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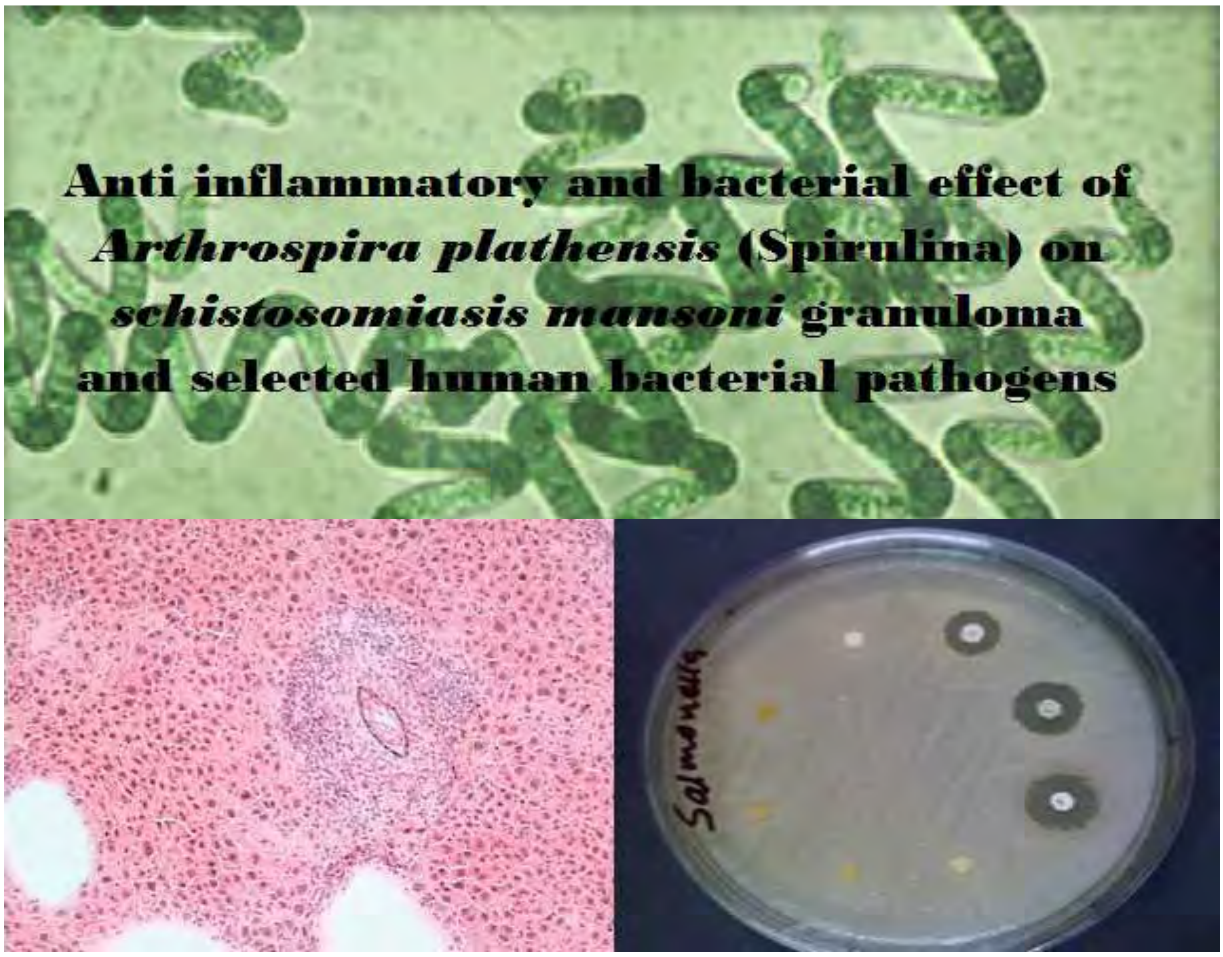

By

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February, 2011

Anti inflammatory and bacterial effect of *Arthrospira plathensis* (Spirulina) on *Schistosomiasis mansoni* and Selected Human Bacterial Pathogens

M. Sc THESIS

A Thesis Submitted to the School of Graduate Studies, Addis Ababa University in Partial Fulfillment of the Requirements for Masters of Science Degree in Biomedical Sciences

Under the supervision of Mekuria Lakew, Associate Professor of Immunoparasitology, in Department of Biology, Faculty of Science, Addis Ababa University.

DECLARATION

Investigator: Abraham Sileshi

Signature: _____

Date of Submission: _____

“This thesis is my own work, has not been presented as a thesis work for a degree in this or any other University and that all sources of material used for the thesis have been duly acknowledged”.

This thesis has been submitted for examination with my approval as University adviser.

Name: Mekuria Lakew (Assoc. Prof. of Immunoparasitology)

Signature: _____

Date: _____

Acknowledgement

My earnest thanks go to my Advisor and mentor, Dr. Mekuria Lakew for his close supervision, very kind assistance, and dedication to science have been a source of inspiration to what I may aspire to do in life for decades to come. Therefore the successes in the work are all due to the concerns and very encouraging assistance of Dr. Mekuria Lakew.

My special thanks and appreciation also goes to the biomedical lab technicians of Biology department, especially W/o Amelework Eyado, for her very consistent help in all my work. My friends; Solomon Taye, Mahlet Habtu, Bereket Haylemeskel, Nigatu Tuasha and Gebeyehu Yihenew I want to thank them for their support in some of the lab works and their helpful ideas.

I would also like to thank Yekatit 12 General Hospital bacteriology laboratory staffs for their support in providing me with pure culture of human pathogenic bacteria (*S. dysenteriae, C. Freundii, P. aeruginosa* and *S. epidermidis)* which were used to test the antimicrobial effect of spirulina.

Finally my special appreciation is also extended to my parents Ato Sileshi Awoke and W/o Zenebech Demisse for their encouragement, financial support for my study and living costs.

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List of abbreviation

AA - Arachidonic acid

ALA - γ linolenic acid

BHA - Butylated hydroxyanisole

BMAA - β-*N*-methylamino-l-alanine

Ca-SP - Calcium spirulan

CC14 - Carbon tetrachloride

COX-2 - Cyclooxygenase –2

C-PCs - C-phycocyanins

DEN - Diethyl nitrosamine

DHA -Docosahexaenoic acid

DMBA -7, 12 –dimethylbenz [α]anthracene

DMSO - Dimethyl sulfoxide

DNP - 2, 4-Dinitrophenol

DS - Dextran sulfate

ED ₋ Exhibition dose

EPA - Eicosapentaenoic acid

GAPDH - Glyceraldehydes phosphate dehydrogenase

GLA - Gamma linoleic acid

GMP - Good manufacturing practice

GST - Glutathione *S*-Transferase

HWE - Hot water extract

Ir V-5 - Irradiated vaccine no. five

LST - Land Surface Temperature

NF-kappa B - Nuclear Factor-KappaB

PBMC - Peripheral blood mononuclear cells

PCNA - Proliferating cell nuclear antigen

PgE2 - Prostaglandin E2

PLA2 - Phospholipase A2

PRV - Pseudo rabies virus

PUFAs - Polyunsaturated fatty acids

PZQ - Praziquantel

RA - Radiation attenuated

SDA - Stearidonic acid

SEA - Soluble egg antigens

Sm14 - Schistosoma *mansoni* fatty acid-binding protein 14

SmEA - *Schistosoma mansoni* egg antigen

SOD - Superoxide dismutase

SSPE - Subacute sclerosing panencephalitis virus

SWAP - Soluble adult worm antigen preparations

TPI - Triose phosphate isomerase

VSV - Vesicular stomatitis virus

Abstract: *Arthrospira platensis* (spirulina) is multicellular filamentous blue green alga that is abundantly found in lakes of high salinity in Ethiopia. Several studies have shown that it has importance in human health and nutrition. The health benefits of spirulina relies mainly on tits anti-inflammatory, anti- oxidant and antimicrobial properties. In the current study *spirulina* was studied for its anti-inflammatory and antimicrobial effects using change in size and number of *S. mansoni* induced granuloma in infected mice and inhibitions of known human bacterial pathogens (*S. typhi*, *S. dysenteriae*, *E. coli*, *S. aureus*, *S. epidermidis*, *C. freundii*, and *P. aeruginosa*) as parameters. *S. mansoni* is a parasitic helmintic infection with high public health importance next to malaria, HIV-AIDS and TB especially in sub Saharan countries. Chronic inflammatory response in response to *S. mansoni* egg trapped in liver tissue is the main pathology responsible for the morbidity and mortality associated with the disease. Methodologies used in the study involve *S. mansoni* infection of Swiss albino mice, treatment of experimental animals with spirulina at two doses 100 and 400 mg/kg through oral, rectal, and oral/rectal. Animal dissection and histological staining of the liver to measure granuloma diameter, and finally antibiotic sensitivity test using disk diffusion method was done. Independent *T*. test comparison of the measurements form the anti-inflammatory test showed that the size of granuloma around an egg from spirulina treated compared to controls was significantly reduced. In association with this, comparison between different treated groups showed that treatment through oral route at a dose of 400 mg/ kg scores the highest percentage reduction both in granuloma size (59.53%) and granuloma number (72%). Result from the antimicrobial study showed that all test organisms except *S. typhi* were susceptible for the water suspension and extracts of spirulina unlike that of the Methanol extract. The possible explanations are discussed. In conclusion and recommendation the anti-inflammatory and antimicrobial effect of spirulina are summarized and future direction of research indicated. In association with this spirulina's role in child health care and fight against child malnutrition and morbidity due to infection is underscored.

Key words: - Spirulina, *S. mansoni*, Granuloma, Anti-inflammatory, Antimicrobia

I. INTRODUCTION

1. Spirulina

1.1. General characteristics

Spirulina is multicellular filamentous blue - green algae that can be rod- or disk shaped. Their main photosynthetic pigment is phycocyanin, which is blue in color. These bacteria also contain chlorophyll A and carotenoids. Some contain the pigment phycoythrin, giving the bacteria a red or pink colour. Spirulina are photosynthetic and therefore autotrophic. Spirulina reproduce by binary fission. The helical shape of the trichomes is characteristic of the genus and is maintained only in a liquid environment or culture medium. The presence of gas-filled vacuoles in the cells, together with the helical shape of the filaments, result in floating mats. The trichomes have a length of 50 to 500 μm and a width of 3 to 4 μm. Cyanobacteria have a cell wall similar to that of Gram-negative bacteria: they contain peptidoglycan, a lysozyme-sensitive heteropolymer that confers shape and osmotic protection to the cell. The body surface of spirulina is smooth and lacks cell wall so it is easily digestible by simple enzymatic systems. (Habib *et al*. 2008).

Spirulina is ancient organism originating some 3.5 billion years ago that has established the ability to utilize carbon dioxide dissolved in seawater as a nutrient source for their reproduction.

1.1.1. Taxonomy

In 1989, these micro-organisms were separately classified into two genera *Spirulina* and *Arthrospira*; this classification is currently accepted (Tomaselli and Tredici, 1996; Sánchez *et al*., undated). *Arthrospira maxima* and *A. platensis* are the most important species in this genus and among these existed taxonomic differences in filaments, vacuoles and external cover or capsule regularity of each filament (Tomaselli, 1997).

1.1.2. Natural habitat, source and growth

1.1.2.1. Ecology

Besides Lake Texcoco (Mexico), the largest spirulina lakes are in Central Africa around Lakes Chad and Niger, and in East Africa along the Great Rift Valley. Lakes Bodou and Rombou in Chad have a stable monoculture of spirulina dating back centuries. It is also a major species in Kenya's lakes Nakuru and Elementeita and Ethiopia's lakes Aranguadi and Chitu (Habib *et al.* 2008). Spirulina thrives in alkaline lakes where it is difficult or impossible for other micro organisms to survive. In natural lakes, the limited supply of nutrients usually regulates growth cycles. New nutrients come from either an upwelling from inside the water bodies, influxes of nutrients from rivers or from pollution. The algae population grows rapidly, reaches a maximum density, and then dies off when nutrients are exhausted. A new seasonal cycle begins when decomposed algae release their nutrients or when more nutrients flow into the lake. Spirulina is found in soil, marshes, freshwater, brackish water, seawater and thermal springs. Alkaline, saline water (>30 g/l) with high pH (8.5–11.0) favor good production of spirulina, especially where there is a high level of solar radiation at altitude in the tropics. *Spirulina platensis* and Spirulina *maxima* thrive in highly alkaline lakes of Africa and Mexico where the cyanobacteria population

is practically mono-specific. The higher the pH and the conductivity of the water, the greater is the likely predominance of *Spirulina* spp. This is the case in the lakes of the Rift Valley of eastern Africa, where pH can reach values close to 11 and sodium carbonate is abundant. *Spirulina platensis* was isolated from waters containing from 85 to 270 g of salt per liter, and optimum growth occurred between 20 and 70 g of salt per liter. A relatively high cytoplasmic pH (4.2 to 8.5) may account for the ability of this micro-organism to utilize ammonia as a source of nitrogen at high alkaline pH values (Sasson, 1997).

1.1.2.1. Culture

Spirulina like most cyanobacteria is an obligate photoautotroph, i.e. it cannot grow in the dark on media containing organic carbon compounds. It reduces Carbon Dioxide in the light and assimilates mainly Nitrates. The main assimilation product of spirulina photosynthesis is glycogen. Spirulina shows an optimum growth between 35 and 37 °C under laboratory conditions. Outdoors, it seems that an increase in temperature up to 39 °C for a few hours does not harm the blue-green alga, or its photosynthetic ability. Thermophilic or thermotolerant strains of spirulina can be cultivated at temperatures between 35 and 40 °C. Such a property has the advantage of eliminating microbial mesophilic contaminants. The minimum temperature at which growth of spirulina takes place is around 15 °C during the day. At night, spirulina can tolerate relatively low temperatures. The resistance of spirulina to ultraviolet rays seems to be rather high (Richmond, 1986).

1.2. Beneficial Aspects of spirulina

1.2.1. Nutritional

The concentrated nutritional profile of spirulina occurs naturally, so it is ideal for those preferring a whole food supplement to artificial nutrient sources. Spirulina has a unique blend of nutrients that no single source can provide. It contains a wide spectrum of nutrients that include B-complex vitamins, minerals, good quality proteins, gamma-linolenic acid and the super antioxidants, beta-carotene, vitamin E and trace elements Anitha *et al*., (2006) and whole spectrum of natural mixed carotene and xanthophylls phytopigments (Habib *et al*., 2008).

According to Habib *et al.*, (2008) the basic biochemical composition of spirulina can be summarized as follows:

Protein: Spirulina contains unusually high amounts of protein, between 55 and 70 percent by dry weight, depending upon the source (Phang *et al*., 2000). It is a complete protein, containing all essential amino acids, though with reduced amounts of methionine, cystine, and lysine, as compared to standard proteins such as that from meat, eggs, or milk; it is, however, superior to all standard plant protein, such as that from legumes (Clement *et al*., 1967).

Essential fatty acids: Spirulina has a high amount of polyunsaturated fatty acids (PUFAs), 1.5–2.0 %, of 5–6 % total lipid. Spirulina is particularly rich in γ-linolenic acid (36 % of total PUFAs), and also provides γ linolenic acid (ALA), Linoleic acid (LA, 36 % of total), Stearidonic acid (SDA), Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and Arachidonic acid (AA) (Pascaud, 1993). These are integral components of neural membrane phospholipids whose

deficiency in humans could affect neurotransmission, membrane-bound enzyme and ion channel activities

Vitamins: Spirulina contains vitamin B1 (Thiamine), B2 (Riboflavin), B3 (Nicotinamide), B6 (Pyridoxine), B9 (Folic acid), B12 (Cyanocobalamin), vitamin C, vitamin D and vitamin E (Clement et al., 1967).

Minerals: Spirulina is a rich source of potassium, and also contains calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium and zinc (Johnson and Shubert, 1975).

Photosynthetic pigments: Spirulina contains many pigments including chlorophyll a, xanthophyll, betacarotene, echinenone, myxoxanthophyll, zeaxanthin, canthaxanthin, diatoxanthin, 3-hydroxyechinenone, beta-cryptoxanthin, oscillaxanthin, plus the phycobiliproteins c-phycocyanin and allophycocyanin (Mary *et al*., 2010). Detailed biochemical composition analyses have been conducted of spirulina grown either under laboratory conditions, collected in natural condition or in mass culture system using different agroindustrial waste effluent. This was found to vary in response to the salinity of the growing medium. Vonshak *et al*. (1996) reported that salt-adapted cells had a modified biochemical composition with a reduced protein and chlorophyll content, and increased carbohydrate content.

1.2.2. Therapeutic and Immunomodulatory

Spirulina has been considered as a supplement in human and animal food. Spirulin is also being widely studied for its possible pharmacological properties. Over the last few years, those properties have been confirmed and additional pharmacological properties found by different workers: antiviral (Hernández-Corona *et al.,* 2002), anti-bacterial (Ozdemir *et al.,* 2004), anti-platelet (Hsiao *et al.,* 2005), anti-cardiotoxic (Khan *et al.,* 2005a and Khan *et al.,* 2005b), hypocholesterolemic (Nagaoka *et al.,* 2005) anti-nephrotoxic (Khan *et al.,* 2006), and anti-hepatoxic (Mohan *et al.,* 2006) effects. In other studies, spirulina has been shown to prevent Cataracts (Haque and Gilani, 2005), acute allergic rhinitis (Mao *et al.,* 2005), Cerebral ischemia (Khan *et al.,* 2005a), (Khan *et al.,* 2005b) and (Wang *et al.,* 2005), vascular reactivity (Mascher *et al.,* 2005) and experimental Parkinson's (Chamorro *et al.,* 2006), and spirulina has also been shown to be effective against Cadmium Jeyaprakash and Chinnaswamy, (2005) and Arsenic toxicities (Saha *et al.,* 2005).

1.2.2.1. Immunomodulatory effect

It is now well established that nutrient deficiency is associated with consistent changes in immune responses such as number of T-cells, lymphocyte response to mitogens and antigens, phagocyte function, secretory IgA antibody response, compliment activity, NK cell activity, and production of cytokines. Excesses nutrient is also associated with impaired immune function. For example, dietary intake of large quantities of fats impairs immune response. In addition, the immune system can be positively or negatively affected by certain phytochemicals found in

conventional foods and foods derived from other plants like algae, mushrooms, and some herbs (Amha, 2002).

Recently, Spirulina has been speculated to be associated with modulation of the host immune system. *S. plantensis* is surmised to potentiate the immune system leading to suppression of cancer development and viral infection (Hirahashi *et al.,* 2002). In mice, Spirulina enhanced IL-1 and antibody production (Hayashi *et al.,* 1994 and Hayashi *et al.,* 1998). Oral administration of a hot water extract of Spirulina was shown to activate the human innate immune system by augmenting the production of interferon and cytolysis in human NK cells (Hirahashi *et al.,* 2002).

Several published studies have shown significant therapeutic effects of Spirulina or its extracts on animals and on humans. Several other studies have also indicated possible effect of spirulina in immunomodulation of host immune system. Among this Yang *et al.,* (1997) and Kim *et al.,* (1998) have indicated the inhibitory effect of spirulina on mast cell- mediated immediate – type allergic reactions by suppressing the release of histamine from rats peritoneal mast cells in a dose dependent fashion. In addition Qureshi and Ali (1996) demonstrated that spirulina exposure enhances the phagocytic function of macrophages in felines showed an increase in secondary antibody response as well as an increase in natural killer cell activity (Qureshi *et al.,* 1996). Saeki *et al*., (2000) reported the results of an investigation on the adjuvant effect of a hot water extract of *Spirulina* in the regression of tumors in tumor-bearing mice. Mao *et al.,* (2000) reported the results of a study that adds evidence for the immunomodulatory effects of *Spirulina*. Using human peripheral blood mononuclear cells (PBMC), they demonstrated that *Spirulina*

stimulated the secretion of Interlukin (IL)-1β, IL-4, and interferon (IFN)-γ to nearly 2.0, 3.3, and 13.6 times basal levels, respectively.

A high molecular weight polysaccharide fraction (Immulina) from *Spirulina* activator NF-kappa B and induces both IL-1 β and TNF-α mRNAs in THP-1 human monocytes (Premalatha *et al.*, 2006). Spirulina inhibits mast cell-mediated immediate-type allergic reactions *in vivo* and *in vitro*. Moreover, spirulina (10 μg/ml) had a significant inhibitory effect on anti-DNP IgE-induced tumor necrosis factor-α production. (Hyung *et al*., 1998).

1.2.2.2. Anti-inflammatory property

Although *Spirulina* can stimulate the production of some of the major proinflammatory cytokines, TNF-*α* and IL-1, there have been several investigations on its ability to inhibit inflammatory reactions. Even more data are available on the anti-inflammatory activities of phycocyanin. There are many reports that the whole spirulina and its Phycocyanin component which constitutes 20% of its dry and is a biliprotein found in blue green algae have anti-inflammatory effect in some animal models of inflammation (Greshwin and Belay, 2008).

Ricardo et al., 1999 have indicated the anti inflammatory effect of C- phycocyanin on acetic acid induced colitis in rats. They have also shown that phycocyanin inhibited inflammatory cell infiltration and reduce colonic damage to some extent. In another study by Ramirez et *al.*, (2002) spirulina have resulted in reduced allergic inflammatory response and histamine reslese from isolated rat mast cells previousely sensitized with ovalbumin in a dose dependent manner. Similarly, phycocyanin also exerted anti- inflammatory and anti- arthritic effects in Zymosan induced atrherithis in mice (Ramirez *et al.*, 2002). Furthermore the biliprotein also inhibited edema and leukoterine B4 and prostaglandin E2 generation in arachidonic acid induced ear edema in mice (Romay, *et al*., 2000). Another study by Recardo *et al* (2003) have indicated the

potential hepatoprotective and anti inflammatory effect of microalgae phycocyanin in galactose amine induced acute liver damage in rats.

1.2.2.3. Anti- oxidant property

There has been increasing attention in recent years to exploit effective natural antioxidants as replacements for synthetic food additives. Natural antioxidants in foods may have great benefits because they have anti-carcinogenic effects and inhibit biologically harmful oxidation reactions in the body (Imlay and Linn, 1988). Many algal products may improve the nutritional quality of foods because of their ability to prevent oxidative damage to cells (Demmig-Adams and Adams 2002). *Spirulina platensis* or *Arthrospira platensis* is a multicellular filamentous cyanobacterium with up to 40% of its total proteins as C-phycocyanins (C-PCs). Several articles have described that PCs have antioxidant and anti-inflammatory properties Romay *et al.* (1998a) and Romay *et al.* (1998b), and further studies have demonstrated that PCBs, which have structural similarities to bilirubins, act as antioxidants and play an important role in antioxidant activities (Bhat and Madyastha, 2000; Hirata *et al.* 2000). In addition to C-phycocyanins (C-PCs) *Spirulina* contains phenolic acids, tocopherols and ß-carotene which are known to exhibit antioxidant properties.

This antioxidant property of spirulina and its components have been proved with different clinical as well as experimental investigations. In one of the earliest studies Manoj *et al*.(1992) reported that the alcohol extract of *Spirulina* inhibited lipid peroxidation more significantly (65% inhibition) than the chemical antioxidants like α-tocopherol (35%), BHA (45%), and β-carotene (48%). The water extract of *Spirulina* was also shown to have more antioxidant effect (76%) than gallic acid (54%) and chlorogenic acid (56%). An interesting aspect of their findings was that the water extract had a significant antioxidant effect even after the removal of polyphenols.

In what appears to be the first report on antioxidant and anti-inflammatory properties of c-phycocyanin Romay *et al*. (1998) showed that phycocyanin was able to scavenge hydroxyl (IC50 = 0.91 mg/ml) and alkoxyl (IC50= 76 µg /ml) radicals with activity equal to 0.125 mg/ml of dimethyl sulfoxide (DMSO) and 0.038 µg/ml of trolox, specific scavengers of those radicals respectively. Phycocyanin also inhibited liver microsomal lipid peroxidation (IC50=12 mg/ml). It is interesting to note that the oxygen-scavenging activity of c phycocyanin was only 3 times lower than that of superoxide dismutase (SOD). The addition of SOD to the phycocyanin did not alter the antioxidant activity of the phycocyanin, suggesting a different mechanism of action. Further studies by the same group revealed the anti-inflammatory activity of phycocyanin in some animal models of inflammation.

The antioxidant effects of two fractions of a hot water extract of *Spirulina* were studied by Zhi-gang *et al*., (1997) using three systems that generate superoxide, lipid, and hydroxyl radicals. Both fractions showed significant capacity to scavenge hydroxyl radicals (the most highly reactive oxygen radical) but no effect on superoxide radicals. One fraction had significant activity in scavenging lipid radicals at low concentrations. Another study by Hanaa *et al*., (2009) has concluded that algal phenoloc extracts could protect the liver against CC14 induced lipid peroxidation *in invitro*. Other recent study by Mark *et al* (2010) indicates that phycocyanorubin is a proximal mediator of PCB'S impact on NADPH oxidase activity.

According to Reddy *et al.*, (2000) c-phycocyanin from *Spirulina platensis* is a selective inhibitor of cyclooxygenase –2 (COX-2) with a very low IC50 COX-2/IC50 COX- 1 ratio (0.04). Interestingly, their study showed that the IC50 value obtained for COX-2 inhibition by phycocyanin was much lower (180 nM) as compared to those for celecoxib (255 nM) and rofecoxib (401 nM), the well-known selective COX-2 inhibitors. The apoprotein component of

phycocyanin was responsible for the inhibition of COX-2 since reduced phycocyanin and phycocyanobilin were found to be ineffective. Therefore, spirulina extract enrich in phenolic antioxidants may provide a promising source of natural antioxidant.

1.2.2.4. Anti- microbial and Probiotic effect of spirulina

It has long been known that normal flora of intestine have many beneficial aspects some of which include; prevention of constipation, preventing diarrhea, stimulation of immune system, lactose intolerance, reduction in cholesterol level in blood and prevention of cancer. Apart from these therapeutic benefiters, Probiotics also offer protection against many opportunistic human pathogens. The stimulatory effect of *Spirulina platensis* was studied on three lactic acid bacteria. The addition of dry biomass of *S.platensis* at various concentrations of 1mg, 5 mg, 10 mg/ml promoted growth of *Lactobacillus acidophilus* up to 171.67% and 185.84% respectively at pH 6.2. The growth of other strains were also enhanced. Simultaneously the antibacterial activity of *S. platensis* was done against three gram negative and three gram positive bacteria. A maximum activity was shown against *Proteus vulgaris*. Other pathogenic bacterial growth was also inhibited. The results show the probiotic efficiency of *S.platensis* for lactic acid bacteria and also a potent antibacterial activity against human pathogenic bacteria (Dola *et al*., 2009). Similarly Tsuchihashi *et al.*(1987) as sited in Amaha, 2002 found that an intake of *Spirulina* at 5% of the diet increased the population of *Lactobacillus* in the caecum of rats by 3 times over a control group of rats not fed *Spirulina*. Parada *et al.* (1998) as sited in Amaha, 2002 have reported a stimulatory effect of extracellular products from algae on lactic acid bacteria including *Lactococcus lactis, Streptococcus thermophilus, Lactobacillus casei, Lactobacillus acidophilus,* and *Lactobacillus bulgaricus*.

Apart from the above mentioned Probiotic effect, Some *Spirulina* species also exhibit antibacterial Ozdemir *et al*., (2004), and antiviral activities (Hernandez-Corona *et al*., 2002). *Spirulina* as many other cyanobacteria species have the potential to produce a large number of antimicrobial substances, such as organic acids, vitamins, and phytohormones, that have shown antimicrobial activity against *Bacillus subtillis*, *Streptococcus aureus*, *Saccharomyces cerevisiae*, and *Candida albicans*. To date, relatively few studies have been undertaken examining the antibacterial activity of *Spirulina* and its extracts. One of them, de Mule *et al*. (1996) as cited in Gershwin and Belay, (2008) tested crude methanolic and aqueous extracts of *S. platensis* on the growth of three microorganisms. In *Candida albicans*, growth was inhibited 17.6% by aqueous extracts and 7.8% by methanolic extracts; in *Staphylococcus aureus* and *Lactococcus lactis* there was growth promotion by both extracts, ranging from 7.5 to 14.7%. Ozdemir *et al.* (2004) demonstrated that extracts of *S. platensis* were active against four Gram-positive and six Gram-negative bacteria and a yeast, *Candida albicans* ATCC 10239. In this study, the methanol extracts of *S. platensis* (comparable to tobramycin (10 μg/disc), especially against *S. faecalis*, *S. epidermidis*, and *C. albicans*) showed more potent antimicrobial activity than the ethanol, hexane, acetone, and chloroform extracts. Another study examined ethanol, acetone, chloroform, hexane extracts of *S. platensis* against some test microorganisms. Aceton, chloroform, and hexane extracts showed more potent antibacterial activity against *P. aeruginosa* and *S. typhimurium* than ethanol extracts, *S. faecalis*, and *E. coli* for acetone extracts *Mycobacterium smegmatis*, *P. aeruginosa* and *S. aureus* for chloroform extracts, *Proteus vulgaris* for hexane extracts. Antimicrobial activity of different pressure liquid extraction (PLE) fractions of *S. platensis* was tested against *S. aureus* ATCC 25923, *E. coli* TCC 11775, *C. albicans*ATCC

60193, and *Aspergillus niger* ATCC 16404. Data obtained demonstrated that the hexane and petroleum ether extracts were slightly more active than ethanolic extracts. Furthermore, aqueous extracts were inactive against the microorganisms tested. However, *C. albicans* was the most sensitive micro-organism to all *Spirulina* PLE extracts (Mendiola *et al.*, 2007).

1.2.2.5. Anti- cancer effect

According to the possible applications of antigenotoxic drugs in cancer treatment, worldwide interest has converged on a wide variety of plant extracts, food supplements or dietary products (Badary *et al.*, 2007). *Spirulina* besides its other therapeutic effects, it has also been indicated to have anti- cancer or anti- tumor effects. Qureshi *et al*. (1996), Pang *et al*. (1998) and Ismail *et al*. (2006) showed that *Spirulina* or its extracts can prevent or inhibit cancer in humans and animals. *In vitro* studies suggest that polysaccharides of *Spirulina* enhance cell nucleus enzyme activity and DNA repair synthesis. Several other studies have also come up with positive results regarding the anticancer effect of spirulina. Mishima *et al.*,(1998) have elegantly demonstrated inhibition of tumor invasion and metastasis by Calcium Spirulan (Ca-SP), a novel polysaccharide isolated from *Spirulina platensis*. Seven intermittent i.v. injections of 100 μg of Ca- SP in mice caused a marked decrease of lung tumor colonization of B-16-BL6 cells in a spontaneous lung metastasis model. Through investigation on protective effect of spirulina plathensis on the induced mammary tumor in rats by benz (a) anthracine (DMBA) and the proliferation of tumor cells by using immmunohistochemical staining for proliferating cell nuclear antigen (PCNA), Mashael, (2008) have concluded that *S. platensis* as a chemotherapeutic agent that causes apoptosis to tumor cells by reducing the number of malignant cells and resists cancer formation. Similarly Pardhasaradhi *et al*. (2003) who studied the effects that *Spirulina* on rat histiocystic

tumor line. They reported that *Spirulina* is a chemotherapeutic agent that causes apoptosis to tumor cells. On a different study Mashael, (2009), through histopathological and immunohistological examination for proliferation of marker Ki67 in livers tissues of rats given Diethylnitrosamine (DEN) in drinking water, they found spirulina to have protective role by reduction of malignant cells and its proliferation in treated group.

1.2.2.6. Anti- viral property

Apart from studies for its possible anticancer, antibacterial, anti-inflammatory properties, and for several medical conditions such as allergies, ulcers, anemia, heavy-metal poisoning, and radiation poisoning. *S. platensis* has been also studied for its antiviral properties, which seem to be related to its sulfated polysaccharide named Calcium Spirulan (Ca-SP). Since then several other investigations on its antiviral effect have been done.

Hayashi *et al*. (1996) isolated a novel sulfated-polysaccharide, calcium spirulan (Ca-SP) from *Spirulina platensis* that inhibits the replication *in vitro* of several enveloped viruses including Herpes simplex type I (HSV-1), human cytomegalovirus (HVMV), measles virus, mumps virus, influenza A virus, and HIV-1 virus. In a later study Hayashi *et al.* (1996) found that the anti-HIV-1 activity of Ca-SP is comparable to that of Dextran Sulfate (DS; a known potent anti-HIV-1 agent), while its anti-HSV-1 activity was four- to five-fold higher than that of Dextran Sulfate. The anti-HIV-1 activity of Ca-SP or DS was five and four times higher in cultures treated with Ca-SP or DS during infection when compared with that in cultures treated with these substances after infection. Ca-SP was found to be superior to DS in possible therapeutic application because 1) enhancement of viral replication at low concentrations, a usual phenomenon in DS, was not observed with Ca-SP, 2) Ca-SP was found to have a much lower anticoagulant effect than DS, 3)

Ca-SP was found to have a much longer half-life in the blood of mice compared to DS, and 4) Ca-SP was four to five times more effective in inhibiting HSV-1 compared to DS.

In another study by Ayehunie *et al.*(1998) reported that an aqueous extract of *Spirulina* (*Arthrospira*) *platensis* inhibited HIV-1 replication in human T-cell lines, peripheral blood mononuclear cells (PBMC), and Langerhans cells. Depending on the cell type used, therapeutic indices (EC50/IC50) ranged between 200 and 6,000. The extract inactivated HIV-1 infectivity directly when pre-incubated with virus before addition to human T-cell lines. These authors found antiviral activity both in the polysaccharide fraction as well as in a fraction depleted of polysaccharides and tannins.

In support of the above studies a recent study by Armida *et al.*, (2002), on Antiviral activity of a hot water extract (HWE) of a commercial preparation of *Spirulina maxima*, against several viral pathogens a microplate inhibition assay revealed that, the HWE inhibited the infection for: herpes simplex virus type 2 (HSV-2), pseudorabies virus (PRV), human cytomegalovirus (HCMV), and HSV-1, and the 50% effective inhibition doses (ED_{50}) were 0.069, 0.103, 0.142, and 0.333 mg/ml for each virus, respectively. For adenovirus the inhibition was less than 20%, and no inhibition was found for measles virus, subacute sclerosing panencephalitis virus (SSPE), vesicular stomatitis virus (VSV), poliovirus 1 and rotavirus SA-11, at concentrations of 2 mg/ml of the HWE. The highest antiviral activity was for HSV-2, with a selectivity index of 128 (Armida *et al.*, 2002).

1.2.2.7. Other therapeutic effects

Clinical as well as experimental trials have shown that Spirulina could also serve as supplementary cure for many other health complications. For example Anitha *et al*., (2006) have shown that spirulina treatment could cause a decreased activity of glucose -6- phosphatese through pentose phosphate shunt which resulted in a highly reduced glutathione to oxidaized glutathione ratio (GSH/GSSG), which is coupled with conversion of NADPH to NADP. This indicate that Spirulina may produce high NADP+, which results in down regulation of lipogenesis and lower risk of the tissues for oxidation stress and high resistance for diabetes. Accordingly, it can be concluded that spirulina has a beneficial effect on plasma insulin, C-peptide and hexokinase activity. Another investigation by Torres *et al*., (1999) has shown the preventive effect of *Spirulina maxima* on fatty liver development induced by carbon tetrachloride, in the rat. According to Takai *et al.*, (1991) a water-soluble fraction of *Spirulina* was found effective in lowering the serum glucose level at fasting while the water-insoluble fraction suppressed glucose level at glucose loading. Study involving 15 human diabetic patients, a significant decrease in the fasting blood sugar level of patients was observed after 21 days of 2 g/day *Spirulina* supplementation (Mani S, Iyer U 1998 as sighted in Amha, 2002). In a double-blind-crossover study versus placebo, Becker *et al.* (1986) have found that a supplementary diet of 2.8 g of *Spirulina* 3 times d-1 over 4 weeks resulted in a statistically significant reduction of body weight in obese out patients.

1.3. Toxicity associated with spirulina

Conduction of several studies on therapeutic properties of Spirulina, such as hypocholesterolemic, immunological, antiviral and anti-mutagenic, has led to more detailed evaluations such as nucleic acid content and presence of toxic metals, biogenic toxins and organic chemicals. A study by Chamorro *et al.*, (1996) indicates absence or presence at tolerable levels of toxic components according to the recommendations of international regulatory agencies. In animal experiments for acute, sub chronic and chronic toxicity, reproduction, mutagenicity, and teratogenicity the algae did not cause body or organ toxicity. In all instances, the Spirulina administered to the animals were at much higher amounts than those expected for human consumption. On the other hand there is scant information of the effects of the algae in humans. A recent clinical report by Elias *et al.,* (2008) showed the first case of acute rhabidomyolysis after ingestion of *Spirulina* (*Arthrospira platensis*) as a dietary supplement. Similarly other minor side effects have been reported from spirulina including headache, muscle pain, flushing of the face, sweating, and difficulty concentrating. These have been described in people taking 1 g of Spirulina daily. Skin reactions and liver damage have also been reported (Iwasa *et al.*, 2002). Skin reactions and liver damage have also been reported (Iwasa *et al*. 2002).

2. Schistosomiasis mansoni

Human schistosomiasis is a disease of high morbidity and mortality caused by three species of trematodes: *S. mansoni, S. japonicum* and *S. hematobium* that belong to the phylum plathyhelminths, family schistosomatidae. Children, because of their water contact behavior and immune status suffer detrimental effects on their growth and development (WHO, 1997). In sub Saharan Africa, as is in many developing countries, is a high socioeconomic burden and a cause for the death of 28,000 people each year (Simon, 2007).

2.1. Immunopathology of schistosomiasis

2.1.1. Pathogenesis

Most chronic morbidity in schistosomiasis is not due to adult worms but is related to the T- Cell dependent immune response of the host which is directed against schistosomes egg trapped in tissues, mainly in the liver and intestine (Frederico *et al*., 2006). The trapped eggs secret a range of molecules leading to delayed type hypersensitivity like granulomatous inflammation involving eosinophils, monocytes, and lymphocytes (Meeusen, 1999). Granuloma is characterized by cellular deposition and causing severe hepatic portal damage. The lesions of chronic schistosomiasis can be divided into those that deposit (granulomas) mass of egg lesions and those representing systemic cellular immune responses (lymphoreticular proliferation, lesions due to immune complexes (Mark *et al.*, 2007).

A single egg liner granuloma of *S. mansoni* in recently infected host is a sharply contoured , rounded cell aggregate composed in descending order of eosinophils, mononuclear phagocytes, neutrophils, plasma cells and sporadic mast cells. Activated macrophages congregate centrally,

close to the egg shell at times forming multi nucleated large langerhans type giant cells. Lymphocytes and plasma cells occupy mainly a peripheral halo. Fibroblasts appear early and gradually replace all other cell types during the lengthy involution of the granuloma (Iramaya, *et al.,* 2008). Much of the morbidity and mortality associated with this disease is attributable directly to deposition of connective tissue elements in affected tissues.

2.1.2. Immunology

A number of recent studies have considered the immunology of schistosomiasis, including the nature of host innate adaptive responses to schistosomiasis and strategies used by the parasite to manipulate such responses. Much of our understanding of mammalian immune response to schistosomiasis is based on the use of gene-disrupted (knock out) mice and immunization of mice, non human primates, or other mammalian host with UV or γ- irradiated cercarial vaccines, with or without a subsequent challenge infection with non attenuated cercaria (Brunet *et al.*, 1998).

In general these studies have established that T-cell mediated immunity is fundamental to acquired resistance to schistosomes (Iramaya *et al.,* 2008). Much of these protection shows to be mediated by activated macrophages and together with study of cytokines suggest that a vaccine that induced macrophage activating Th-1 cytokines (γ- interferon (IFN-γ) and IL-12) may be beneficial in preventing schistosomiasis (Donald, *et al.,* 2008). However, repeated vaccination with irradiated cercaria produced incremental increase in Th-2 mediated (IL-4, and IL-5 predominance) protection which was transferable to non vaccinated animals (Luciana *et al.,* 2001). Studies using B-cell deficient and cytokine deficient mice demonstrate that successful

anti schistosome vaccination required induction of strong Th-1 and Th-2 responses (Donald, et *al.*, 2008). Following infection by normal or radiation attenuated cercaria, the predominant early immune response was Th-1 mediated and aimed at the adult worm. Following egg deposition in tissues, the Th-1 response was diminished being replaced by a prominent Th-2 mediated phase (Luciana *et a*l., 2001). Indeed it appears that egg antigens are able to directly suppress Th-1 response, a phenomenon which also occurs in humans. The Th-2 response results in an increase serum IL-5, massive bone and blood eosinophilia, and a granulomatous response aimed at the egg, resulting in collagen deposition, tissue fibrosis and disease manifestation of Schistosomiasis (Mark *et al*., 2007). The precise role of eosinophils in the mouse model of infection remains undetermined. The complexity of immune regulation and T- Cell regulation in schistosome infection in mice model is recently highlighted a specific role for CTLA- 4+ but not CD-25+ cells in the regulation of Th-2 responses in helminth infection (Iramaya, *et al*., 2008). Furthermore, where as cytokine interplay during the development of protective immunity to the radiation attenuated (RA) schistosome vaccine has been characterized extensively over the recent years, the role of co-stimulatory molecules in the development of cell mediated immunity is much less well understood (Haseeb *et al.*,2001). The importance of CD40\CD154 in vaccine induced immunity was shown that CD154 -\- mice exposed to RA schistosomes developed protection to challenge infection suggesting that protective immunity to the RA schistosome vaccine is CD154 dependent but is independent of (IL-12 orchestrated) cellular immune mechanism (Donald *et al*., 2008).

2.2. Chemotherapy against *schistosomiasis mansoni*

The history of chemotherapy of schistosomiasis has been characterized by a prolonged monopoly of antimonials followed by a relatively brief but exiting period, during which events have significantly modified the control approach significantly. Metrifonate, oxamiquine and praziquantel all appear in world health organization's list of essential drugs and currently spearhead attempts to control this important disease through reduction in morbidity at the community level (Nils, 2002). Among all the drug discovery trials, the most important development in the chemotherapy of schistosomiasis has been the discovery of Praziquantel, a prazinoisoquinoline compound active against cestodes and trematodes, including all schistosoma species (Doenhoff *et al*., 2000) which makes it a drug of choice for treating schistosomiasis. However, there are significant limitations associated with large scale usage of praziquantel in schistosomiasis control strategies. Among these limitations the major ones are-

- Praziquantel cannot be the perfect anti-schistosomal drug since it does not affect young stages of the parasite (Chris *et al*., 2001).
- Relative cost of the drug to the cost of other controls is very high and unbearable to the per capita health expenditure in sub Saharan Africa. Additionally, the emergence of HIV\AIDS epidemic with its economic burden, the need to address other health issues and low economic performance have made it difficult for those countries to invest in drug for schistosomiasis (Utzinger *et al*., 2003)
- Given the enormous burden of disease related to schistosomes, relying solely on existing mass and repeated treatment of exposed population with praziquantel is unlikely to be feasible (Nils, 2002).

- Even if it is occasional, there are reports of schistosome strains with various degrees of tolerance to praziquantel, and similarly resistance as a result of drug pressure cannot be ruled out (Michael *et al.*, (2002), Stephen *et al.*, (2003), Chris *et al.*, (2001) and Getinet *et al.*, (2002)).

2.3. Vaccines against *schistosomiasis mansoni*

Looking to the act that inspite of remarkable chemotherapeutic progresses and the existence of effective molecules such as the acylated quinoline pyrazine praziquentel (PZQ), there is still a spread of schistosomiasis into new areas (Steinmann *et al*., 2006). Mass treatment does not prevent re-infection. This occurs rapidly in exposed population following chemotherapy the prevalence returns to the base line level within 6 to 8 months. In high transmission areas, interruption with chemotherapy results in severe (rebound morbidity) in terms of hepatosplenic disease (Utzinger *et al*., 2003). That is to say the spread of the disease is in progress against all efforts of control makes the need for protective and curative vaccines much desired (Utzinger *et al.*, 2003).

Despite the claims and publication of numerous potentially promising vaccine antigens it is the 28-kda GST from *S. mansoni* on animal models and *S. hematobium* (Donald *et al*., 2008) on humans that have entered clinical trials. Understanding of the immune response to schistosome infection both in animal models and in humans, suggest that development of vaccine is possible (Iramaya *et a*l., 2008). Schistosome vaccines will be an integrated approach to chemotherapy and health education (Mahmoud *et al*., 2005).

2.3.1. Strategies for anti -schistosome vaccine development.

Schistosomes do not replicate with in there mammalian hosts. Consequently a non-sterilizing vaccine could significantly decrease human pathology and disease transmission. Vaccination against schistosomes can be targeted towards the prevention of infection and\or reduction of parasite fecundity (Bergquist and Colley, 1998). The migrating schistosomulum stage is likely to be the major vaccine target of protective immune response (Robert *et al*., 2002). However schistosome eggs are responsible for both pathology and transmission. A vaccine targeted at parasite fecundity and egg viability could also be appropriate (Edward, 2003).

Among vaccine development approaches tested so far, the major ones are –

- Radiation attenuated cercarial vaccine.
- Live, attenuated, cryopreserved schistosomulum vaccine against *S. japonicum* in buffalos to reduce zoonotic transmission to humans in china. If successful, the veterinary vaccine could provide a paradigm for the development of an anti-schistosome vaccine for human use (Donald *et al*., 2008).
- Recombinant vaccines through isolation of large number of schistosome antigens (utilizing almost complete genome sequence) and additional candidates through proteomic approaches (Robert *et a*l., 2002). However antigen identification and successful protection are of little value if recombinant proteins cannot be produced easily and cheaply with good manufacturing practice (GMP). Even the best protective results are no guarantee for ultimate success, and the scale up of antigen production can be a bit challenging as any immunological investigation (Edward, 2003).

- DNA vaccines generate both T and B- cells immune responses and are thus particularly appealing for schistosome vaccine development. The preparation and production of DNA vaccines are convenient and cost effective. They can be used in the field without a cold chain. Another advantage of applying DNA vaccines compared to other approaches is the possibility of targeting the in vivo expressed recombinant antigen to different cell compartments (Mahmoud *et al.*, 2005). Furthermore, methods such as prime boost regimens and the use of adjuvants such as IL-12 in combination with DNA vaccine can enhance its protective effectiveness (Donald *et al.*, 2008 and Luis *et al.*, 2008).

2.3.2. Major vaccine candidates and their protective efficacies

Table below summarizes the chemical property, protection level in mice, and the status in 2002 for the most promising vaccine candidates discovered in the last 10 years as well as those that were independently tested under the umbrella of TDR\WHO committee (Robert *et al.*, 2002).

Table 1- Overview of priority vaccine candidates (Robert *et al*., 2002)

Candidate antigen	Mol.weight (KDa)	Chemical property	Protection in mice (%)	Status in 2002
Paramyosin	97	Muscle protein	30	GMP production
Irradiated vaccine no. five(Ir V-5)	62	Muscle protein	50 -70	Prepare re/test DNA construct
Glyceraldehydes phosphate dehydrogenase (GAPDH)	37	Enzyme		Protection studies
Glutathione- S- transferase (GST)	28	Enzyme	40 – 60	Phase 3 trials
Triose phosphate isomerase (TPI)	28	Enzyme	30 – 60	Scaling up research
23 kDa membrane antigen	23	Integrated membrane antigen	40 – 50	Prepare re/test DNA construct
Sm14	14	Fatty –acid binding protein	65	GMP production

2.3.3. Vaccine delivery and adjuvant selection

Traditional approach in vaccination have been the use of Frunds' complete adjuvant when antigens are first delivered in the mouse model, and often particularly licensed (or have the potential for licensing) for human use, have been used to formulate an antigen (Robert *et al*., 2002). Among those less conventional or less widely explored as adjutants for schistosome vaccines include, live salmonella, tetanus toxin, filamentous phages, recombinant

mycobacterium bovis BCG, IL-12, nanoparticles, and various methods of mucosal delivery (Donald *et al.*, 2008).

2.3.4. Limitations in vaccine development prospects

The current schistosome vaccine candidates have not proved yet to be the most effective or characterized by reproducible efficacy. Difficulties in obtaining good expression levels in scale up production according to good laboratory practice (GMP) (Edward, 2003). Standards for the limited number of antigens selected have turned out to be another major obstacle. Some front line candidates have suffered from difficulties in scale up production according to GMP standards and have been dropped (Corrêa-Oliveira *et al*., 2000).

Therefore in order to overcome these and other related difficulties in schistosome vaccine development prospects, it is important to identify new target antigens and to explore alternative vaccination strategies to improve vaccine efficacy (Donald *et al.*, 2008).

3. Tested pathogenic bacterial species and associated drug resistance

In the current study we selected a commonly occurring bacterial infections; Gram-positive (*S. aureus, S. epidermidis*) and five Gram-negative (*S. dysenteriae, S. typhi, C. freundii, E. coli, P. aeruginosa*,). Among these pathogens the first three are known to cause different respiratory, gastrointestinal, systemic as well as skin diseases in man. Two are normal flora of the intestine (*E. coli, C. freundii*) with pathogenic strains, while three nosocomial ones (*S. aureus, S. epidermidis* and *P. aeruginosa*), an emerging opportunistic (*P. aeruginosa*), and another associated with food born/ intoxication (*S. typhi*).

Several studies have shown that these bacterial pathogens have developed resistance to many of antibiotics which are currently on use. For instance C. *freundii* strains have inducible ampC genes encoding resistance to ampicillin and first-generation cephalosporins (Joanna *et al*., 2003) and some degree of multiple reistance due to plasmid-encoded resistance genes (Yu-Min *et al.*, 2006), resistance to penicillins, tetracyclines, aminoglycosides, erythromycins, etc by *S. aureus* due to production of β-Lactamase and plasmids which can be transmitted by transduction and conjugation (Howard, 1999), resistance to antibiotics such as methicillin, novobiocin, clindamycin, and benzyl penicillin by *S. epidermidi,* Masaru *et al*., (2009), resistance to many antibiotics that are effective against Gram-positive organisms (Heike and Reinhard, 2005) by pathogenic variable *E. coli* strains, failure of ampicillin, doxycycline, and trimethoprim-sulfamethoxazole to eliminate *Shigella dysenteriae* form the gut (Bhattacharya et al., 2003), multiple drug resistance by *S. typhi* and inability to treat *P. aeruginosa* with a single drug therapy Gamal *et al*., (2008).

Antimicrobial resistance is one of the biggest challenges facing global public health. Antimicrobial drugs have saved many lives and eased the suffering of many millions. However, because poverty, ignorance, poor sanitation, hunger and malnutrition, inadequate access to drugs, in developing countries have tremendously limited the benefits of these drugs in controlling infectious diseases (Byarugaba, 2004). The development of resistance in pathogens that are responsible for the death of millions has worsened the situation often with very little resource to provide reliable treatments. The emergence of multi-drug-resistant isolates in tuberculosis, acute respiratory infections and diarrhea, often referred to as diseases of poverty, has had its greatest toll in developing countries.

Patient education, prescriber education, resistance and antimicrobial use surveillance, treatment, preventing spread, and research have been suggested as possible strategies for controlling resistance (Carlos et al., 2008). Basic and clinical research provides the fundamental knowledge necessary to develop appropriate responses to antimicrobial resistance emerging and spreading in hospitals, communities, farms, and the food supply (Carlos *et al*., 2008). Major scientific accomplishments throughout the years have contributed much to the understanding of the fundamental biological processes of antimicrobial resistance within microbes and the resulting impact on humans, animals, and the environment. This knowledge base provides us the opportunity to influence these processes and outcomes.

4. Gap of knowledge

- Although the exact mechanisms responsible for the anti-inflammatory and anti-oxidant properties are not clearly known several studies have shown the anti-inflammatory and antioxidant properties of Arthrospira platensis (spirulina). However, no study has been done to see its effect on granulomatous responses induced by pathogens like the schistosomiasis, tuberculosis, HBV.
- Spirulina have been implicated to have several nutritional and health benefits which are well studied and documented. Among the potential medical applications of spirulina is its antimicrobial property which is proved with different scientific experiments. However, antimicrobial effect of spirulina against human intestinal, nosocomial and other bacterial pathogens has not been well studied.

Therefore, the purpose of the current study is to learn the effects of spirulina on hepatic granuloma induced by *S. mansoni* and bacterial pathogens of the gut, nose and other parts of the body as described in the objective below:

II. OBJECTIVES

General objectives:

- To evaluate the anti-inflammatory and anti- infectious effect of *Arthrospira plathensis* on *Schistosomiasis mansoni* granuloma using *Swiss albino* mice.
- To evaluate the antimicrobial property of *Arthrospira platensis* on human pathogenic bacterial species of the gut.

Specific objectives:

- ✓ To establish *Arthrospira platensis* monoculture in the laboratory.
- ✓ To maintain *Biomphalaria pfefferi in* the laboratory.
- ✓ To maintain *Schistosomiasis mansoni* life cycle in the laboratory between *B. pfefferi* and Swiss Albino mice.
- ✓ To see the effect of different extracts of spirulina treatment on the size and number of liver granuloma.
- ✓ To examine the antibiotic effect of spirulina on human pathogenic bacteria.

III. MATERIAL AND METHOD

1. Laboratory Maintenance of life cycles

1.1. Lab-maintenance of *Biomphalaria pfefferi* life cycle

Lab-maintenance of *Biomphalaria pfefferi* life cycle was performed according to Lewis *et al.*, (1986). *Biomphalaria pfefferi,* snails were collected from *S. mansoni* transmission foci in two locations; (Wondogenet town and Loke village around Bishangari lodge) in the Oromia region. The collected snails were transported to Addis Ababa University, Biomedical science laboratory using pads of moist cotton. The snails were then cleaned using deionized water and small brushes in order to remove invertebrates sheltered in the shell and dirt from their natural habitat. Egg masses were collected from floating foams kept in aquaria and transferred into a blue green algal culture of *Nostoc muscorum* in petri dishes. The hatching young snails were collected and transferred into plastic plates when they get to 3mm diameter and fed there with cooked lettuce and protein rich fish food. Following this they were transferred in to aquaria of different size with proportion of 30 -50 snails in 3 liter of thermostat heated (28^0c) deionized water. The aquarium was kept at 25 -28^0c room temperature with alternating 12 hours light and 12 hours dark. The snails were feed with boiled salad (lettuce) and protein rich fish food (Tetramine).

The quality of the water was monitored by Daphnia species that were seeded in the same aquaria to serve as a bio-sensor. The aquaria water was changed at interval of about 10 days and protein enrichment was done on the last 3 days before water change.

1.2. *S. mansoni* life cycle

S. mansoni life cycle was maintained between the snail intermediate host *Biomphalaria pfefferi* and Swiss albino mice the definitive host following procedures indicated by Lewis *et al*., (1986).

Cercarial shedding was done from the field collected and/or laboratory breed and infected snails by illuminating them with florescent lamp for an approximately one and half hour by putting individual snail on a shedding chamber filled with deionized water.

After one and half hours of illumination, the chambers were checked for the presence and species of the cercaria using a dissecting microscope at 4X magnification power in order to avoid mixing of *S. mansoni* with bird schistosome. Cercarias form different chambers were pooled in to a test tube to allow mixing of sexes. The total number of cercaria was determined from counts made in1ml iodine killed cercaria.

The pooled cercarial containing solution was homogenized to create a uniform cercarial suspension. The quantity which was found to contain enough cercaria for heavy infection was used to infect Swiss albino mice percutaneously through two hours of exposure. Finally infected mice were transferred into the animal house and followed for about eight weeks. Starting from the fourth week post infection, mice were checked for the presence of *S. mansoni* eggs in their feces using direct microscopy.

By the end of the 7^{th} week all the mice that were positive for *S. mansoni* were scarified using cerebral dislocation. Harvest of egg was done from the liver of infected mice through maceration and repeated washing with 1X PBS.

Hatching of the recovered *S. mansoni* eggs was done by changing the salinity of medium containing the *S. mansoni* eggs (PBS) into fresh water and additional stimulation of the hatching process with 2 -3 drops of aquaria water that contain snail stool and other waste and 15 -20 minute illumination with florescent lamp.

The hatching medium was checked for the presence of miracidia, and then approximately 5 - 7 Miracidiae was transferred with Pasteur pipette in to individual shading chambers on which individual snails which bred in the laboratory were placed. 7 days later the snails were checked for sporocystes that appear on the antenna and their foot pad. Those that bear sporocyst were selected and fed with protein rich fish food (Tetramine) for 28 days which is required for the snails to start shedding cercaria.

1.3. Maintenance (culture) of *Arthrospira platensis* (Spirulina)

The algal sample was collected from Lake Chitu by filtering the lake water with algal phytonet, and transported to Addis Ababa University, Biomedical science laboratory on the day of collection over ice. The algal sample was washed with distilled water for several times to avoid any impurities and other invertebrates. Algal sample was divided into two. A portion was used for direct selection of individual spirulina filaments under microscope after serial dilution. The selected clones were cultured first on 12 well plates and then inoculated into Erleyrmayer flasks as recommended by (Aiba and Ogawa 1977, Schlösser 1994) by mixing 500 ml of solution I, 500 ml of solution II, 1ml of trace metal solution and 1ml of Cyanocobalimin stock solution. The protocol and media contents are presented in Table-2 below. Simultaneously, the other portion of the sample was purified, lyophilized, powdered, and stored as dry product for latter use at $4\ ^{0}$c.

Table 2- **Modified spirulina culture medium (Aiba and Ogawa 1977, Schlösser 1994)**

Component	**Stock Solution**	**Quantity**	**Molar Concentration in Final Medium**
Solution I	500 mL	---	---
$NaHCO_3$	---	13.61 g	1.62×10^{-4} M
Na_2CO_3	---	4.03 g	3.80×10^{-5} M
K_2HPO_4	---	0.50 g	2.87×10^{-6} M
Solution II	500 mL	---	---
$NaNO_3$	---	2.5 g	2.94×10^{-5} M
K_2SO_4	---	1.0 g	5.74×10^{-6} M
NaCl	---	1.0 g	1.71×10^{-5} M
$MgSO_4\ 7H_2O$	---	0.2 g	8.11×10^{-7} M
$CaCl_2\ 2H_2O$	---	0.04 g	2.72×10^{-7} M
$FeSO_4 7H_2O$	---	0.01 g	3.60×10^{-8} M
$Na_2EDTA\ 2H_2O$	---	0.08 g	2.15×10^{-7} M
Trace metals solution			
$Na_2EDTA\ 2H_2O$	---	0.8 g	2.15×10^{-6} M
$FeSO_4\ 7H_2O$	---	0.7 g	2.52×10^{-6} M
$ZnSO_4.7H_2O$	1.0 g L^{-1} dH_2O	1 mL	3.48×10^{-9} M
$MnSO_4\ 7H_2O$	2.0 g L^{-1} dH_2O	1 mL	8.97×10^{-9} M
H_3BO_3	10.0 g L^{-1} dH_2O	1 mL	1.62×10^{-7} M
$Co(NO_3)_2\ 6H_2O$	1.0 g L^{-1} dH_2O	1 mL	3.44×10^{-9} M
$Na_2MoO_4\ 2H_2O$	1.0 g L^{-1} dH_2O	1 mL	4.13×10^{-9} M
$CuSO_4\ 5H_2O$	0.005 g L^{-1} dH_2O	1 mL	2.00×10^{-11} M
Cyanocobalimin Stock Solution			
Cyanocobalomin (vit. B_{12})	---	5 mg	3.69×10^{-9} M

The spirulina culture was used as a continuous culture through daily shaking and harvesting once in a week. The algal yield was lyophilized, powdered, and stored as a dry product until used.

A.

B

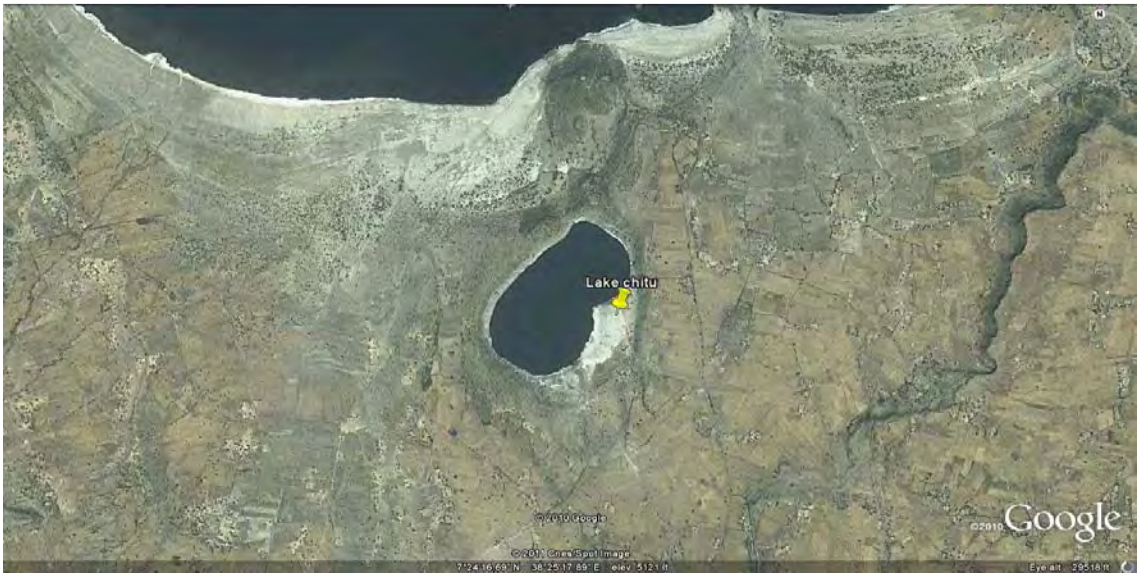


Figure: 1 A. Laboratory culture of spirulina B. Lake Chitu: *Arthrospira platensis* (spirulina) collection site, Southern Ethiopia.

2. Optimization

From a preliminary experiment conducted in order to optimize doses, sectioning temperature, sectioning thickness and Granuloma measurments used in the actual experiment were determined.

Spirulina for the treatment of the experiment was prepared by suspending the previously harvested, filtered, washed, lyophilized and powdered spirulina product with sterile 1X PBS at three known concentrations (50, 100 and 200 mg/kg). Then the spirulina and the PBS solution was sonicated, filter sterilized and, stored until used.

Three set of preliminary experiments (pre- infection spirulina intervention, spirulina intervention at zero day of infection, and post- infection spirulina intervention following experimental design that is not included in this paper.

For life cycle maintenance percutaneous infection was used where as for the experimental infection, tail immersion method was employed.

At the eighth week of the experiment, mice under group 1-7, 13 and 14 were scarified and the forth quadrant of liver removed for histological examination. At the eighth week mice under groups 8, 9, 10, 11 and 12 were sacarified and liver was collected. And it was then covered with mounting media, kept in the microtone at -28 ^{0}c for 20 minutes. Sectioning at 5µm thickness was found to be good for our purpose. The sections were transferred into frozen microscopic glass slides and stained with Hematoxyline and Eosine.

The prepared slides were examined under microscope at 100x magnification and pictures were taken using connected computer. Finally the pictures was transferred into Adobe Photoshop CS3 software and measured at picture resolution of 72 PPi and Dimension of 640 x 576 mm.

Plasma samples from all experimental mice of all groups was collected from each mice once a week by bleeding the tail tip and centrifuging it at 2,500 revolutions per minute for three to four minutes.

Data collected from the preliminary study was used to optimization the study protocol in terms of infection dose, treatment dose, route, sectioning temperature, and sectioning thickness for the actual experiment and are not presented in the thesis.

3. Test for the anti- inflammatory effect off spirulina

One day earlier to the starting day of the experiment, 28 five week old female mice were collected and categorize into 7 groups of four each. The next day spirulina for the treatment of the experimental animals was prepared by suspending the previously cultured, washed, lyophilized and powdered spirulina product with sterile 1X PBS at 100, 400, and 800 mg/kg, two selected doses, based on the earlier preliminary experiment. The diluted product was then sterile filtered using a micro filter of 2 µm pore size just before use.

Snails used in the maintenance of the *S. mansoni* infection were stimulated to shed, cercariae were pooled and cercarial number was calculated using the previously indicated method. From the pooled cercarial suspension 70 -75 cercarial containing volumes (1.5-2 ml) were taken and

made ready for infection brought to 9ml with deionized water and let the mice infected for one hour using tail immersion.

Following the infection, mice were treated following the experimental protocol indicated in the table below.

Table 3- Experimental protocol for the anti- inflammatory effect off spirulina test

Spirulina Intervention	**Rout**	**Dose**	**Intervention days**	**Group No**
Post-infection	Oral	100 mg/kg	2^{nd} ,3^{rd} and 4^{th} weeks post infection	1
		400 mg/kg	2^{nd} ,3^{rd} and 4^{th} weeks post infection	2
	Rectal	100 mg/kg	2^{nd} ,3^{rd} and 4^{th} weeks post infection	3
		400 mg/kg	2^{nd} ,3^{rd} and 4^{th} weeks post infection	4
	Oral/Rectal	400 mg/kg	2^{nd} ,3^{rd} and 4^{th} weeks post infection	5
Day Zero	Oral	400 mg/kg	0 day, 2^{nd} ,3^{rd} and 4^{th} weeks post infection	6
- Ve. Control	----------	----------	----------	7

At the sixth weeks of infection, all experimental animals were scarified and the fourth quadrant of the liver was removed, mounted, cryostat sectioned at a thickness of 5µm using a Microtone at a temperature of (– 28^0C). The sections were then transferred into frozen microscopic glass slides and stained with Hematoxylin and Eosin. The prepared slides were examined under microscope using 10x magnification power and pictures were taken using a computer connected. Finally the picture taken was transferred into Adobe Photoshop CS3 software and measured at picture resolution of 72 PPi and Dimension of 640 x 576 mm.

4. Test for the antimicrobial effect of spirulina

4.1. Collection of pathogenic bacterial test strains

Standard pathogenic bacterial test strains: *S. aureus*, *S. epidermidis*, *E. coli*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Shigella dysenteriae* were collected from Ethiopian Health and Nutrition Research institute, and biomedical science laboratory at Addis Ababa University and clinical isolates of the pathogenic bacterial strains were collected from Yekatit 12 General hospital.

4.2. Preparation of algal sample for the antimicrobial test

Algal samples collected from Lake Chitu and lab cultured clones were used as a source for the algal test. The algal product from the two sources were dried, powdered, weighted, diluted with water and 80% methanol and sonicated to facilitate the removal of active components into the solvent medium. The resulting solution was then sterilized using micro filters of 2 µm mesh size and directly used for the antimicrobial sensitivity test.

Further fractionation of the extracts was done using column chromatography packed with reverse phase silica gel. Fractionates were collected in 1ml epindorf tubes and used for the test.

Figure 2- Fractionation of different spirulina extracts using modified column chromatography

4.3. Test of the extracts for antimicrobial sensitivity

Sterile Whatman paper discs with 6mm diameter were used for the investigation of the antibiotic activities of the different algal extracts. The discs were impregnated in different concentrations of the algal products: the crude at 1,5,10 and 20 mg/ml, water extract at 20, 25, 30, 35 mg/ml and the alcohol extract at 10, 20, 30 and 40 mg/ml concentrations. The discs were carefully placed on Muller Hinton agar on which the test organisms were seeded and incubated at 37^0c for 24 hours. Standard antimicrobial disks (Gentamicin, Vancomycin, Ciprofloxacin, Chloramphenicol and Neomycin) and solvent impregnated disks were used as positive and negative controls respectively.

Investigation of the antimicrobial property of the extracts and fractionates was done in triplicates using Kirby-Bauer disk diffusion test. Effect of the extracts tested was determined by measurement of the diameter of zone of inhibition on the plates after 24 hours of incubation using slide caliper.

IV. RESULT

Result on anti-inflammatory activity of spirulina

Table 4- Independent T. test comparison between treated and non treated groups based on Morphometric analysis (means ± SD) of schistosomal hepatic granulomas stained with Hematoxylin-eosin.

Groups	**Morphometric characters**	
	Diameter	**Number**
Oral/100mg/kg	55.48 ± 9.29	8.50 ± 1.92
Control	91.54 ± 5.68	10.5 ± 1.25
p. value	0.001	0.097
Oral/400mg/kg	37.04 ± 12.26	3.00 ± 1.41
Control	91.54 ±5.68	10.5 ± 1.25
p. value	0.000	0.000
Rectal/100mg/kg	69.03 ± 4.87	6.75 ± 0.96
Control	91.54 ± 5.68	10.5 ± 1.25
p. value	0.001	0.002
Rectal/400mg/kg	78.31 ± 13.21	5.00 ± 0.82
Control	91.54 ± 5.68	10.5 ± 1.25
p. value	0.115	0.000
Oral/recta/400mg/kg	82.75 ± 3.82	6.00 ± 1.16
Control	91.54 ±5.68	10.5 ± 1.25
p. value	0.043	0.001
Group 6	65.00 ± 9.48	8.75 ± 0.50
Control	91.54 ±5.68	10.5 ± 1.25
P. value	0.003	0.025

As it is presented on table one, when treated groups were compared with the non treated group based on granuloma size at 0.05um level of significance, all except the group treated at 400mg/kg through rectal route of administration were found to be significantly different from measurement taken from the control group. Additionally comparison between treated and non treated group based on number of granuloma, all except the group treated at 100mg/kg through oral route of administration were found to be significantly different.

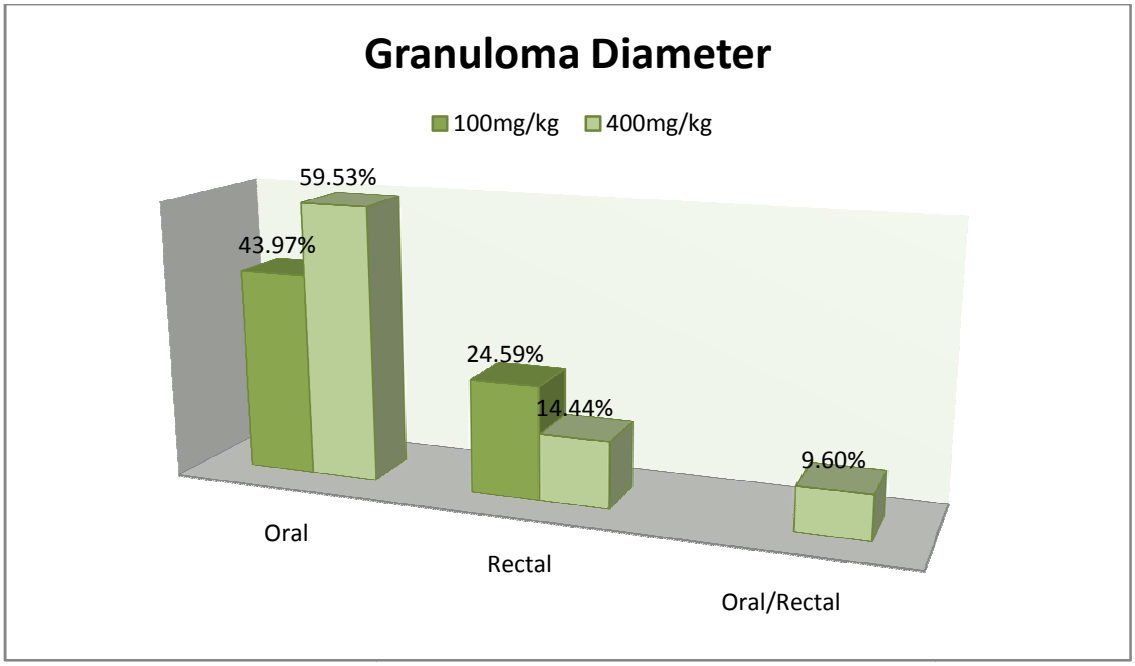


Figure 3- Bar- graph showing percentage reductions in Granuloma diameter under different treatment dose and route of administration

Based on the percentage reduction, 400 mg/kg and 100 mg/kg resulted higher in oral and rectal routes respectively. Similarly when we compare the three treatment routes at a dose of 400 mg/kg, the oral and oral/rectal route was found to have the highest and the least respectively

through leaving the rectal route with moderate reduction percentage. Similarly comparison at a dose of 100 mg/kg showed a better result through oral route than the rectal.

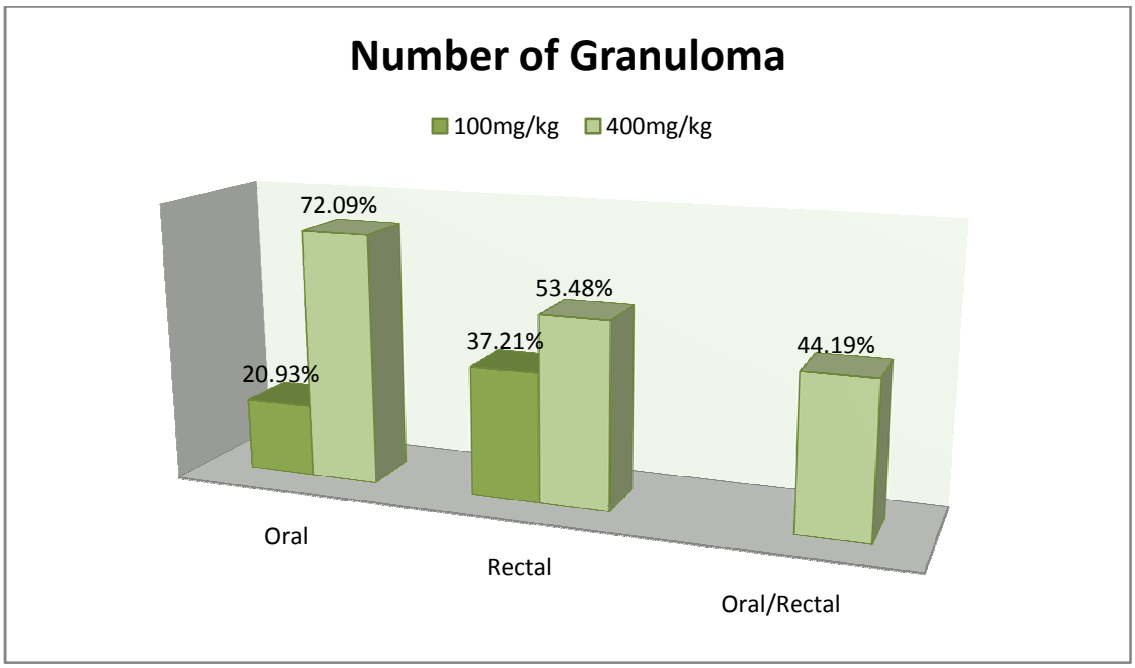


Figure 4- Bar- graph showing percentage reduction in number of Granuloma under different treatment dose and route of administration

Based on the percentage reduction, 400 mg/kg resulted higher in both routes.

When we compare the three treatment routes at a dose of 400 mg/kg, the oral and oral/rectal routes were found to have the highest and the least respectively through leaving the rectal rout with moderate reduction percentage. Contrary to this, comparison at a dose of 100 mg/kg showed a better result through rectal route than the oral.

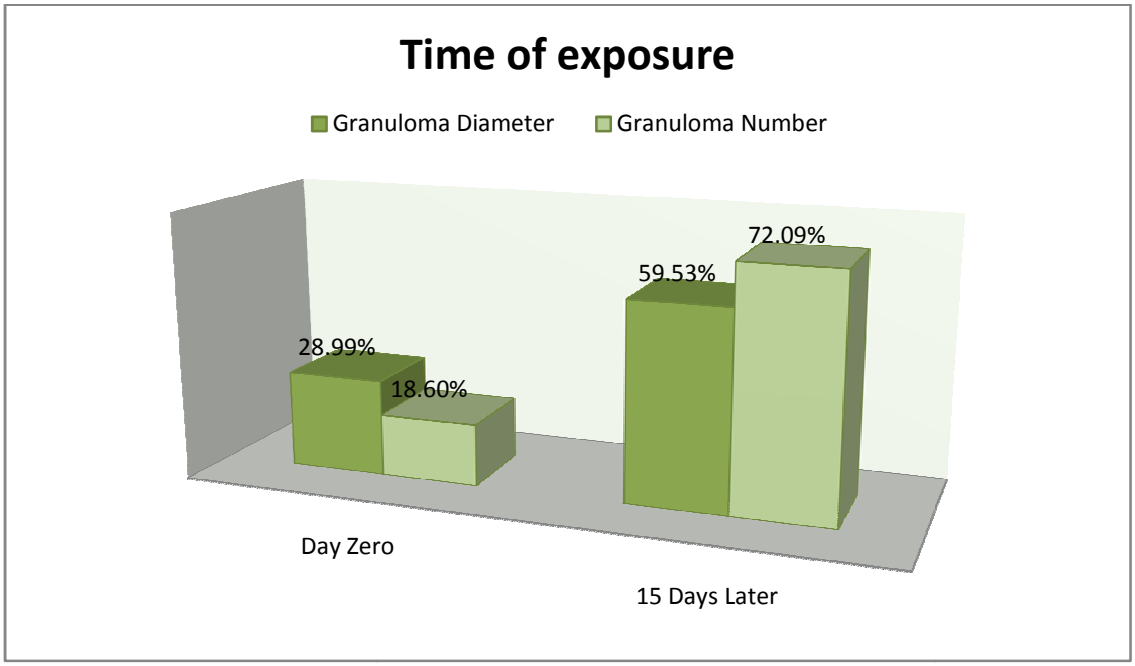


Figure 5- Bar**-graph showing effect of first time of spirulina exposure on percentage reduction of granuloma diameter and number**

When we consider time of exposure as a determinant factor for the diameter and number of granuloma, treating the animals 15 days post infection resulted a higher percentage reduction in both granuloma diameter and number with significant increment on granuloma number.

B. Independent T. test comparison of mean granuloma diameter and granuloma number between experimental groups

Table 5- T. test comparison of experimental groups based on treatment dose

Groups	**Morphometric character**	
	Granuloma diameter	**Granuloma number**
Dose Comparison	**(Mean ± SD)**	
Oral/ 100mg/kg	55.48 ± 9.29	8.50 ± 1.915
Oral/400mg/kg	37.04 ± 12.26	3.00 ± 1.414
p. value	0.002	0.004
Rectal/100mg/kg	69.03 ± 4.87	6.75 ± 0.975
Rectal/400mg/kg	78.31 ± 13.21	5.00 ± 0.816
p. value	0.007	0.032

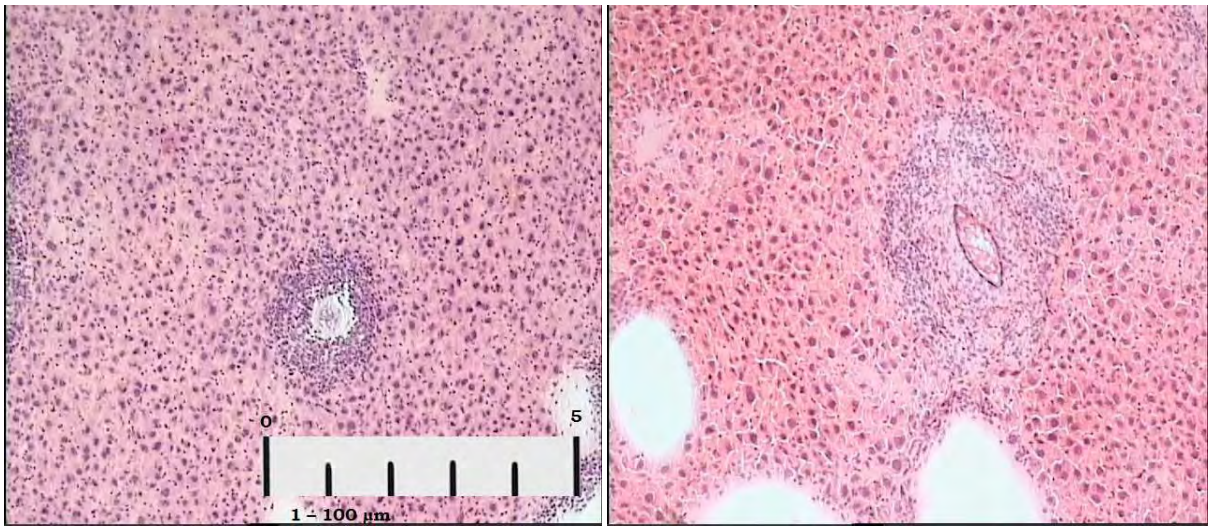
Table 6- T. test comparison of experimental groups based on treatment routes

Groups	Morphometric characters	
	Granuloma diameter	Granuloma number
Route comparison		
Oral/ 100mg/kg	55.48 ± 9.29	8.50 ± 1.915
Rectal/100mg/kg	69.03 ± 4.87	6.75 ± 0.975
p. value	0.000	0.153
Oral/400mg/kg	37.04 ± 12.26	3.00 ± 1.414
Rectal/400mg/kg	78.31 ± 13.21	5.00 ± 0.816
p. value	0.000	0.050
Oral/400mg/kg	37.04 ± 12.26	3.00 ± 1.414
Oral/Rectal/400mg/kg	82.75 ± 3.82	6.00 ± 1.155
p. value	0.000	0.017
Rectal/400mg/kg	78.31 ± 13.21	5.00 ± 0.816
Oral/Rectal/400mg/kg	82.75 ± 3.82	6.00 ± 1.155
p. value	0.882	0.207

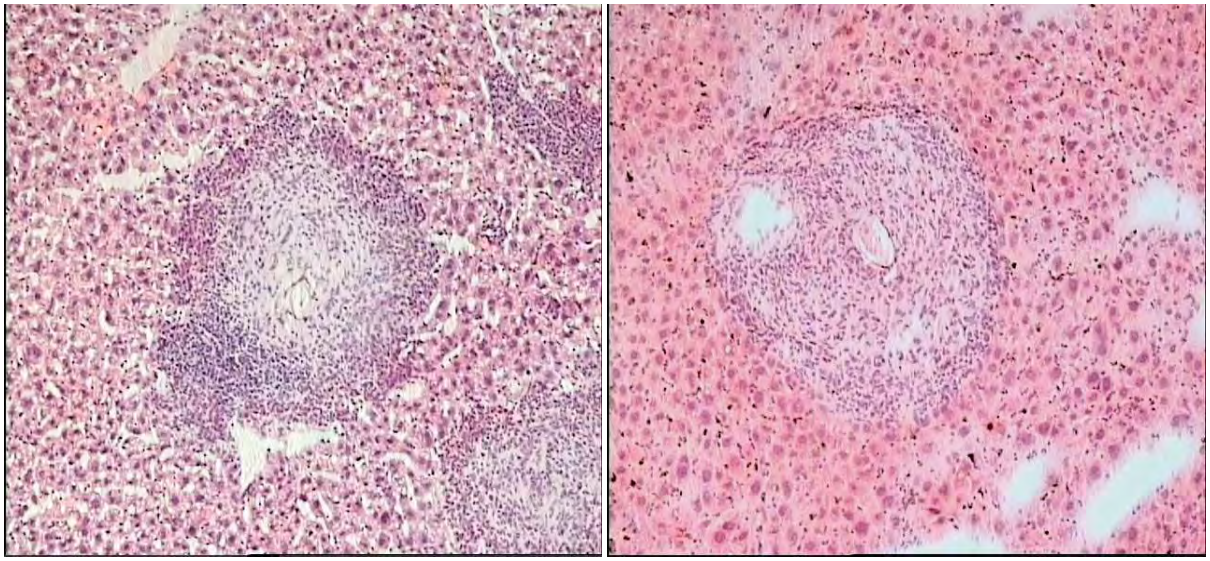
Table 7- T. test comparison of experimental groups based on time of exposure

Groups	Morphometric characters	
	Granuloma diameter	Granuloma number
Time of exposure comparison		
Oral/400mg/kg/15 days later	37.04 ± 12.26	3.00 ± 1.414
Oral/400mg/kg/day zero	65.00 ± 9.48	8.75 ± 0.500
p. value	0.000	0.000

A **B**



C **D**



E **F**

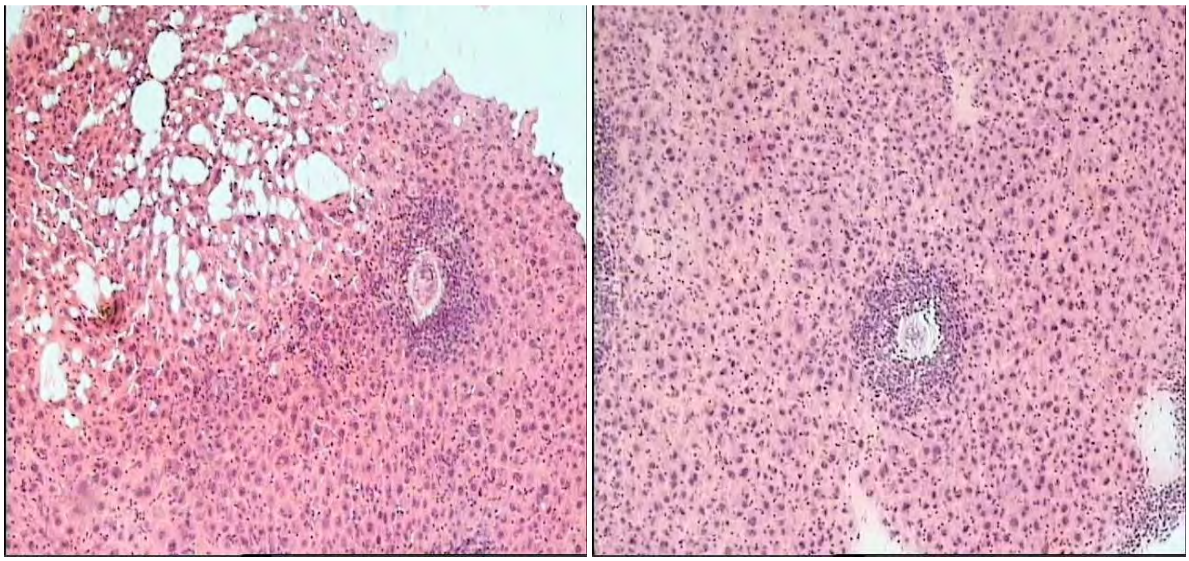


Figure 6- Liver granuloma A. **Scaled liver granuloma** B**. S. mansoni egg trapped in liver granulomatous tissue.** C**. &** D**. Liver granuloma from untreated mice** E. F. **Liver granuloma from mice treated with spirulina. (All picture taken at 10X magnification)**

Result on antimicrobial activity of spirulina

Table 8- Zone of inhibition (mm) of bacteria against water suspension of crude *A. plathensis*

Bacterial Sps.	Diameter of zone of inhibition in (mm) at a given concentration				
	1mg/ml	5 mg/ml	10mg/ml	20mg/ml	Standard
S. typhi	-	-	-	-	22
S. dysenteriae	-	-	-	11	21
C. freundii	-	-	-	8	21
S. aureus	9	12	13	15	22
E. coli	-	-	-	7	21
P. aeruginosa	-	-	-	-	12
S. epidermidis	-	-	-	-	38

Effect of crude spirulina water suspension on pathogenic bacteria: The antibacterial activity of *A. plathensis* was seen on 7 known pathogenic bacteria. As shown in the table 1 above, it has inhibited the growth of 4 species (*S. dysenteriae, C. freundii, S. aureus,* and *E. coli*). Among all the tested species S. *aureus* was found to be the most susceptible with 9mm of inhibition zone at the lowest tested concentration of 1mg/ml and increased with the gradient of concentration. The rest three species; *P. aeruginosa, S. epidermidis* and *S. typhi* were resistant at all tested concentrations. Antibiotic drug that results the highest inhibition zone on an individual bacteria was taken as standard (positive control).

Table 9- Bacterial inhibition zone against water suspension of A. *plathensis* collected from wild

Bacterial Sps.	Diameter of zone of inhibition(mm)				
	10mg/ml	20 mg/ml	30mg/ml	40mg/ml	Standard
S. typhi	-	-	-	-	22
S. dysenteriae	8	8	8	9	21
C. freundii	8	8	9	9	21
S. aureus	14	15	16	16	22
E. coli	7	7	9	9	21
P. aeruginosa	-	-	-	-	12
S. epidermidis	-	-	-	-	38

Effect of water suspension of crud wild spirulina on pathogenic bacteria: The wild spirulina has an effect on four of the bacterial species tested (*S. dysenteriae, C. freundii, S. aureus* and *E. coli)* at 10, 20, 30, and 40 mg/ml concentrations while the rest of the bacterial species: *S. typhi, S. epidermidis*, and *P. aeruginosa* remained resistant.

Table 10- Bacterial zone of inhibition (mm) against water extract of laboratory cultured *A. plathensis*

Bacterial Sps.	Diameter of zone of inhibition in (mm)				
	20mg/ml	25 mg/ml	30mg/ml	35mg/ml	Standard
S. typhi	-	-	-	-	22
S. dysenteriae	-	-	-	-	21
C. freundii	-	-	-	-	21
S. aureus	-	-	-	-	22
E. coli	5	6	6	6	21
P. aeruginosa	8	8	9	10	12
S. epidermidis	7	9	11	11	38

Effect of spirulina water extract on pathogenic bacteria: The antimicrobial activity of water extract of spirulina was seen on three of the tested species. *P. aeruginosa, E. coli* and *S. epidermidis* were found to be susceptible in a more or less dose dependent manner. *S. typhi, S. dysenteriae, C. freundii* and *S. aureus* show resistance at all tested concentrations.

Table 11- Bacterial inhibition zone (mm) against Methanol extract of *A. plathensis*

Bacterial Sps.	Diameter of bacterial inhibition zone in (mm)				
	10mg/ml	20 mg/ml	30mg/ml	40mg/ml	Standard
S. typhi	-	-	-	-	22
S. dysenteriae	-	-	-	-	21
C. freundii	-	-	-	-	21
S. aureus	-	-	-	-	22
E. coli	-	-	-	-	21
P. aeruginosa	-	-	-	-	12
S. epidermidis	-	-	-	9	38

Effect of alcohol extract of spirulina on pathogenic bacteria: The antimicrobial activity of the alcohol extract of spirulina was seen only on *S. epidermidis* at the highest tested concentration of 40 mg/ml. The remaining test species; S. *typhi*, *S. dysenteriae*, *C. freundii*, S. *aureus*, *E. coli*, and *P. aeruginosa* showed no susceptibility at all concentrations.

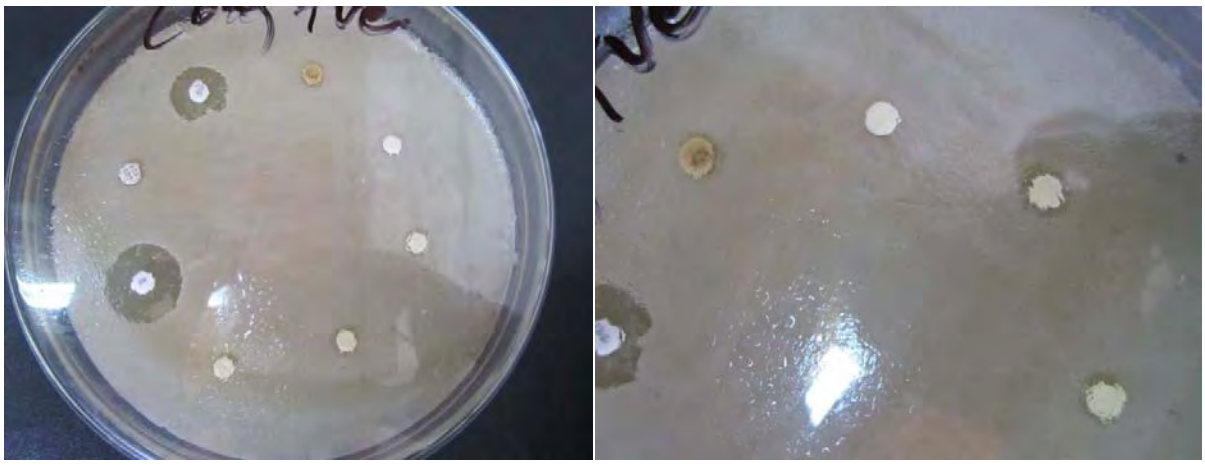


Figure 7-Zones of inhibition due to standard antimicrobial sensitivity disks and different spirulina extracts tested

V. DISCUSSION

Anti-inflammatory action of spirulina

Independent T. test comparisons of results from the spirulina treated and none treated groups at (*P. value* 0.05) indicate that all treated groups except the group which were treated at 400mg/kg through rectal route of administration to have statistically significant differences in the size of liver granuloma.

Since granulomas are composed of several cell types and extracellular matrix components, the action of spirulina on these elements is pleotropic and difficult to evaluate in vivo. However, granuloma sizes in those experimental animals treated with spirulina showed significant decreases, probably due to dampened Th2 response and enhanced production of IL-2 and IFN- γ induced by *S. plathensis* as described by Tomohiro, *et al*., (2002) which synergize to inhibit acute granulomatous and fibrotic response (Boros and Whiitfield, 1999) or it could also be due to elevated level of IL-12 as a result of spirulina treatment (Tomihiro *et al*., 2002) which have been described to have a strong regulatory role in the lung granulomatous response of naïve or sensitized mice (Wynn *et al*., 1995). Other explanation for this could be due to antioxidant property of *Spirulina* (Romay, and Gonzalez, 2000, Hirata, *et al*., 2000. Miranda, *et al*., 1998) anti- inflammatory activity of spirulina (Romay, *et al*., 1998[1]. And Romay, *et al*., 1998[2]) and this anti-inflammatory effect of phycocyanin may result partly from inhibition of Prostagladi E2 (PGE2) production and a moderate inhibition of phospholipase A2 (PLA2) activity (Romay, *et al*., 2000). Similarly reduction in the granuloma size have also been discussed to be due to radical scavenging activity of spirulina and its role for reduced level of NO (Bhat and Madyastha 1998. La Flamme *et al*., 2001).

Results from the histopathology have also revealed that size of liver granuloma varies between different treatment doses and routes. As it can be seen from the tables presenting percentage reduction in granuloma size and number, 400 mg/kg and 100 mg/kg resulted higher in oral and rectal routes respectively. Similarly comparison based on treatment routes at a dose of 400 mg/kg, the oral and oral/rectal routes were found to have the highest and the least respectively through leaving the rectal route with moderate reduction percentage. And comparison at a dose of 100 mg/kg showed a better result through oral route than the rectal. Our result argues with similar studies on anti-inflammatory and antioxidant properties of spirulina which showed that effects associated with spirulina treatment to be dose dependent (Romey *et al*., 2000. Sabina *et al*., 2009. Shih *et al*., 2009. Narendra *et al.,* 2010). Similarly treatment of *S. mansoni* infected mice at different doses of standard drug artemether shown to have variable effect on liver weight as well as number of worms recovered (Susana *et al*., 2004).

As to that of treatment dose, different study results have come up with different results using variable routes for administration of spirulina in experimental animal models. Despite the variation most suggest that the oral route of administration as is with our finding to be the most effective (Johny *et al*., 2010. Rimbau *et al.,* 1999; Rasool *et al*., 2006)

Considering time of exposure as a deterministic factor for the diameter and number of granuloma, treating the animals 15 days post infection resulted a higher percentage reduction in both granuloma diameter and number. In our opinion this variation could be related to time dependent differential immunomodulatory effect of spirulina given at day zero and 15 days later. It could also be due to differential susceptibility of different schistosome life stages. However

there is no clear explanation yet, for the time dependent effect of spirulina put forward for further studies except a study by Liu et al., 2000 that reported *Spirulina platensis* inhibit cell viability of human leukemia K562 in a dose and time dependent manner. In another study Simsek *et al.*, 2007 showed that oral supplementation of *Spirulina platensis* on peripheral blood cells in rats time dependently change the Packed Cell Volume and Red Blood Cell count.

Antimicrobial activity of spirulina

The result from the present study on the antimicrobial activity of spirulina showed that spirulina has inhibitory effect on the growth of 6 bacterial species except no effect on *S. typhi*.

As it has been presented on table one and two the water suspension of both the laboratory cultured and spirulina collected from the wild exhibited antimicrobial activity against four of the tested bacterial specie (*S. dysenteriae, C. freundii, S. aureus* and *E. coli*). However the two suspensions show deference in their minimum inhibitory concentrations on *E. coli* which was 10mg/ml in the wild spirulina and 20mg/ml for the laboratory cultured spirulina. In association with this it was found that S. *aureus* is the most susceptible with 9mm of inhibition zone at a test concentration of 1mg/ml. In our opinion these differences could be due to variation in concentration of the active secondary metabolites between the two sources. This difference on concentration of secondary metabolites can be explained by absence of strain variation in the clones and expected to be abundant in the wild and responsible for increased production of the active substances in the wild environment (Trischman *et al*., 2004 and Shiqing and Shao., 2007).

From table 3 it is inferred that there is dissimilarity between the water suspension and extract of spirulina in their antimicrobial activity. With the exception of *E. coli*, all test organisms that were susceptible in the suspension showed no susceptibility to the extract. Similarly those which were susceptible to the extract were not affected by the water suspension of spirulina. The discrepancy seen here can be explained by the difference in purity and concentration of active substances that are water soluble which expected to be high in the water extract and low in the suspension. We believe that these active substances could be responsible for the inhibitory activity of the water extract on the growth of P. *aeruginosa* and *S. epidermidis* which is not seen among effects of the water suspension. Similarly the selective activity of the water suspension on *S. dysenteriae* and *C. freundii* could be due to the presence of water insoluble active substances in the suspension and their absence in the extract.

Extracts of *Spirulina platensis* obtained by different solvents exhibited different degrees of antimicrobial activity on both Gram-positive and Gram-negative organisms goes with similar works elsewhere. The results presented in table 3 and 4 revealed that the methanol extract resulted no antimicrobial activity in all microbial organisms tested except week or negligible activity towards *S. epidermidis* at highest test concentration of 40 mg/ml. However the water extract was active against *E. coli*, *P. aeruginosa* and *S. epidermidis* at all the test concentrations. The variation in the antimicrobial activity of these two spirulina extracts could be due to differential solubility of the active components in these two solvents. These results go in harmony with those obtained by other workers (Ozdemir *et al*., 2001 And Rania *et al.*, 2008; Mala *et al*., 2009) they found that water extract of *Spirulina plathensis* showed a moderate antimicrobial activity against *K. pneumonia,* and *P. aeruginosa* . Furthermore all tested

organisms were resistant to methanol. In contrary to this research result by Guven *et al*., (2004) and Mendiola *et al*., (2007) reported that the methanol extract exhibited the highest antimicrobial activity. These disparities could be due to differences in extraction procedure, test concentrations used or factors associated with culture medium which influence the production of biologically active secondary metabolites (Dola *et al*., 2009).

Several studies have shown that the currently tested bacterial pathogens have developed resistance to many of antibiotics which are currently on use. For instance C. *freundii* strains have inducible ampC genes encoding resistance to ampicillin and first-generation cephalosporins (Joanna *et al*., 2003) and some degree of multiple reistance due to plasmid-encoded resistance genes (Yu-Min *et al.*, 2006), resistance to penicillins, tetracyclines, aminoglycosides, erythromycins, etc by *S. aureus* due to production of β-Lactamase and plasmids which can be transmitted by transduction and conjugation (Howard, 1999). Similarly resistance to antibiotics such as methicillin, novobiocin, clindamycin, and benzyl penicillin by *S. epidermidi*, Masaru *et al*., (2009), resistance to many antibiotics that are effective against Gram-positive organisms (Heike and Reinhard, 2005) by pathogenic variable *E. coli* strains, failure of ampicillin, doxycycline, and trimethoprim-sulfamethoxazole to eliminate *Shigella dysenteriae* form the gut (Bhattacharya et al., 2003), multiple drug resistance by *S. typhi* and inability to treat *P. aeruginosa* with a single drug therapy Gamal *et al*., (2008). Therefore, the present study could highlight a possible alternative approach to tackle a problem of drug resistance by these pathogens.

VI. CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

In the present study the possible anti-inflammatory and antimicrobial effect of *Arthrospira plathensis* has shown that

- ✓ Arthrospira plathensis (spirulina) treatment of S. mansoni infected mice reduced the size of liver granuloma.
- ✓ Spirulina treatment of *S. mansoni* infected mice has an effect on the number of liver granuloma.
- ✓ The effect of spirulina treatment on the size and number of granuloma is dependent on the route of administration, treatment dose and time of exposure (treatment).
- ✓ Treatment of *S. mansoni* infected mice with spirulina through oral route has a better effect on both the size and number of liver granuloma.
- ✓ Water suspension of crude *Arthrospira plathensis* collected from the wild and laboratory cultured have inhibitory effect on the growth of *S. dysenteriae, C. freundii, S. aureus*, and *E. coli.*
- ✓ Water extract of laboratory cultured spirulina has inhibited the growth of *P. aeruginosa, E. coli* and *S. epidermidis.*
- ✓ Methanol extract of spirulina have a negligible effect on the growth of human pathogenic bacteria.

RECOMMENDATIONS

- Possible mechanism of action of spirulina in comparison with liver granuloma formation and inhibition of bacterial growth should be investigated.
- Pharmacological screening, further fractionation and isolation of active ingredient and structural elucidation and further pharmacological investigation on the active ingredients of spirulina need be done.
- Attention should be given by government, NGOs, researcher's etc. to systemically evaluate this medically and nutritionally important algae and other phytoplankton in order to develop safe, effective, affordable and accessible products since majority of our population depend on herbal medicine.
- Bacterial resistance has been the main factor responsible for the increase of morbidity, mortality and health care costs of bacterial infections, there for an improved knowledge of the composition, analysis, and properties of *Spirulina platensis* with respect to antimicrobial compounds would assist in efforts for the pharmaceutical application of this blue green algae.
- Attention should be given by government and NGOs working on child health should consider integration of spirulina in their fight against child malnutrition and morbidity due to Schistosomiasis.

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