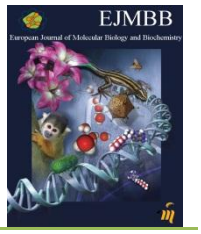
e - ISSN - 2348-2206
Print ISSN - 2348-2192

European Journal of Molecular Biology and Biochemistry

Journal homepage: www.mcmed.us/journal/ejmbb




# ANTIMICROBIAL INVESTIGATION OF WHEAT GRASS (*TRITICUM AESTIVUM L.*) AGAINST *ESCHERICHIA COLI*

**Deshwal VK* and Deepshikha**

Department of Microbiology, BFIT Group of Institutions, Sudhowala, Dehradun, India.

**Article Info**

*Received 11/08/2017*
*Revised 16/09/2017*
*Accepted 28/11/2017*

**Key words:-** *Triticum aestivum* L., *E. coli*, antibacterial.

**ABSTRACT**

Aim of this study was to evaluate the antibacterial activity of wheat grass (*Triticum aestivum L.*) against *E. coli*. *E. coli* was isolated from the urine of UTI infected patient. Urine sample was culture on EMB agar medium and these pure culture strains were characterized according to Bergey's Manual of determinative bacteriology. The 50% water extract of wheat grass (*Triticum aestivum L.*) was prepared and observed its antimicrobial activity against isolated *E. coli*. Maximum inhibition zone (28.66 mm) was seen by extract (concentration 50µg/ml) of 5 days old wheat grass and extract of wheat sprouts showed 27.66 mm inhibition zone against *E. coli*. 100 µg/ml water extract of wheat grass showed 160% more inhibition zone against *E. coli* as compared to 50 µg/ml Norfloxacin antibiotics. Present study confirmed that wheat grass (*Triticum aestivum* L.) significantly inhibited the growth of *E. coli* and can be good alternative of chemical antibiotics.

## INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of which based on their use in traditional medicine. It has been noted that the original source of many important pharmaceuticals currently in use have been plants used by indigenous people [1,2]. Herbal medicine or phyto-medicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes [3].

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 [4,5]. Plants contain thousands of constituents which are valuable sources of new and biologically active molecules having antimicrobial properties [6].

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. Wheat grass juice contains almost all the nutrients the body requires and is considered to be a complete food [7]. *T. aestivum* showed rich source of Vitamins A, C, E and B complex, including B12 [8]. *T. aestivum* showed anti-inflammatory, antioxidant, anticarcinogenic, immune modulatory, laxative, astringent, diuretic, antibacterial, anti-aging properties and use in acidity, colitis, kidney malfunctions, atherosclerosis and swelling has been shown to be beneficial [9]. Similarly**,** wheat grass juice used for treatment to cure some disease like Cooley's anemia and haemolytic anemia [10], asthma and some allergenic infections [11], inflammatory bowel diseases [12]. It has demonstrated anti-cancer properties both *In-vitro* and *In-vivo*; [13-14]. Further, wheat grass extract has a high content of bioflavonoids which may add towards anti-microbial effects [15]. Wheat grass juice showed antimicrobial effect against certain pathogens [16-17].

*E. coli* is the most common cause of urinary tract infection (UTI) [2,18]. These Uropathogenic *Escherichia coli* (UPEC) is the most common etiologic agent, responsible for 80 to 85% of community- acquired UTIs

Corresponding Author

**Deshwal VK**
Email: - vishal_deshwal@rediffmail.com

[19]. Further, *E. coli* is common causative organism of UTI and was declared as multiple drug resistant [20]. Aqueous extract of *Triticum aestivum* (wheat grass) antimicrobial activity against *E. coli*, *E. faecalis*, *B. subtilis*, *S. aureus* [21].

In recent years, it has become a public health concern because of the development of multiple antimicrobial resistant strains, emphasizing the importance of continuous monitoring of the pathogen. Therefore, the present study aimed to investigate *in vitro* study of antimicrobial investigation of wheat grass (*Triticum aestivum L.*) against *Escherichia coli.*

## MATERIAL AND METHODS

### Isolation of microorganism

Urine sample was collected in a sterilized container from a patient suffering from urinary tract infection. The mid-stream urine was collected after carefully cleaning the genitalia and mid-stream urine was collected because the first portion of urine may contain most of contaminants. Pathogens were isolated and counted by standard plate method and EMB agar medium was used for isolation of *E. coli*. The plates were incubated at 37$^{o}$C for 24-48h.

### Characterization of pathogens

*E. coli* was characterized according to Bergey's Manual of Determinative Bacteriology [22].

### Preparation of 50% aqueous extract

Each of 15 gm sample (wheat grass) was taken in a sterile mortar and pestle, and macerated to form a coarse paste. In a conical flask, 50% aqueous solvent was prepared by mixing 50 ml of acetone and 50 ml of distilled water (1:1 ratio). Each sample was put in separate flask and the flask was plugged properly and covered with aluminum foil. This was kept in a water bath at a temperature of 50-60 deg C, for 7-8 hours. After the boiling was done, the mixture in the flask was allowed to pass through Whattman filter paper no. 1. A filtrate was obtained. This filtrate was put in a dried beaker and kept in water bath open, till all the filtrate evaporated leaving behind a film of plant residue. The beaker was weighed before and after the evaporation of the filterate, to know that how much residue was obtained. This residue is used further for the analysis and antimicrobial study.

### Antibacterial activity of plant extract

Antibacterial activity was performed by disc diffusion method. The microorganism was activated by inoculating a loopful of the strain in nutrient broth and incubated at 37$^{o}$C at 120 rpm. After 24 hrs, 0.2ml of inoculum ($10^{8}$ cells/ml) was spread on Mueller Hinton agar media. With help of sterile forceps 7 mm sterilized filter paper discs were placed in the centre of the plate. One plate was labelled for each organism, and single extract was put in one plate. 100 µl of extract was dispensed on the centre of the disc. The plates were carefully placed in the incubator in an upright position at 37°C for 24 hours. Inhibition zones size was measured using a regular scale. Diameter of disc was subtracted from the zone of inhibition so obtained. The procedure was repeated three times to confirm reproducibility of the results.

## RESULTS AND DISCUSSION

*E. coli* strains were isolated from the urine of UTI infected patient. *E. coli* showed the green metallic sheen on selective media i.e. EMB agar medium. Therefore, we selected only 3 strains which showed green metallic sheen on EMB agar medium and named as EC-S1, EC-S2 and EC-S3. Microscopic examination of isolated strains was done by Gram's staining and observed that all three strains were Gram negative rods. Further, these strains were characterized by various biochemical tests. All isolated *E. coli* strains showed Indole test positive, Methyl red test positive, Nitrate reduction test positive and TSI acidic slant/ acidic butt and gas production (Table. 1). All these biochemical tests showed that isolated strains were *E. coli*. A similar result has been observed [23].

The 50% water extract of wheat grass (*Triticum aestivum L.*) was prepared and observed its antimicrobial activity against isolated *E. coli*. Maximum inhibition zone (28.66 mm) was seen by extract (concentration 50µg/ml) of 5 days old wheat grass and extract of wheat sprouts showed 27.66 mm inhibition zone against *E. coli*. Further, results suggested that as growth of plant is increased than decrease in antimicrobial activity against *E. coli* (Table 2). Further, we evaluated the antimicrobial activity of wheat grass with different concentrations of antibiotics against *E. coli.* Water extract of wheat grass showed antimicrobial activity against *E. coli*. 100 µg/ml water extract of wheat grass showed 160% more inhibition zone against *E. coli* as compared to 50 µg/ml Norfloxacin antibiotics. 50 µg water extract showed 20 mm inhibition zone and 25 µg/ml water extract showed 10 mm inhibition zone against *E. coli* (Table 3). Similar antimicrobial activity of medicinal plants has been studied [24-27]. Similarly, antimicrobial activity of wheat grass extracts of five different solvents (water, ethanol, methanol, ethyl acetate and hexane) against food borne pathogens. Similarly, aqueous extract of *Triticum aestivum* (wheat grass) showed maximum antimicrobial activity against *E. coli* (30.6 mm), *E. faecalis* (26.4 mm), *B. subtilis* (25.7 mm) and *S. aureus* (20.8 mm) diameter inhibitory zone in aqueous extract [28].

**Table 1. Biochemical characterization of *Escherichia coli.***

| Serial no. | Name of Biochemical test | EC-S1 | EC-S2 | EC-S3 | Final Result |
|---|---|---|---|---|---|
| 1. | MR | + | + | + | Positive |
| 2. | VP | - | - | - | Negative |

| | | | | | |
|---|---|---|---|---|---|
| 3. | Indole | + | + | + | Positive |
| 4. | Citrate | - | - | - | Negative |
| 5. | $NO_3$ reduction | + | + | + | Positive |
| 6. | Urease | - | - | - | Negative |
| 7. | TSI | A/A, G | A/A, G | A/A, G | A/A, G |
| 8. | $H_2S$ | - | + | - | Negative |

**Table 2. *In vitro* antibacterial activity of 50% water extract of wheat grass against *E. coli*.**

| Samples on different days | Mean of WGJ (50µg/ml) | Norflox antibiotic (100 µg /ml) |
|---|---|---|
| Sprouts | 27.66 mm | 41 mm |
| 5th Day old wheat grass | 28.66 mm | 41 mm |
| 10th Day old wheat grass | 16 mm | 40 mm |
| 15th Day old wheat grass | 16 mm | 41 mm |

**Table 3. Comparative antibacterial study of wheat grass and Norfloxacin against *E. coli***

| S. no. | Different concentration of Norfloxacin/ Wheat Grass(WG) | Inhibition zone against *E. coli* in diameter (mm) |
|---|---|---|
| 1. | 100 µg Norfloxacin/ ml | 40 mm |
| 2. | 50 µg Norfloxacin/ ml | 20 mm |
| 3. | 100 µg WG/ml | 32 mm |
| 4. | 50 µg WG/ml | 20 mm |
| 5. | 25 µg WG/ml | 10 mm |
| 6. | Blank | No zone |

## CONCLUSIONS

Present study shows that wheat grass (*Triticum aestivum* L.) significantly inhibited the growth of *E. coli* and it's medicinal value improve its application. Use of antibiotic has side effect to human so it is necessary search for new antimicrobial substance. Medicinal plants are good alternative of chemical antibiotics.

## STATEMENT OF HUMAN AND ANIMAL RIGHTS

All procedures performed in human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

## ACKNOWLEDGEMENT

Nil

## CONFLICT OF INTEREST

No interest

## REFERENCES

1. Balick MJ, Cox P. (1996). Plants, people and culture. *The Science of Ethanobotany, Scientific American Library*, New York, USA, 219.
2. Deshwal VK, Vig K, Tripathi PK. (2014a). *In vitro* synergistic effect of antibacterial activity of *Piper nigrum* and *Rauwolfia serpentina* against certain pathogens. *Euro J Mol Biol and Biochem*, 1(4), 128-133.
3. Barrett B, Kiefer D, Rabago D. (1999). Assessing the risks and benefits of herbal medicine, An overview of scientific evidence, *Altern Ther Health Med*, 5, 40-49.
4. Kothari S, Jain AK, Mehta SC and Tonpay SD. (2008). Effect of fresh *Triticumaestivum*L. grass juice on lipid profile of normal rats. *Ind J Pharmacol*, 40(5), 235-236.
5. Mujoriya R. (2011). A study on wheat grass and its nutritional value. *Food Sci Quality Manag*, 2, 1-9.
6. Das A, Raychaudhuri U, Chakraborty R. (2012). Anti-microbial effect of edible plant extracts on the growth of some food borne bacteria including pathogens. *Nutra foods*, 12, 83-88.
7. Murali M, Nair AS, Kumar NS. (2016). *in vitro* anti-microbial activities of wheat grass. *J Pharmaceut Sci Innov*, 5(6), 201-204.
8. Ashok SA. (2011). Phytochemical and Pharmacological screening of wheat grass Juice (*Triticum aestivum* L.). *Int J Pharmaceutical Sci Review Res*, 9(1), 029.
9. Rana S, Kamboj JK, Gandhi V. (2011). Living life the natural way – Wheatgrass and Health. *Funct Foods in Heal Dis*, 11, 444-456.
10. Fernandes CJ, Donovan DJ. (2005). Natural antioxidant therapy for patients with hemolytic anemia. *Ind Pediatrics*, 42, 618-619.

11. Andrew F, Sarah L, Weiss S, Britton J. (2000). Dietary Vitamin E, IgE Concentrations and Atopy. *The Lancet*, 9241, 1573-1574.
12. Shah S, (2007). Dietary Factors in the Modulation of İnflammatory Bowel Disease Activity. *Medscape Gen Medi*, 9(1), 60.
13. Alitheen, NB, Oon C.L, Keong YS, Chaun, TK, Li HK, Yong HW. (2011). Cytotoxic effects of commercial wheatgrass and fibre towards human acute promyelocyticleukemia cells (HL60). *Pak J Pharmaceu Sci,* 24(3), 243-250.
14. Arya P, Kumar M. (2011). Chemoprevention by Triticum aestivum of mouse skin carcinogenesis induced by DMBA and croton oil-association with oxidative status. *Asian Pacific J Cancer Preven*, 12(1), 143.
15. Kapil S. (2012). The Elixir of Life, Wheatgrass Juice, *Popul Book Depot.*, 302-310.
16. Pallavi K, Kumarswammy G, Shruthi. (2011). Pharmacognostic investigation and antibacterial activity of *Triticum aestivum*. *J Pharmacy Res*, 4(10), 3355-3359.
17. Pehlivanoğlu H, Gündüz HH, Özülkü G, Demirci M. (2015). An Investigation of Antimicrobial Activity of Wheat Grass Juice, Barley Grass Juice, Hardaliye and Boza. *Int Interdiscipl J Scienti Res*, 2(1), 8-14.
18. Jacobsen SM, Stickler DJ, Mobleym HLT, Shirtliff ME. (2008). Complicated Catheter-Associated Urinary Tract Infections Due to *Escherichia coli* and *Proteus mirabilis*. *Clin Microbiol Rev*, 21(1), 26-59.
19. Ronald AR, Nicolle LE, Stamm E, Krieger J, Warren J, Schaeffer A, Naber KG, Hooton TM, Johnson J, Chambers S, Andriole V. (2001). Urinary tract infection in adults: research priorities and strategies, *Int J Anti Agents*, 17, 343-348.
20. Sabir S, Anjum AA, Ijaz T, Ali MA, Khan MR, Nawaz M.(2014). Isolation and antibiotic susceptibility of E. coli from urinary tract infections in a tertiary care hospital. *Pak J Med sci*, 30(2), 389-392.
21. Rajaraman K, Balamurugan V, Susindran P, Venkatesan S, Sundaresan A, Vasanthi K. (2016). Preliminary phytochemical analysis and antibacterial activity of *Triticum aestivum* (Wheat grass) aqueous extract. *J Medical Health Research*, 1, 1.
22. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. (1994). Bergey's manual of determinative bacteriology, Williams and Wilkins press, Baltimore.
23. Lakshmi R, Jayavardhanan KK. (2016). Isolation and identification of major causing bacteria from bovinemastitis. *Int J Applied Pure Sci Agri*, 2, 45- 48.
24. Deshwal VK, Manisha, Chatterjee N, Md. Siddiqui, Srivastava P, Sharma M. (2014b). A Synergism between aqueous extract of leaves of the *Murraya koenigii* and Norfloxacin against *Klebsiella pneumonia*. *Int J Pharma Toxico*, 4(3): 171-174.
25. Deshwal VK, Vig K, Tripathi PK. (2014c). *In vitro* synergistic effect of antibacterial activity of *Piper nigrum* and *Tribulus terrestris* against certain pathogens. *Curr Pharma Clin Res*, 4(3), 163-168.
26. Deshwal VK. (2013a). Antimicrobial investigation of *Piper nigrum* L. against *Salmonella typhi*. *J Drug Deliv Therap*, 3(3), 100-103.
27. Deshwal VK. (2013b). Antibacterial investigation of black pepper against *Shigella dysenteriae*. *Journal of Plant Development Sciences*. 5(1): 89-90
28. Sundaresan A, Selvi A, Manonmani HK. (2015). The Anti-Microbial Properties of *Triticum aestivum* (Wheat Grass) extract. *Int J Biotechn for Wellness Indu*, 4, 84-91.