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PHYTOCHEMICAL ANALYSIS, ANTIBACTERIAL ACTIVITY AND FTIR ANALYSIS OF WHEAT AND RAGI AGAINST BACTERIA CAUSING NOSOCOMIAL INFECTION

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ABSTRACT:

In the present study phytochemical profile, antibacterial activity and FTIR of various extracts were evaluated. Qualitative phytochemical analysis of the extracts showed the presence of alkaloids, flavonoids and phenols in the aqueous extract of wheat and presence of alkaloids, tannins and phenols in the aqueous extract of ragi. Antibacterial properties were studied using kirby Bauer disc diffusion technique and the extracts showed antibacterial activity on *Enterococcus feacalis* and *Staphylococcus aureus.* FTIR analysis shows some differences in functional groups of various extracts of wheat and ragi. Finally, the presence of phytochemicals, antibacterial activity and functional groups concludes that these extracts have a broad spectrum of antibacterial potential and phytochemical properties which makes it as a candidate for bio prospecting of antibiotic drugs.

KEY WORDS:

Wheat (*Triticum aestivum*), Ragi (*Eleusine coracana*), Solvent extraction, Phytochemical analysis, Antibacterial activity, FTIR.

INTRODUCTION:

Plants contain numerous of constituents and are valuable sources of new and biologically active molecules having antimicrobial properties that are important for drug designing against diseases. Antibiotic resistance has become a global concern and clinical efficiency of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens. So, there is an urgent need to develop new antimicrobial compounds which are more active against newer and re-emerging infectious diseases. Wheat one of the members of Gramineae family, has been known for very efficient medicinal values and several therapeutic drugs that includes bran and germ, therefore, provides protection against various diseases such as constipation, heart diseases, appendicitis, obesity, diabetes and diseases of the colon called diverticulum. Wheat is an easily grown plant in the world and the young stems had proven effective for the treatment of biliousness, intoxication and removing skin blemishes, antipyretic, antihidrotic and sedative. *Triticum aestivum* has also been used against cough, sore throat, malaise, spasmic pain, abdominal coldness and constipation. Wheat is also known to have anticancer and antimicrobial properties. Due to bacterial expression of resistance to antibiotics, the development of new antiseptics and antimicrobial agents are of growing interest from different crops and cereals. A wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoid have been found to have antimicrobial properties in vitro. Wheat extract has a high content of bioflavonoids which may add towards antimicrobial effects. Finger millet is also called as 'ragi' which is consumed without dehulling is also a principal food grain of people belonging to low-income groups. It is rich in polyphenols and tannins and reported as a good source of antioxidants. Phenolics are the most abundant secondary metabolic products of plants. Plant polyphenols are an extensively used in cancer research due to their potent antioxidant properties and have been implicated for disease resistance in various research. The presence of phenolic acids in cereal grains has been confirmed in several studies. The polyphenolic content in cereals is usually less than 1% of dry matter and the main polyphenols in cereals are phenolic acids and tannins whereas flavonoids are present in small quantities. Polyphenols are the most important phytochemicals of the millet because of their nutraceutical potentials such as antioxidant activity, anti-inflammatory, anticarcinogenic, antimicrobial, antidiarrhoeal, antiulcer, and anti-cardiovascular properties. The acidic methanol extracts from the seed coat showed high antibacterial and antifungal activity compared to whole flour extract due to high polyphenols content in seed coat. In the present study phytochemical analysis, antibacterial activity and FTIR of wheat and ragi using various extract were determined.

MATERIAL AND METHODS:

1) COLLECTION OF SAMPLES:

Wheat (*Triticum aestivum*) and Ragi (*Eleusine coracana*) were purchased from Uzhavar sandhai in Coimbatore. The samples were grinded finely.

2) SELECTION OF MICROORGANISMS:

The Clinical Pathogens such as *Salmonella sp., Klebsiella sp., Enterococcus feacalis, Staphylococcus aureus and E. coli* were collected from the Bioline Laboratory, R S Puram, Coimbatore.

3) SOLVENT EXTRACTION:

20 gram of Wheat and Ragi powder was dissolved in 200 ml of solvent (Methanol, Hexane and Aqueous) in a conical flask, mixed and tightly plugged. It was kept in rotary shaker for 48 hours at a minimum rpm. Then it was filtered using Whatman filter paper, filtrate was evaporated in a petri dish at room temperature for 2-3 days until it evaporates. Then the dried samples were resuspended with a sterile Deionised (DI) water and stored in vials at 4˚c.

4) PHYTOCHEMICAL ANALYSIS:

I. TEST FOR ALKALOIDS (Hager's test)

2 ml of extracts was mixed with few ml of dilute Hydrochloric (HCl) Acid and filtered. The filtrate was added with few drops of Hager's reagent (Aqueous solution of Picric acid). A yellow precipitate indicates the presence of Alkaloids.

II. TEST FLAVONOIDS (Alkaline reagent test)

To a 2ml of extracts, few drops of NaOH solution was added, a yellow colour solution was formed. Then add few ml of diluted Hydrochloric (HCl) Acid which turns yellow colour solution into a colourless solution, which indicates the presence of Flavonoids.

III. TEST FOR TANNIS (Braymer's test)

A small amount of extract was mixed with 2ml of ferric chloride (FeCl3) and the colour change was recorded. The formation of green grey / dark blue colour indicates the presence of Tannins.

IV. TEST FOR SAPONINS (Foam test)

The extract and the distilled water were mixed as same volume and the mixture was shaken vigorously. The formation of a layer of foam indicates the presence of Saponins.

V. TEST FOR PHENOLS (Ferric chloride test)

To a 2 ml of extract, 1 ml of ferric chloride (FeCl3) solution was added. Deep blue-black colour indicates the presence of Phenols.

5) ANTIBACTERIAL ACTIVITY:

Select a pure culture plate of one of the organisms to be tested. Aseptically emulsify a colony from the plate in the sterile saline solution. Mix it thoroughly to ensure that no solid material from the colony is visible in the saline solution. Take a sterile swab and dip it into the broth culture. Gently squeeze the swab against the inside of the tube to remove excess fluid in the swab. Take a sterile Mueller-Hinton agar (MHA) plate Use the swab with the test organism to streak an MHA plate for a lawn of growth. After the streaking is complete, allow the plate to dry for 5 minutes. The well was punctured with a well cutter, then 30µl of extracts were loaded in the well. Streptomycin (10µg) was used as a control. Carefully invert the inoculated plates and incubate for 24 hours at 37° C. After incubation, use a metric ruler to measure the diameter of the zone of inhibition. The zone of inhibition was observed and tabulated.

Commented [KP1]:

6) FTIR:

Before FTIR analysis begins, the sample is prepared for testing using either the attenuated total reflectance (ATR), Nujol or other technique. Enough sample is required to obtain an absorption spectrum. The FTIR Spectrometer generates a graph in the form of an absorbance spectra, which shows the unique chemical bonds and the molecular structure of the sample material. This absorption spectrum will have peaks representing components present. These absorbance peaks indicate functional groups (e.g., alkanes, ketones, acid chlorides). Different types of bonds, and thus different functional groups, absorb infrared radiation of different wavelengths. The analytical spectrum is then compared in a reference library program to identify components or to find a “best match” for unknown material using the cataloged spectra for known materials. The results were plotted in the graph and tabulated.

RESULT AND DISCUSSION:

PHYTOCHEMICAL ANALYSIS:

The phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids and phenols in wheat and the presence of alkaloids, flavonoids, phenols and tannins in ragi. Flavonoids were detected in the methanol and aqueous extracts of wheat and in the methanol extract of ragi. Alkaloids were detected in the methanol and aqueous extracts of wheat and Ragi. Phenols were detected in the methanol extracts of ragi and aqueous extracts of wheat and Ragi. Tannins were detected only in the aqueous extracts of Ragi. These active constituents have been used as antibiotics, insecticidal, anthelmintic and antiseptic in the pharmaceutical industry.

TABLE-1: PHYTOCHEMICAL ANALYSIS OF WHEAT AND RAGI EXTRACT

S. No.	PHYTOCHEMICALS	WHEAT (METHANOL) EXTRACT	RAGI (METHANOL) EXTRACT
1.	Alkaloids	+	+
2.	Flavonoids	+	+
3.	Tannins	-	-
4.	Saponins	-	-
5.	Phenols	-	+
PRESENT (+) ABSENT (-)			

TABLE-2: PHYTOCHEMICAL ANALYSIS OF WHEAT AND RAGI EXTRACT

S. No.	PHYTOCHEMICALS	WHEAT (AQUEOUS) EXTRACT	RAGI (AQUEOUS) EXTRACT
1.	Alkaloids	+	+
2.	Flavonoids	+	-
3.	Tannins	-	+
4.	Saponins	-	-
5.	Phenols	+	+
PRESENT (+) ABSENT (-)			

ANTIBACTERIAL ACTIVITY:

The extracts were tested against *Klebsiella sp., Enterococcus faecalis, Salmonella sp., Staphylococcus aureus* and *E. coli*. The methanol and aqueous extracts of wheat did not show any activity on *E. coli*. The hexane and methanol extract of wheat did not show any activity on *Klebsiella sp*., The hexane extract of Ragi did not show any activity on *Salmonella sp.*, and *E. coli.* The methanol extract of ragi did not show activity on *Salmonella and Klebsiella.* The aqueous extract of ragi did not show activity only on *Salmonella sp.*, Streptomycin which is used as a control shows activity on all the organisms tested.

TABLE-3: WHEAT EXTRACTS

S.No.	ORGANISM	ZONE OF INHIBITION (mm)			
		HEXANE EXTRACT	METHANOL EXTRACT	AQUEOUS EXTRACT	CONTROL (STREPTOMYCIN)
1.	*Salmonella sp.,*	-	10	20	16
2.	*Klebsiella sp.,*	-	-	5	13
3.	*Enterococcus faecalis*	40	30	32	40
4.	*Staphylococcus aureus*	20	40	30	40
5.	*E. coli*	14	-	-	23

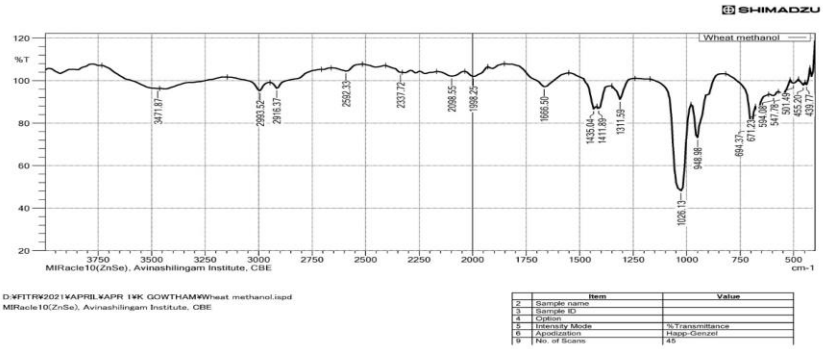
TABLE-4: RAGI EXTRACTS

S.No.	ORGANISM	ZONE OF INHIBITION (mm)			
		HEXANE EXTRACT	METHANOL EXTRACT	AQUEOUS EXTRACT	CONTROL (STREPTOMYCIN)
1.	*Salmonella sp.,*	-	-	-	16
2.	*Klebsiella sp.,*	40	-	15	13
3.	*Enterococcus faecalis*	30	26	20	40
4.	*Staphylococcus aureus*	22	40	38	40
5.	*E. coli*	-	18	9	23

FTIR:

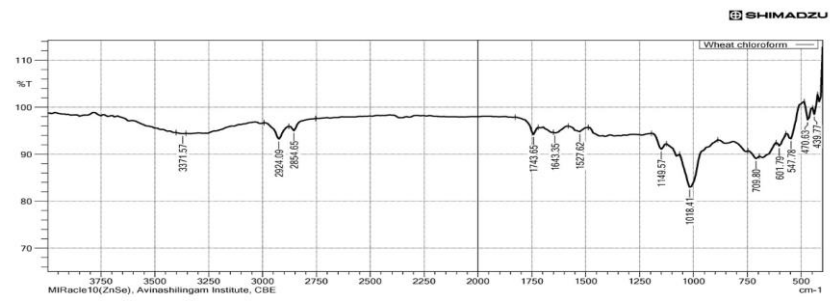
The Methanol and Chloroform extract of Wheat has Alcohol, Alkane, Thiol, Isothiocyanate, Aromatic compound, Conjugated ketone, Carboxylic acid and Alcohol, Alkane, Thiol, Isothiocyanate, Aromatic compound, Conjugated ketone, Carboxylic acid as functional groups. Methanol and Chloroform extract of Ragi has Alcohol, Alkane, Thiol, Isothiocyanate, Conjugated ketone, Carboxylic acid, Sulfate and Alcohol, Alkane, Esters and α-lactone, Inine\oxime, Nitro compound as functional groups.

GRAPH 1- WHEAT (METHANOL)



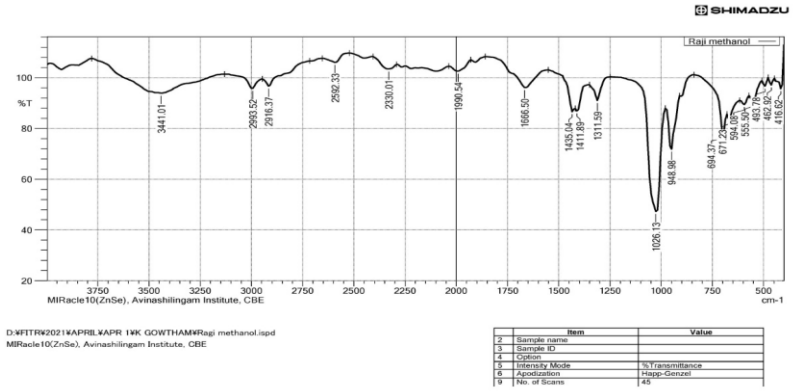
	Item	Value
2	Sample name	
3	Sample ID	
4	Option	
5	Intensity Mode	%Transmittance
6	Apodization	Happ-Genzel
9	No. of Scans	45

GRAPH 2 – WHEAT (CHLOROFORM)



	Item	Value
2	Sample name	
3	Sample ID	
4	Option	
5	Intensity Mode	%Transmittance
6	Apodization	Happ-Genzel
9	No. of Scans	45

GRAPH 3 -RAGI (METHANOL)



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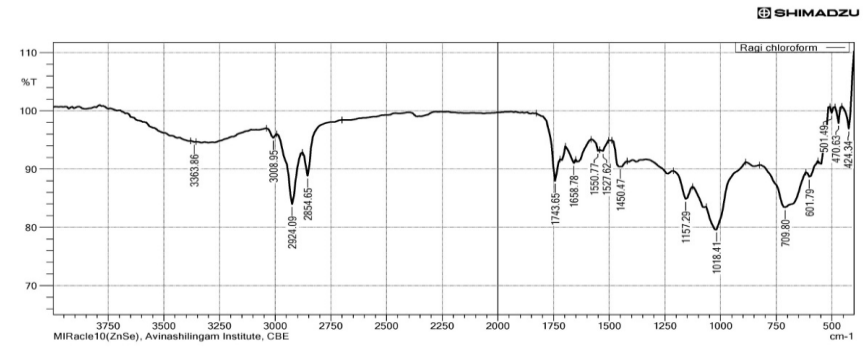
5	Intensity Mode	%Transmittance
6	Apodization	Happ-Genzel
9	No. of Scans	45

GRAPH 4 -RAGI (CHLOROFORM)



	Item	Value
2	Sample name	
3	Sample ID	
4	Option	
5	Intensity Mode	%Transmittance
6	Apodization	Happ-Genzel
9	No. of Scans	45

Table 5: FTIR

SAMPLES	FUNCTIONAL GROUPS	WAVE NUMBER RANGES
Wheat (Methanol)	Alcohol, Alkane, Thiol, Isothiocyanate, Aromatic compound, Conjugated ketone, Carboxylic acid	3471,2993,2916,2592,2337, 2098,1998,1666,1435
Ragi (Methanol)	Alcohol, Alkane, Thiol, Isothiocyanate, Conjugated ketone, Carboxylic acid, Sulfate	3441,2993,2916,2592,2303, 1998,1666,1435,1411
Wheat (Chloroform)	Amine, Alkane, α, β unsaturated ester, Imine\Oxime, Nitro compound, Aliphatic ether	3371,2924,2854,1743,1643, 1527,1149,1018
Ragi (chloroform)	Alcohol, Alkane, Esters and α-lactone, Inine\oxime, Nitro compound	3363,3008,2924,2854,1743, 1658,1550,1450,1157

SUMMARY AND CONCLUSION:

This study showed the presence of various phytochemicals in the various extracts of wheat and ragi. The antibacterial efficacy of *Triticum aestivum* and *Eleusine coracana* against pathogenic microorganisms could be assigned to number of phytochemicals such as flavonoids, alkaloids, tannins and phenols and also the presence of various functional groups. These findings concludes that it can be used as an alternative to antibiotic drugs against the bacteria causing nosocomial infection.

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CONFLICTS OF INTEREST:

The author has no conflicts of interest to publish this Research article in this journal.

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