid in each extract

3.3.4 Test for alkaloids

0.2 g of the selected plant samples was added in each test tube and 3 ml of hexane mixed in it, shaken well and filtered. 5 ml of 2% HCl was taken and poured into a test tube having the mixture of plant extract and hexane. The test tube having the mixture was heated, filtered and a few drops of picric acid added into the mixture. Formation of yellow color precipitate indicated the presence of alkaloids.

3.4 Determination of Antibacterial Activity of the different plant extracts.

The different plant extracts were prepared and tested for their activity against four bacterial species (Gram positive bacteria: Bacillus subtilis, Gram negative bacteria: Escherichia coli, Salmonella typhimurium and Staphylococcus aureus). Strains were obtained from Biological Sciences Department, Faculty of Science, Egerton University. Antibacterial activity was screened by using the disc diffusion bioassay and the diameter of inhibition zones was compared with those obtained by the standard antibacterial agent; gentamicin. The bacterial isolates of Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium were first grown in a nutrient broth for 24 hours before use. 500µl of the sub culturedbroth after preparation of 0.5 McFarland solutions was transferred to the sterile petri plates containing Mueller Hinton Agar and spread evenly to achieve uniformity. Sterilized filter paper discs (6mm) saturated with solutions of gentamicin was used as a positive control. After preparation of a bacterial lawn on the surface of the petri dish, a well was created within the agar. The different concentrations of the aqueous extract were added to the wells. The concentrations used were in the ratio of 10>7.5>5.0>2.5. The plates were then incubated for 24 hours at a temperature of 37°C in a CO2 incubator. The diameter of the clear inhibition zones surrounding the well saturated with the aqueous extract were taken as a measure of the inhibitory power of the sample against the particular bacterial organisms. Standard antibacterial agents were used as positive controls (gentamicin).

3.4.1 Calculations for the different concentrations conducted.

10g of the plant extract were dissolved in 100mls of dilute water. The original concentration of the aqueous extract was 10 To determine the different concentrations the formula applied was; (Required concentration× Required volume)÷Original concentration Starting with 7.5;

7.5= (7.5×100)/10

75mls Therefore, 75 ml of the aqueous extract was derived from the original concentration and added to the mark of 100.

5.0;
5.0= (5.0×100)/10
50mls

50 ml of the aqueous extract was derived from the original concentration and added to the mark of 100

2.5;
2.5= (2.5×100)/10

25mls 50 ml of the aqueous extract was derived from the original concentration and added to the mark of 100

4.0 RESULTS AND DISCUSSION

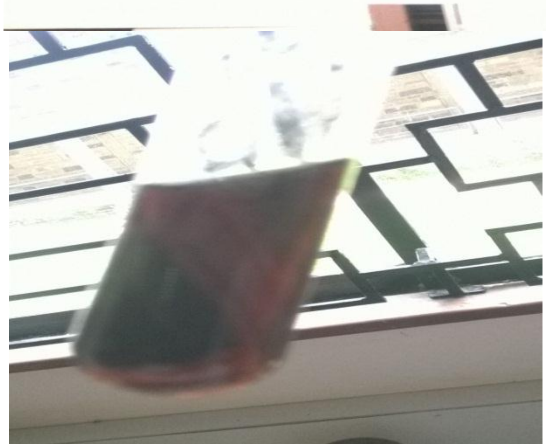
In the qualitative analysis of the phytochemicals, the plant extract tested indicated presence of different compounds. It is an indication of the presence of a few secondary metabolites in the plant Eichhornia crassipes. Kandukuriet et al reported presence of triterpenoids, and alkaloids.

Preliminary phytochemical screening of different extracts of Eichhornia crassipes

Test	Aqueous Extract
Phlobatannins	-
Terpenoids	+
Flavonoids	+
Alkaloids	+

Antibacterial Activity of Eichhornia crassipes

	Types of Isolates and Diameter of Zone of inhibition(mm)			
	E.coli	S.typhimurium	B.subtilis	S.aureus
Concentration				
10	0	0	9	8
7.5	0	0	0	6
5.0	0	0	0	0
2.5	0	0	0	0



Flavonoids
Terpenoids

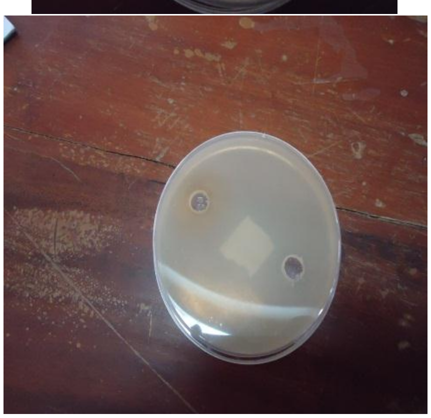
Alkaloids

Bacillus subtilis10Bacillus subtilis 7.5 Bacillus subtilis 5.0

Bacillus subtilis 2.5 Escherichia coli 2.5
Escherichia coli 5.0

Escherichia coli 7.5 Escherichia coli 10
Salmonella typhimurium 5.0

Salmonella typhimurium 7.5 Salmonella typhimurium 10.0
Salmonella typhimurium 2.5



Staphylococcus aureus 2.5 Staphylococcus aureus 5.0
Staphylococcus aureus 7.5

Staphylococcus aureus 10.0

In the study, the activity of the four bacterial isolates was determined by the disc diffusion method. The aqueous extracts, though low, showed mild inhibitory effects on Salmonella typhimurium, Bacillus subtilis, and Staphylococcus aureus. There was no activity against Escherichia coli.

5.0 CONCLUSION

Phytochemicals are a diverse and large group of chemical constituents of natural origin. The study done indicates that E. crassipes has different types of the phytochemicals. The different composition could be attributed to the antimicrobial properties against some selected species of bacteria.

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