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Research Article

IN VITRO ANTI-MICROBIAL ACTIVITIES OF WHEAT GRASS

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ABSTRACT

Wheat grass refers to the young grass of the common wheat plant, *Triticum aestivum* L. that is freshly juiced or dried into powder for animal and human consumption. Green Blood Therapy is the use of wheat grass juice (WGJ) to cure multiple diseases. The name "green blood" of wheat grass is attributable to its high chlorophyll content which accounts for about 70% to its total chemical constituents and also to its close structural similarity to Haemoglobin. Wheat grass juice contains almost all the nutrients the body requires and is considered to be a complete food. The present study intends to provide an overview of the antimicrobial activities of the crude fresh leaf extracts of *T. aestivum* with special emphasis on their pharmacological actions. Wheat grass chloroform extract with DMSO was assayed in vitro by agar cup method against two clinical gram negative isolates of bacteria such as *E. coli* and *Klebsiella pneumonia* and two clinical fungal isolates such as *Candida albicans* and *Aspergillus niger*. The chloroform extracts of wheat grass was found to have high antifungal activity than antibacterial activity. The extract had shown more inhibition against *E. coli and A. niger*. On the basis of the results obtained, the present study concludes that wheat grasses are rich in phytochemical constituents exhibiting antimicrobial properties. Thus the present study supports to a certain degree, the usage of traditional medicinal plants in human and animal disease therapy and reinforce the ethno botanical approach to screening plants as potential sources of bioactive substance is successful.

KEYWORDS: In vitro, Wheat grass, Antimicrobial activity, Agar cup Method, Green Blood Therapy, *Triticum aestivum*.

INTRODUCTION

Anti-microbial is the act of killing or inhibiting or suppressing microorganisms from their multiplication or growth. There are certain phytochemicals that are known to have anti-bacterial properties which include flavonoids and alkaloids. Most of these phytochemical constituents are potent bioactive compounds found in wheat grass, parts of which are precursors for the synthesis of useful drugs.[1]Das *et al.,* (2012) found that 80% acetone extracts of wheat grass were effective against five food borne microorganisms, including the fungus *Aspergillus niger,* a common contaminant of food.[2]Due to bacterial expression of resistance to antibiotics, the development of new antiseptics and anti-microbial agents are of growing interest now.[3]Plants contain thousands of constituents which are valuable sources of new and biologically active molecules having antimicrobial properties.[4]A wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, are found to have anti-microbial properties invitro.[5, 6]These natural products are of great concern as a source of safer and/or more effective alternatives to synthetically produced anti-microbial agents.[7] Wheat grass extract has a high content of bioflavonoids which may add towards anti-microbial effects.[8, 9] The increasing failure of chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential anti-microbial activity.

The complete phytochemical investigation of medicinal plants of India have to be explored as these secondary metabolites are of immense important and contribute to the medicinal activity of the plants.[10]Saponins are considered to be a key ingredient in Chinese medicine and are believed to be responsible for the biological effects which include anti-microbial, anti-inflammatory and haemolytic effect.[11]Kothari (2008) states that many of tannin containing plants are used in medicine as astringents.[12] It can be employed for the treatment of burns as they precipitate the proteins of exposed tissue to form a protective covering. It has been found to have anti-viral, anti-bacterial, anti-inflammatory, anti-ulcer and anti-oxidant properties for therapeutic applications. Studies have proven that many of the phyto compounds possess anti-inflammatory, anti-diabetic and anti-microbial activities.[13]

The harvesting period of wheat grass appeared to play a major role in the presence or absence of active anti-microbial ingredients. The extracts of wheat grass were found to possess anti-bacterial activity against some of the major food borne pathogens used in this study. The anti-microbial activity was clearly dependent on the harvesting period of the wheat grass as well as the solvent used for extraction. Pallavi *et al.,* (2011) reported activity in acetone wheat grass juice extracts against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* [14] Das *et al.* (2012) reported activity in 80% acetone extracted samples against four bacteria: *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Shigella flexneri* and one fungus *Aspergillus niger.* In vitro studies was conducted for the antibacterial activity of *Acacia farnesiana* Linn *and Pergularia daemia* Linn against two bacterial strains *(Staphylococcus aureus ,* Gram +ve MTCC 737*;Pseudomonas aeruginosa,* Gram –ve MTCC 1688). Highest sensitivity of both the bacterial strains for the aqueous extract of *Acacia farnesiana* may be due

to the presence of Phenol, flavonoids, alkaloids, steroid, triterpenoids, and tannins which are said to have potential anti-microbial actions.[15]

Wheat grass is cost efficient and a source to provide all kinds of nutrients like vitamins, proteins, minerals, antioxidants and medicinal benefits for a healthy and rejuvenating body. Wheat grass has high concentration of chlorophyll, minerals (calcium, potassium, iron, magnesium, sodium and sulphur), and 17 forms of amino acids, Vitamins (A, B, C, E and K) and active enzymes. It stimulates metabolism and also restores alkalinity to the blood. It's the chlorophyll content in wheat grass that detoxifies the body and strengthens immunity.

MATERIALS AND METHODS

Preparation of plant extract for anti-microbial screening

The collected leaves were washed thoroughly in tap water and finely chopped. Extracts were prepared by grinding fresh leaf samples with chloroform (1:1) and subjected to mechanical shaking at 100 rpm for 48 hours at room temperature. Supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume. For anti-microbial screening the concentrated, dried leaf extract was dissolved in 10 % dimethyl sulfoxide (DMSO) and were stored at 4 ^{0}C for further use.

Test Organisms: - Antibacterial activity was evaluated against two selected gram negative pathogens such as *Escherichia coli* and *Klebsiella pneumonia* whereas anti-fungal against two clinical fungal isolates such as *Candida albicans* and *Aspergillus niger* (as recommended by the National Committee for Clinical Laboratories Standards, NCCLS), purchased from Biogenix Research Centre, Valiyavila, Thiruvananthapuram. In order to access the biological significance and ability of the plant part, the minimal inhibitory activity was determined by Agar Cup Plate Assay Method**.** [16]

Anti-bacterial activity: - Petri plates containing 20ml of Muller Hinton medium were seeded each with 24hrs old culture of bacterial strains such as *Escherichia coli* and *Klebsiella pneumonia* (growth of culture adjusted according to McFards Standard, 0.5%). Wells of approximately 10mm diameter were bored using a well cutter and 25µl, 50µl and 100µl of the extracts were added to the wells from a stock concentration of 0.1g/1ml. The plates were then incubated at 37°C for 24 hours. Anti-bacterial activity was assayed by measuring the diameter of the inhibition zone in millimetres formed around the wells. Streptomycin (standard anti-bacterial agent, concentration: 20mg / ml) was used as a positive control.

Anti-fungal activity: - Anti-fungal activity was also determined by Agar Cup Method. Potato Dextrose agar plates were prepared and overnight grown isolates of fungi such as *C. albicans* and *A. niger* were swabbed. Wells of approximately 10mm diameter were bored using a well cutter and extracts of 25 µl, 50 µl and 100 µl concentrations were added and the zones of inhibition were measured after overnight incubation which were then compared with that of standard antibiotics. Clotrimazole (standard antimycotic agent, concentration: 10mg / ml) was used as a positive control.

RESULT AND DISCUSSION

Our approach involved the germination, harvest, extraction and anti-microbial evaluation of the chloroform leaf extracts of *Triticum aestivum* L.

ANTI-BACTERIAL ACTIVITY

Anti-bacterial activity was assayed in vitro by Agar Cup Method against two clinical gram negative isolates of bacteria such as *E. coli* and *K. pneumonia*. Standard antibiotics were tested for their activity and their zones of inhibition were recorded. Table1 shows the zone of inhibition produced by the extracts on Muller Hinton agar against respective bacterial strains.

Table 1: Zone diameter of inhibition of Chloroform leaf extract of *Triticum aestivum* L.

Test Organisms	Zone of inhibition in mm			Positive Control
	Concentration of leaf extracts (µl)			
	25	50	100	
E. coli	10	13	18	45
K. pneumonia	-	10	13	40

Sample Stock: 1mg/ml DMSO

Among the varying concentration of extracts of *T. aestivum* L. leaf, maximum inhibition was shown against *E. coli* compared to *K. pneumonia* (Table1) (Plate 1& 2)

The sequence of anti-bacterial activity of leaf extract against *E. coli* exhibited a fairly good activity in all the three different concentrations with 10, 13 and 18 mm zones of inhibition respectively. Whereas *K. pneumonia* exhibited no activity in 25µl concentration but produced a 10 and 13 mm zones of inhibition in 50 and 100µl concentrations of the leaf extracts. Anti- bacterial activity was expressed at varying degrees with the difference in concentration of leaf extract as higher concentration shows highest anti-bacterial activity. The result obtained shows that wheat grass exhibit anti-bacterial property and this might be considered sufficient for further studies for the evaluation of possible anti-microbial activity of other extracts of the plant (Table1) (plate 1 & 2).

Anti-bacterial activity of the chloroform leaf extract of *Triticum aestivum* L. against *E. coli* and *Klebsiella pneumonia*

Plate 1

Plate 2

Plates 1 & 2 showing the zone of inhibition produced by the chloroform leaf extracts of *Triticum aestivum* L**.** at 25µl, 50µl & 100µl concentrations against *E. coli* and *K. pneumonia* .

ANTI-FUNGAL ACTIVITY

The anti-fungal activity of *Triticum aestivum* L. (leaf chloroform extract with DMSO) was assayed invitro by Agar Cup Method against two clinical fungal isolates such as *Candida albicans* and *Aspergillus niger* (Table 2, Plate 3 & 4) shows the zone of inhibition produced by the extracts on Muller Hinton agar against respective fungal strains.

Table 2: Zone diameter of inhibition of Chloroform leaf extract of *Triticum aestivum* L.

Test Organisms	Zone of inhibition in mm			Positive Control
	Concentration of leaf extracts (µl)			
	25	50	100	
C. albicans	10	10	15	30
A.niger	10	16	22	34

Concentration of Sample Stock: 1mg/ml DMSO

Table2 shows the zone of inhibition produced by the extracts against the respective fungal strains. *A.niger* exhibited maximum activity when compared to *C. albicans* in all concentrations. The sequence of anti-fungal activity of plant extract against *A.niger* produced a 10 mm inhibition zone in 25µl, 16mm in 50µl and a 22 mm inhibition in 100µl concentrations of leaf extract respectively. Thus the chloroform leaf extracts of *T. aestivum* L. was found to have high anti-fungal property.

Anti-fungal activity of the chloroform leaf extract of *Triticum aestivum* L. against *Candida albicans* and *Aspergillus niger*

Plate 3

Plate 4

Plate 3 & 4 showing the zone of inhibition produced by the chloroform leaf extracts at 25µl, 50µl & 100µl concentrations against *Candida albicans* and *Aspergillus niger*

CONCLUSION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts. Antioxidant and antimicrobial properties of various extracts from many plants have recently been of great interest in both research and in food industry, because of their possible use as natural additives to replace synthetic antioxidants and antimicrobials with natural ones. The present study highlights the possible use of *T. Aestivum* (L) leaf extracts as a source of antioxidants and as antibacterial agents that can be used to prevent enteric diseases. *T. aestivum* L. chloroform extracts had shown better anti-fungal activity than anti-bacterial activity. Hence it can be concluded that WGJ would direct to the establishment of some compounds that could be used to invent new and more potent anti-microbial drugs of natural origin. Therefore future research should be addressed on the application of using Wheat grass as natural remedy and to protect against infectious diseases.

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