



## “WHEAT GRASS- A PERFECT FOOD AND ITS ANTI-MICROBIAL PROPERTIES FROM THE DIFFERENT SOLVENT EXTRACT”

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Article Received on  
11 July 2016,  
Revised on 01 Aug 2016,  
Accepted on 22 Aug 2016,  
DOI: 10.20959/wjpps20169-7654

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### ABSTRACT

Wheatgrass juice is nature's finest medicine and a complete source of protein, supplying all of the essential amino acids, and more. Wheatgrass (*Triticum aestivum*) refers to young grass of the common wheat plant, which belongs to Poaceae family. This is the most commonly found herb in India. This plant is believed to have many nutritional values; it has been shown to have anti-inflammatory, antioxidant, anti-carcinogenic, immune-modulatory, laxative, astringent, diuretic, antibacterial and anti-aging properties. Wheatgrass extracts being natural medicine can be extremely valuable for treating various sicknesses from minor scratches and blazes to genuine

infections. Plant products are of increasing interest in the search for new drugs and medicines in the treatment of diseases. There is a vast scope of research and innovations for wheat grass (*Triticum aestivum*) and its formulations as highly effective antimicrobial agents.

**KEYWORDS:** Wheat grass, Growth, Extraction, Immunity, Antimicrobial activity, Bioactive Compound, Observation.

### INTRODUCTION

Wheatgrass juice is nature's finest medicine. It is our signature elixir here at Hippocrates. It is a powerful concentrated liquid nutrient. Two ounces of wheatgrass juice has the nutritional equivalent of five pounds of the best raw organic vegetables. For example, wheatgrass has twice the amount of Vitamin A as carrots and is higher in Vitamin C than oranges! It contains the full spectrum of B vitamins, as well as calcium, phosphorus, magnesium, sodium and potassium in a balanced ratio.<sup>[1]</sup>



**Figure showing the healthy Wheatgrass**

Wheatgrass is a complete source of protein, supplying all of the essential amino acids, and more. It has about 20% of total calories coming from protein. This protein is in the form of poly peptides, simpler and shorter chains of amino acids that the body uses more efficiently in the blood stream and tissues. In addition to flooding the body with therapeutic dosages of vitamins, minerals, antioxidants, enzymes, and phyto-nutrients, wheatgrass is also a powerful detoxifier, especially of the liver and blood. It helps neutralize toxins and environmental pollutants in the body. This is because Wheatgrass contains beneficial enzymes that help protect us from carcinogens, including Superoxide Disumates (SOD), that lessens the effects of radiation and digest toxins in the body. It cleanses the body from head to toe of any heavy metals, pollutants and other toxins that may be stored in the body's tissues and organs.<sup>[2]</sup>

The wheatgrass juice must be consumed fresh – within fifteen minutes of juicing for the best results. The juice should always be taken undiluted and on an empty stomach so the nutrients can be absorbed more efficiently. Powdered and freeze dried wheatgrass supplements are nowhere near as effective as fresh wheatgrass juice. Our study revealed that wheatgrass supplements and freeze dried powders are only two percent as effective as fresh juiced wheatgrass when it is consumed within fifteen minutes. These nutrients quickly begin to oxidize (break down) shortly after juicing. When it is consumed fresh it is a living food and has bio-electricity. This high vibration energy is literally the life force within the living juice. This resource of life-force energy can potentially unleash powerful renewing vibrations and greater connectivity to one's inner being. These powerful nutrients can also prevent DNA destruction and help protect us from the ongoing effects of pre-mature aging and cellular breakdown. Recent research shows that only living foods and juices can restore the electrical charge between the capillaries and the cell walls which boosts the immune system. When it is fresh, wheatgrass juice is the king of living juices. Among other things wheatgrass juice is

particularly high in chlorophyll. Wheatgrass cleanses and builds the blood due to its high content of chlorophyll. Chlorophyll is the first product of light and therefore contains more healing properties than any other element. All life on this planet comes from the sun. Only green plants can transform the sun's energy into chlorophyll through the process of photosynthesis.<sup>[2]</sup>

### **A Perfect Food**

Wheat– grass (*Triticum aestivum*) means “full of life”. It contains all the nutritional value one can expect from organic foods. It also includes the right mix of minerals. 30ml juice of wheat–grass is claimed to be nutritionally equivalent to one kilogram of whole leafy vegetables. Wheat– grass juice is a complete food that can be taken orally with no toxic side effects. It is an important nutritional supplement as it is bio-available from most of the vitamins, minerals, proteins, carbohydrates, enzyme and amino acids needed by the body. It has no cholesterol and is a natural body purifier. Wheat– grass helps detoxify the body with high chlorophyll content. The enzymes contained in Wheat– grass aid the digestion and assimilation of nutrients, and contribute to the body's life-force.<sup>[2]</sup>

## **MATERIALS AND METHODS**

### **The Dosage Tips**

There are few dosage tips for taking the wheat grass. Normally Healthy people should take once ounce (25-30 ml) of Wheat –grass juice per day and increase slowly to 90-100 ml. For Healthy person, the therapeutic dosage, drink 2 ounces each three times a day. The juice should be consumed soon after it is extracted. The best time to take this juice is morning hours on an empty stomach. Do not eat or drink anything for at least one hour before and after. If someone cannot take it in the morning, take any time when the stomach is empty.<sup>[5-7]</sup>

### **Growth of Wheat Grass: Humble Healer**

Wash 100gm of wheat grains well, soak them and put them in a container (glass, mud or steel). Soak them for 8-10 hours; cover the container with a lid. Drain the water and keep the grains for 12-15 hours to sprout. Choose a warm and shady place. Spread the sprouts in soil (you can also use earthen pots or wooden trays with 3 inches of mud), mixed with cow dung or compost manure. Sprinkle water twice a day for seven days until the grass grows 5-7 inches high. Cut the grass as close to the root as possible and after cutting the grass, empty the pot and expose it to sunlight. Using fresh soil, repeat the process. Wheat –grass juice can cure Liver problems, Arthritis, Gout, Cystitis, Ulcerative colitis, Constipation, Chronic skin

disorders, Tooth decay, Wounds, skin problems, cuts boils and many more as The Ultimate Remedy.<sup>[9-12]</sup>

### **Extraction of Wheat – Grass Juice**

Wash the grass thoroughly and cut each blade into small pieces. Grind the grass either in a mixer or stone –grinder. Extract the juice and strain with a fine piece of cloth. Drink the juice then and there (its efficiency and medical value get lost after three hours). But it can store it in a refrigerator. Mixing with 3% lemon juice and 2% ginger juice, it can keep it up to 15-20 days at 5 - 10°C.

### **The Protector of Immunity**

Chlorophyll (in Wheat– grass) and hemoglobin (in red blood cells) are molecularly similar. The only difference is that the central element in chlorophyll is magnesium and in hemoglobin it is iron; so chlorophyll has been shown to build red blood cells quickly after ingestion. Chlorophyll can be extracted from all plants, but Wheat – grass is superior because it has been found to have over 100 elements needed by humans. Wheat- grass can acts as an antibacterial and it can be used inside and outside the body. Washes drug deposits and neutralizes toxins in the body. Normalizes high blood pressure by dilating the blood pathways throughout the body and stimulates healthy blood tissues (cell growth). It protects the body from carcinogens (cancer-causing substances) and helps to strengthen the immune system.<sup>[13-14]</sup>

## **RESULT**

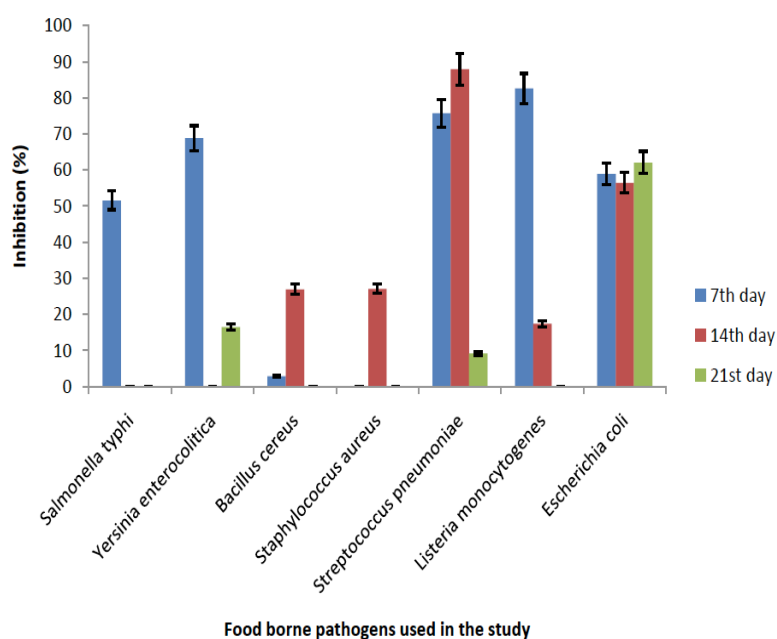
### **Antimicrobial Activity of Different Solvent Extracts of Wheat Grass**

To find out the best extracting solvent, the wheat grass extracts from different solvents were screened, using agar well diffusion method. Clear zones were observed for all pathogens studied. But *Yersinia enterocolitica*, *Staphylococcus aureus* and *Listeria monocytogenes* were observed to be most sensitive pathogens.<sup>[15]</sup>

### **Quantitative Estimation of Antibacterial Activity**

Quantitative estimation of the viable biomass after treatment with wheatgrass extracts indicated that wheat grass extracts had differential effects on each microorganism under study. Methanol extract of wheat grass had maximum inhibitory effect on *Salmonella paratyphi*. With *Yersinia enterocolitica*, hexane extract gave maximum inhibition of 72.6% followed by ethyl acetate (61.4%). *Bacillus cereus* was more resistant to any type of extract

of wheat grass. Maximum of 20% inhibition was observed with ethyl acetate extract of wheat grass. *Staphylococcus aureus* showed 47.6 and 45% inhibition respectively with ethanol and methanol extracts of wheat grass. Ethyl acetate and hexane extracts showed no inhibition of *Staphylococcus aureus* while aqueous extract of wheat grass showed only 11 % inhibition of growth. *Streptococcus pneumoniae* showed inhibition with all types of extracts. Of these, hexane extract inhibited the growth of *Streptococcus pneumoniae* by 91% followed by ethyl acetate and methanol extracts and ethanol extract showed the lowest inhibitory activity of 28%. Growth of *Listeria monocytogenes* was also inhibited by extracts of wheat grass with all solvents studied. Ethanol extract had lowest inhibition (39%) while hexane extract inhibited the growth of *Listeria monocytogenes* completely. Also Ethyl acetate, aqueous and methanol extracts showed 61.3, 49.3 and 46.6 % inhibition of growth respectively.

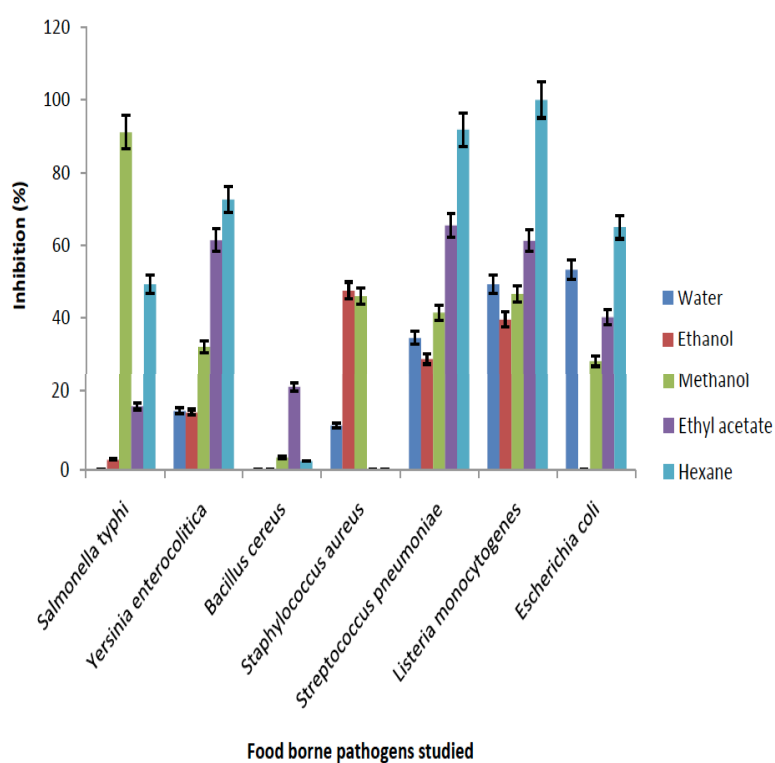


### Age of Wheat Grass and Antimicrobial Activity

The age of the wheat grass appeared to play a major role in the presence or absence of active antimicrobial ingredient. The hexane extract from 7 days old wheat grass showed inhibitory activities against few food borne pathogens used. Highest inhibition of 82.56% was observed with *Listeria monocytogenes* followed by *Streptococcus pneumoniae* (75.69%). No activity was observed against *Staphylococcus aureus*. Only 2.84% inhibitory activity was observed against *Bacillus cereus*.

7 days old wheat grass had antibacterial activity against *Salmonella paratyphi* while 14 days and 21 days old wheat grass had no activity against *Salmonella paratyphi*. Similarly, 7 days

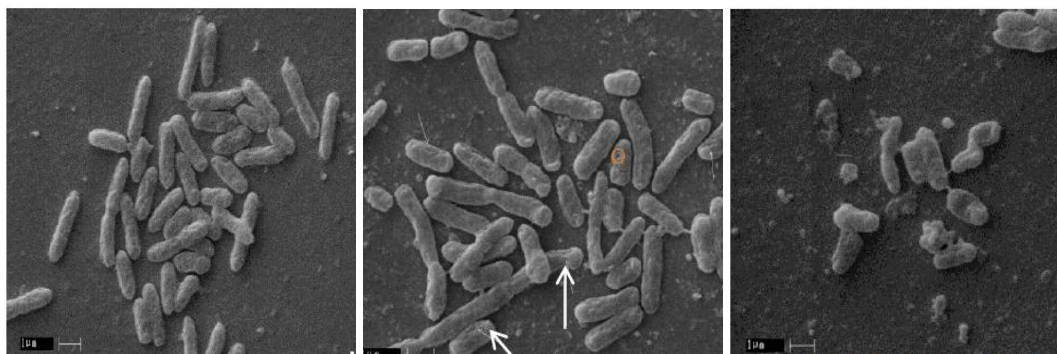
old wheat grass had activity against *Yersinia enterocolitica* while hexane extract of 14 days old wheat grass had no activity against *Yersinia enterocolitica*. Activity of hexane extract of 21 days old wheat grass was very low (16%). With *Streptococcus pneumoniae*, activity of hexane extract of 7 days old wheat grass was 75.69%. The activity increased with hexane extract of 14 days old wheat grass (87.90%) and decreased with hexane extract of 21 days old wheat grass (9.14%). Hexane extract of 21 days old wheat grass had highest activity against *Escherichia coli* (62.02%).



### Bacterial Morphology Changes Observed in SEM

On comparing the surface morphology in control cells of gram positive and gram negative strains with 16<sup>th</sup> and 24<sup>th</sup> hour (after treatment with antimicrobial) respectively, it can be inferred that there was visible action of hexane extract on bacteria surface. When the cellular permeability gets altered, the cell dies eventually. Other changes in the morphology of 16<sup>th</sup> hour sample of both gram positive and gram negative were the bifurcation of few cells (absent in control) and flattening of cells at the centre leading to dumb-bell shape formation in *Yersinia enterocolitica*. Also coagulation of cells got initialized in the 16<sup>th</sup> hour in gram positive cells compared to gram negative cells (which required 24 hr to coagulate). This also supported the fact that gram negative bacteria studied were slightly more resistant compared to gram positive bacteria. This may be due to the impermeability of bio-actives caused by

LPS (Lipopolysaccharides) in their membrane. Overall shrinkage in cell size was also observed with increasing time of exposure. The 24<sup>th</sup> hour cells of *Yersinia enterocolitica* showed clear fragmentation of cells.



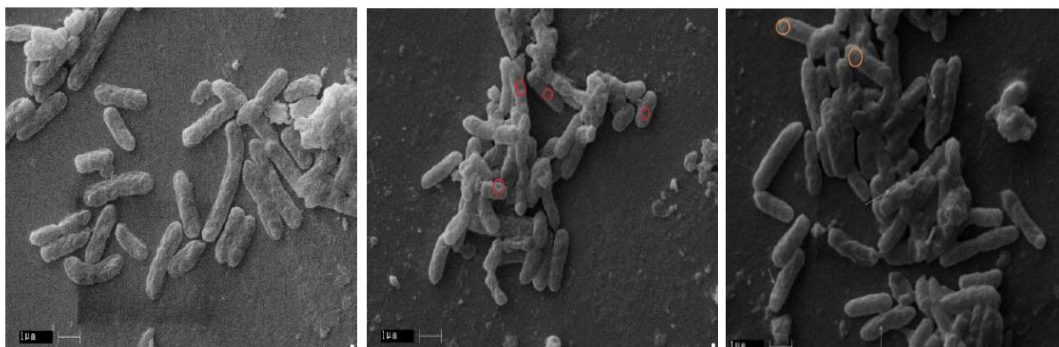
**Figures showing the Different Gram negative bacteria of *Yersinia species***

### **Determination of MIC (Minimum Inhibitory Concentration)**

The minimum inhibitory concentration (MIC) is the concentration at which an antibacterial agent experiences the complete inhibition of micro-organism growth. MICs are considered as golden standard for determining the susceptibility of microorganisms to antimicrobial compounds. In our studies, it was observed that for *Listeria monocytogenes*, MIC was 168.6uL with an IC<sub>50</sub>= 84.30uL and with *Yersinia enterocolitica*, MIC was 113.14uL with an IC<sub>50</sub>=56.57uL.

### **DISCUSSION**

The Human diet is enriched with young parts of plants (so called green foods), which can improve nutrient balance intake in natural way. Wheatgrass (*Triticum aestivum*) refers to young grass of the common wheat plant, which belongs to *Poaceae* family. This is the most commonly found herb in India. This plant is believed to have many nutritional values; it has been shown to have anti-inflammatory, antioxidant, anti-carcinogenic, immune-modulatory, laxative, astringent, diuretic, antibacterial and anti-aging properties. Its use in acidity, colitis, kidney malfunctions, atherosclerosis and swelling has been shown to be beneficial.<sup>[16,17]</sup> Wheatgrass packs a nutritional punch, including (per 3.5 grams) 860 mg protein, 18.5 mg chlorophyll, 15 mg calcium, 38 mg lysine, 7.5 mg vitamin C and an abundance of micronutrients, such as B complex vitamins and amino acids.<sup>[17]</sup>



**Figures showing the Different Gram positive bacteria of *Listeria species***

Phytochemical constituents of wheatgrass include alkaloids, carbohydrates, saponins, gum and mucilages. Its water soluble extractive value is found to be greater than its alcohol soluble extractive value.<sup>[3]</sup> Wheat grass juice is high in vitamin K, which is a blood clotting agent. Wheatgrass leaf extract improves the digestive system, and promotes general well-being.<sup>[18-20]</sup> It has higher nutritive value than broccoli and spinach.<sup>[21]</sup> Its high levels of enzymes and amino acids work like a natural cleanser to detoxify the liver, eliminate toxic heavy metals from the blood stream, rid the body of waste matter and slow down the aging process.<sup>[22]</sup> With these beneficial effects of wheat grass extracts, in our study we attempted to study the antimicrobial effect of wheat grass extracts. We obtained antimicrobial activity of aqueous extract of wheat grass against some of the food borne bacterial pathogens. Pallavi *et al.*(2011) reported activity in acetone WGJ (Wheatgrass juice) extracts against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.<sup>[8]</sup> Das *et al.* reported activity in 80% acetone extracted samples against four bacteria: *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella flexneri* and one fungus: *Aspergillus niger*.<sup>[23]</sup> However, Padalia (2010) found acetone and methanolic extracts did not show any antibacterial activity while fresh and undiluted wheatgrass juice exhibited mild antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium* and *Kleibsell pneumoniae*. It is interesting to note that in the study by Padalia (2010) the efficacy of fresh WGJ declined after 2 hours. Fresh undiluted wheatgrass juice was then screened against bacteria: *Escherichia coli* NCTC 10418; *Staphylococcus aureus* NCTC 6571 and *Streptococcus mutans* NCIMB 702062. None of the extracts tested displayed any kind of antimicrobial activity against selected pathogens.<sup>[22]</sup>

## CONCLUSION

Inactivation of pathogenic microorganisms or microorganisms that cause food spoilage is one of the basic purposes of food preservation methods. The objective of this study was to



explore the benefits of wheatgrass extract with respect to its antibacterial potential. The extracts of wheat grass were found to possess antibacterial activity against some of the major food borne pathogens used in this study. The antimicrobial activity was clearly dependent on the age of the wheat grass as well as the solvent used for extraction. Thus wheatgrass extract would be a novel antimicrobial agent. More optimisation can be carried out to obtain extract exhibiting very high antimicrobial activity. The Wheatgrass extracts can also be used as a health tonic with the additional nutritional benefits they provide. Wheatgrass extracts being natural medicine can be extremely valuable for treating various sicknesses from minor scratches and blazes to genuine infections. Plant products are of increasing interest in the search for new drugs and medicines in the treatment of diseases. We conclude by saying that there is a vast scope of research and innovations for wheat grass (*Triticum aestivum*) and its formulations as highly effective antimicrobial agents.

#### ACKNOWLEDGEMENT

We are really thankful to the Hon'ble Chancellor and Vice-Chancellor Sir for providing us research facility at School of Biological & Chemical Sciences, MATS University, Raipur(CG). We owe our deep gratitude towards all the Faculty Members and the Colleagues of the MATS School of Biological & Chemical Sciences. Our sincere thanks towards our parents for supporting us with directly and indirectly in different ways for this microbiological research work.

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