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Research Article

Pharmacognostic, Ethnopharmacological, Phytochemical Screening of *Echinochloa Frumentacea* For Its Anti-Bacterial Activity

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ABSTRACT

Bacterial infection is highly spread in all over the world at a large scale and very common in now a day. microbial infections are a very severe and dangerous infection which can cause harmful effects. *Echinochloa frumentacea* is a millet, capable to show antibacterial activity. It is known as Sawa rice, Shyama rice, vratkechawal and belongs to Poaceae family. It is cultivated in Garhwal region of Uttarakhand. Highly nutritional rich and full of dietary fibres (12.8%), Proteins (10.1%), Carbohydrates (68.8%), fat (3.9%), Irons(5mg), Phosphorus(281mg) are present in millet. Extraction of millet was done by Soxhlet apparatus and observe that the ethanolic extraction of millet has yield percentage comparison to aqueous extraction. Anti-bacterial testing was done by the Disc diffusion method and check the effectiveness of plant extract by calculating zone of inhibition against both strain of bacteria E. coli. Ethanolic extraction of millet have high zone of inhibition comparison to aqueous against EHEC (Enterohemorrhagic bacteria) and ETEC (Enter toxicogenic bacteria). For the testing of antibacterial activity of millet *Echinochloa frumentacea*, 2 different extraction was used, Ethanolic and Aqueous extraction. The yield percentage of ethanolic extraction was higher than aqueous extraction used Disc diffusion method and also measure the zone of inhibition. By performing the phytochemical test, indicated the presence of glycosides, phenolic compounds, flavonoids, amino acids etc. according to the zone of inhibition report, Ethanolic extraction of millet has higher antibacterial activity on both bacteria strain of E. coli (EHEC and ETEC). MIC for ethanolic extraction on EHEC was 0.5mg/ml, for aqueous 1mg/ml, for ethanolic extraction on ETEC was 0.5mg/ml, for aqueous 1mg/ml.

Keywords: *Echinochloa frumentacea*, Soxhlet apparatus, Disc diffusion method, Zone of inhibition, *Escherichia coli*, Anti-bacterial method.

INTRODUCTION: -

Bacterial infection is highly spread in all over the world at a large scale and very common in now a day. microbial infections are a very severe and dangerous infection which can cause harmful effects. It is caused by the invade of microbes in the body. Most commonly found in world and highly responsible to cause infection disease. Cell wall is made up of Peptidoglycan, also capable to synthesize their RNA, proteins and DNA. On the basis of staining 2 types of bacteria – gram positive or gram-negative bacteria. (Doron.et al 2008) Most of the bacteria are not harmful and even more helpful. Some can cause life threatening complications. Certain body parts are frequently the targets of bacterial infection. The substances or drug which are used to overcome the bacterial infection, antibacterial drugs. They show antibacterial activity by working on 2 different mechanisms, by killing the bacterial or by inhibiting the growth of bacteria. They can work by inhibiting cell wall synthesis, inhibit protein synthesis or inhibit folate or DNA replication. (Kadner R.J et al., 2024)

Escherichia coli is a gram-negative bacterium. German Physician Thedor Escherich was discovered the E. coli in 1885. They are able to evolve into smooth, colorless colonies on non-selective medium. 1-2 mm in diameter nutritional agar after 18 hours temp. 15-45^{o}C. it is a normal flora of mouth and intestine. (Depta P., Swain K.S et al., 2020) It is excreted in faeces of man and animals in very large number and contaminate soil and water infection, caused by consuming contaminated water and food. It can cause 4 type of clinical syndrome – urinary tract infection, diarrhoea and dysentery, Pyogenic infection and septicaemis. It can also cause Pneumonia, Bacteremia and peritonitis, urinary tract infection. (Van S. et al., 2017) E. coli is mainly cause Nosocominal infections including Catheter associated UTIs and Ventilator associated Pneumonia. Commonly found in vegetables, in soil and in water, in undercooked meats. There are 5 strains of E. coli which are responsible to cause different diseases. like: - *Enterotoxigenic Escherichia coli (ETEC), Enterohemorrhagic Escherichia coli & Shigatoxin Escherichia coli (EHEC), Enter-invasive E. coli (EIEC), Entero-pathogenic Escherichia coli (EPEC), Entero-aggregative Escherichia coli (EAEC). Enterotoxigenic Escherichia* coli ingested into the human body for causing diseases. it can cause watery diarrhoea and commonly found in food and water. It is a single organism causing traveller's diarrhoea. Dehydrating diarrhoea illness in infants and children. (Choudhary S.T. 2019) Fever, nausea with or without vomiting, loss of appetite, headache, muscle ache and bloating can also occur. *Enterohemorrhagic E. coli* responsible for bloodydysentery and increased the risk of haemolyticuremic-syndrome (HUS). It can produce Shiga toxin and vero-toxin, which mediates dysregulation of the gut epithelial membrane's membrane ion channels, resulting in considerable water loss and ion loss. Kidney, gastrointestinal the most often impacted organ in HUS is the digestive tract, yet there has also been evidence of involvement in the CNS, pancreas, skeletal system, and heart studied. (King K.C et al., 2023)
The study of global burden disease (GBD) 2019, avai-lable across age group and in terms of gender between 1990 and 2019. Study is related to diarrhoea disease – number of deaths, mortality rate (per 100,000 populations) in 2019 in India for each group or sex. The total number of deaths of all ages is 632,344 in 2019 (95% uncertainly interval 358,561- 1,056,036) and the mortality rate is 45 per 100,000 population. Entero-toxicogenic E. coli is responsible to cause death 26690 in children and 22668 in all ages and Enterohemorrhagic E. coli cause death, 1556 in children and 3393 in all ages. E. coli is responsible to cause fatalities from hemolytic uremic syndrome, severe dehydration, anemia, mental abnormalities such disorientation, and kidney failure. Stool culture and stool inspection procedures are used to test for E. Coli infections. (Wisplinghoff H, Seifert H, et al., 2003)
Echinochloa frumentacea is a millet, capable to show antibacterial activity. It is known as Sawa rice, Shyama rice, vratkechawal and belongs to Poaceae family. It is cultivated in Garhwal region of Uttarakhand. Highly nutritional rich and full of dietary fibres (12.8%), Proteins (10.1%), Carbohydrates (68.8%), fat (3.9%), Irons(5mg), Phosphorus(281mg) are present in millet. It is able to show lots of pharmacological activity like Antidiabetic, Anti-inflammatory, Anti-oxidants etc. (Rogers, K. et al., 2024), (Sizar O, Leslie SW, et al., 2024) his plant is available in India, Pakistan and Nepal. Plant is tall and grow 220cm high, fast growth and complete life cycle 45-60 days. Roots are fibrous and shallow. (Borkar Vijay et al.2020) Essential amino acids are present in plant are Isoleucine, Leucine, Lysine, Methionine, Threonine, Valine, Histidine, Tryptophan. Main polyphenols tannin and flavonoids are responsible for body immune system. (Valsanker K. et al.,2020) Many invitro methods are working to test antibacterial activity of any plant extract and disc diffusion method is one which we are using in our research work.(Kaur Hardeep, Sharma Shilpa et al., 2020)

MATERIAL AND METHODS: -

1.Procurement and Authentication of plant: - *Echinochloa Frumentacea* is a millet and commonly found in Uttarakhand. Millet was procured and collected from Garhi cantt. Dehradun and get authentication from Shaheed Durga Mall Govt. Post Graduate College, Doiwala, Dehradun. After the authentication, we dry the seeds at the room temp. and stored in air tight container save from moisture content. Millet seeds were collected, washed in water, dried in shade, packed in air tight container, save from moisture content. It prevents the degradation of bioactive component in plants due to sunlight. The dried leaves were ground into fine powder and further use for extraction.

2.Selection of solvent: Both solvent Ethanol and Aqueous has contained the soluble phytochemical during extraction, which are responsible for antibacterial activity. (Fatima R et al., 2023) So, both of the solvent, Ethanol and Aqueous was used in the extraction process. (Plaskova A. 2023)

3.Extraction of plant: - For the extraction of drug, Soxhlet extraction method was used.[14] Millet seeds were collected, washed in water, dried in shade, and make coarse powder by using pestle mortar. Two different extractions of millet have used for the testing of anti-bacterial activity of millet.(Sander LC. 2017) The first one is Ethanolic extraction and second is Aqueous extraction. For both extractions, Soxhlet apparatus was used. It uses solvent reflux and Siphon principle to continue extraction the solid matter by pure solvent, saves efficacy and efficiency of extraction. (Dai J. et al., 2010)

For Ethanolic extraction: - Measure 50gm of coarse powder of *Echinochloa Frumentacea* seeds and packed in thimble. Take 500 ml of ethanol as solvent. Ratio of drug: solvent is 1:10 and temperature for the extraction is 50^{0}C. this process has taken 42-46 hours. After completing the extraction process. Dry the extract by using water bath at 80-90^{0}C temperature. After drying it, weigh the dry extract and the weight of dry extract is 3.37 gm. (Alara, O. R. et al., 2021)
The yield percentage which was found after the ethanolic extraction of millet seeds was 6.74%.

Fig.(a) Ethanolic extraction

For aqueous extraction: - weigh 50gm of coarse powder of seeds *E. Frumentacea* and covered in thimble. Take 500 ml of distilled water as solvent. Ratio of drug: distilled water is 1:10 and temperature 60-70^{0}C. this process has taken 30-36 hours for extraction. After completing the extraction process. Dry the extract by using water bath at 80-90^{0}C temperature. After drying it, weigh the dry extract and weight is 2.67gm.
The Yield percentage which was found after the aqueous extraction of millet was 2.67%.

Fig.(b) Aqueous extraction

4.Selection of Bacteria: - For testing antibacterial activity of millet *E. Frumentacea*, two strains of bacteria *E. coli, Enterotoxigenic Escherichia coli (ETEC), Enterohemorrhagic Escherichia coli (EHEC). E. coli* grows well on non-selective media forming smooth colourless colonies 2-3mm in diameter in 18-hour nutrient agar temp. 15-45^{0}C. E. coli is normal intestinal flora of man and animals. It can cause urinary tract infection,

Pneumonia, diarrhoea etc. ETEC can cause watery diarrhoea and present in food and water. EHEC responsible to cause bloody dysentery and also increased risk of haemolytic uremic syndrome (HUS). For the growth of bacteria, prepare nutrient agar media. The composition for the media is nutrient agar, beef extract, peptone sodium chloride, distilled water. Collect all the ingredient, weigh and mixed with water. Heat the solution in water bath until it become transparent. After that cool down the mixture and add LB – media (Luria – Bertani broth) by using sterile inoculating loop. Incubate the culture media in incubator for 48 hours.

5. Phytochemical analysis: -

S.No	Phytochemical Test	Procedure	Result
1.	Alkaloids	Dragendroff test: - Few ml filtrate + 1 ml reagent	Redish brown colour
2.	Alkaloids	Hager'stest:1ml of filtrate+ 1-2ml Hager'sreagent	Creamy white precipitate
3.	Cardiac glycoside	Brominewater: - a little amount of bromine water and plant extract	Yellow precipitate
4.	Amino-acids	One drop of ninhydrin solution (10 mg ninhydrin + 200 ml acetone) plus two milliliters of filtrate	A purple-coloured solution
4.	Phenols	(i) Lead acetate solution: 1 milliliter of plant extract with 3 milliliters of 10% lead acetate (ii)Iodine test: - 1ml extract + few drops of dil. Iodine solution	Bulky white precipitate A transient red colour
5.	Flavonoids test	Lead acetate: 1 milliliter of plant extract plus a few drops of 10% lead acetate unite to form a yellow precipitate.	A yellow precipitate
6.	Anthocyanins	HCL test: - 2ml 2N HCL (+ few ml ammonia)- pink red solution which turns blue after adding ammonia	Pink red solution which turns blue violet after addition

Table (1) Phytochemical test (Shaikh R. et al., 2020)

6. Anti-bacterial method: - To perform the antibacterial test of millet *Echinochloa frumentacea* on 2 strains (ETEC and EHEC) of bacteria E. coli. For the testing, disc diffusion method has used.(Balouiri M. 2015) Grow the bacteria by using LB media (Luria-media broth) after growing the bacteria apply antibiotic plant extract with the help of swab. Incubate the plate for 24 hours. Sterilizing the area with disinfected and burner.(CLSI, 2012) Dipping a sterile cotton swab into inoculum and removing excess media by pressing swab on to wall of tube. Swabbing the surface area of agar plate that has been inoculated with targeted bacteria by rotating plates. Allow the plates to dry for 5mins so the media absorb inoculum properly.(CLSI, 2004)

Enterohemorrhagic E. coli: -

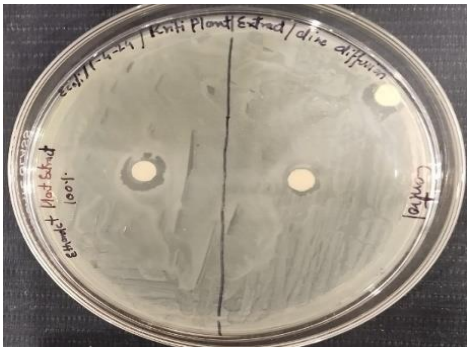


Fig.(c) Disc diffusion method Ethanolic plant extract on EHEC

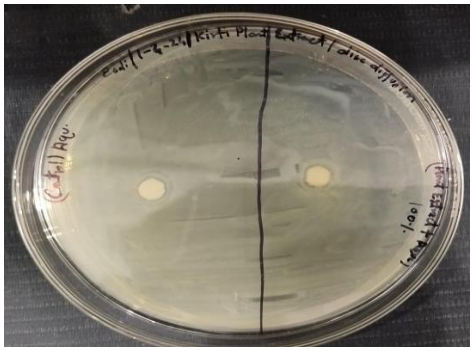


Fig.(d) Disc diffusion method of aqueous plant extract on EHEC

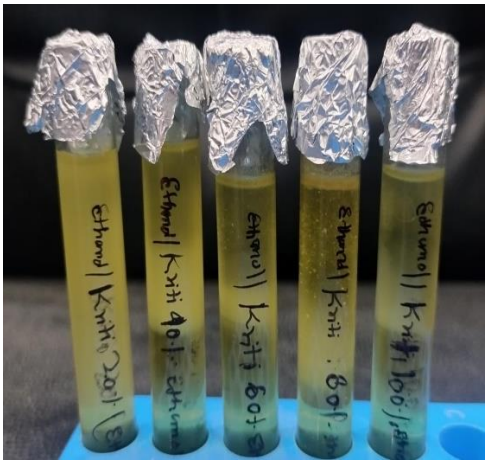


Fig.(e)MIC of ethanolic extraction of millet

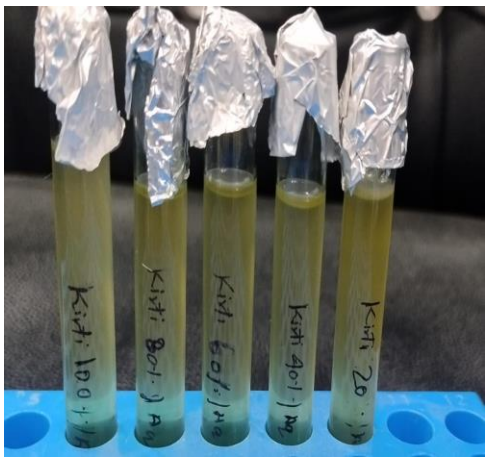
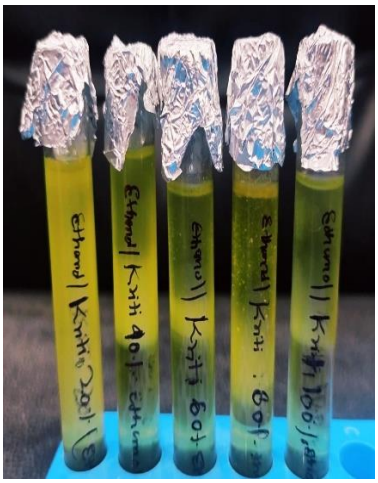


Fig.(f) MIC of aqueous extraction of millet

Enterotoxicogenic E. coli: -



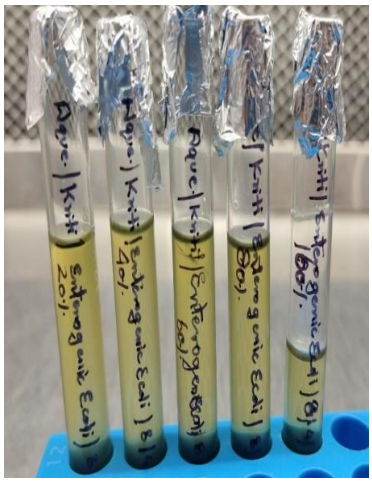


Fig.(g)Disc diffusion method. Fig.(h)MIC of ethanolic extraction. Fig.(i)MIC of aqueous ext.

Zone of inhibition: - Zone of inhibition is the surrounding area of anti-microbial agent where anti-microbial agent is applied. It is size of area where microbes cannot grow. For the measurement of zone of inhibition, hold agar plate upside down over black surface. For measuring the diameter of zone of inhibition, use ruler for measuring in millimetre. The efficacy of antibiotics or other antimicrobial agents against bacteria is evaluated using this method. (Balouiri, M. et al.,2016) More expansive zones suggest that the bacteria are more vulnerable to the chemical.

Minimum inhibitory concentration (MIC): - The lowest dose of a substance—typically a drug—that stops bacteria or fungi from growing visibly in vitro is known as the minimum inhibitory concentration (MIC). The stock of plant extract of different concentration was prepared in 5ml DMSO. Plant extract with different quantity, added to DMSO for preparing different concentration of DMSO which contains plant extract.(Taillefer, B., Grandjean, M et al., 2023) Each test tube has contained different concentration (20%, 40%, 60%,80%,100%). Each test tube, contained bacterial inoculum and plant extract were incubated at 37°C in incubator. After 24 hours the test tube was examined visually for turbidity.[20] The lowest concentration of leaf extract at which there was no visual growth of bacteria and showed 100% transmission of light, there is no bacterial growth. Concentration should be reported as MIC of that antibiotic for particular strain. (Mahira, A. Jain, W. Khan et al., 2019)

RESULT & DISCUSSION: -

Two different extractions were performed for the evaluation of anti-bacterial activity of millet seeds. The yield percentage of ethanolic extraction was more than Aqueous extraction.

Phytochemical analysis: -

S.No	Phytochemical test	Procedure	Result
1.	Alkaloids	Dragendroff test: - Few ml filtrate + 1 ml reagent – reddish brown colour appears.[15] Hager's test: - Few ml of filtrate+ 1-2ml Hager's reagent – creamy white precipitate.	Negative
2.	Cardiac glycoside	Brominewater: - a little amount of bromine water and plant extract[15]	Positive
3.	Amino acids	One drop of ninhydrin solution (10 mg ninhydrin + 200 ml acetone) plus two milliliters of filtrate[15]	positive
4.	Phenols	(i)Lead acetate: - Lead acetate solution: 1 milliliter of plant extract with 3 milliliters of 10% lead acetate – bulky white precipitate.[15] (ii)Iodine test: - 1ml extract + dew drops of dil. Iodine solution – a transient red colour	Positive Positive
5.	Flavonoids	Lead acetate: 1 milliliter of plant extract plus a few drops of 10% lead acetate unite to form a yellow precipitate.[15]	positive
6.	Anthocyanin	HCL test: - 2ml 2N HCL (+ few ml ammonia)- pink red solution which turns blue after adding ammonia	positive

Table No. (2) showing the results of Phytochemical test

Fig. (J) Phytochemical testing

Zone of inhibition of test plant extracts: -

Micro-organism	Concentration	Ethanolic Extraction	Aqueous extraction
Enterotoxigenic E. coli	1mg/ml	12mm ±0.5	10mm±0.5
Enterohemorrhagic E. coli	1mg/ml	11mm± 0.5	10mm±0.5

Table (3) Zone of inhibition of plant extract

Minimum inhibitory concentration: -

MIC was done by the broth dilution method or turbidity method, diluting broth in the 5ml of DMSO which contained plant extract. MIC of ethanolic extraction of millet on EHEC was 0.5mg/ml, aqueous extraction was 1mg/ml and MIC of aqueous extraction on ETEC was 0.5mg/ml, aqueous extraction on ETEC was 1mg/ml.

CONCLUSION: -

For the testing of antibacterial activity of millet *Echinochloa frumentacea*,2 different extraction was used, Ethanolic and Aqueous extraction. The yield percentage of ethanolic extraction was higher than aqueous

extraction. For evaluating the antibacterial activity of millet, used Disc diffusion method and also measure the zone of inhibition. By performing the phytochemical test, indicated the presence of glycosides, phenolic compounds, flavonoids, amino acids etc. according to the zone of inhibition report, Ethanolic extraction of millet has higher antibacterial activity on both bacteria strain of E. coli (EHEC and ETEC). MIC for ethanolic extraction on EHEC was 0.5mg/ml, for aqueous 1mg/ml, for ethanolic extraction on ETEC was 0.5mg/ml, for aqueous 1mg/ml. So, the millet has contained good anti-bacterial activity, can further move for its spectroscopy studies.

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