Research article

Evaluation of antibacterial activity of various extracts of germinated seeds of *Hordeum vulgare*

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Abstract

The purpose of the study is to use various techniques to describe and assess the antibacterial property of germinated seeds of Hordeum vulgare with modification. Thin-layer chromatography was used to analyze the grain after and before alteration, and conventional chemical techniques were used to identify the phytochemical component. Three techniques were used to determine the plant extract's antibacterial activity (well, disc, and OD of broth culture). According to the findings, the extract included four phytochemical components before being modified and one compound after. The extract was found to include a variety of polar molecules with various retardation factors, according to the TLC profile data. Results of antibacterial activity demonstrate that bacterial resistance varies and that various approaches result in various reactions. The greatest findings were from the OD of the broth culture; plant extract prevented the growth of two of these pathogenic bacteria after modification, while it inhibited the growth of five of the pathogenic bacteria before modification. H. vulgare has no antimicrobial activity when compared to Ofloxacin because its antibacterial activity requires several components

Keywords: - Antibacterial Activity, *Hordeum vulgare*, extraction, thin layer chromatography,

Introduction

Hordeum vulgare, commonly known as barley, is a cereal grain that is widely used in a variety of food products. It is known for its high nutritional value and can be found in many countries around the world[1]. In recent years, there has been an increased interest in using barley for its medicinal properties, particularly its potential as an antibacterial agent. This study aims to evaluate the antibacterial activity of various extracts of germinated seeds of Hordeum vulgare against several bacteria[2]. The different extracts used in this study include aqueous extract, ethanolic extract, and methanolic extract. The antimicrobial activity of these extracts was tested against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis[3]. The results of this study will provide insight into the potential of Hordeum vulgare as an antimicrobial agent[4].

Methods and Materials

Evaluation of antimicrobial activity

The antimicrobial screening of extract LA, LM and LP was done from biological assay division, CSIR –Central Drug Research Institute, Jankipuram extension,Lucknow, against their antibacterial ESKSAP pathogen panel.

Pathogenic bacteria

E. coli (ATCC 25922), *A. baumanii* (BAA 1605), *S. aureus* (ATCC 29213), *P. aeruginosa* (ATCC 27853) and *K. pnemoniae* (BAA 1705) were isolated from different sources in suitable media.

Antibacterial activity

The disc diffusion technique was used in order to evaluate the antibacterial activity. After the discs were created, a volume of 20 µl containing plant extracts of varying concentrations was inserted into each disc[5]. On the Muller Hinton agar, the discs containing the organisms were inserted, and swabs were taken from them (bacteria). The diameter of the inhibition zone that had developed around the disc was measured in order to determine the level of antibacterial activity that had been produced[6].

Medicinal uses of *Hordeum vulgare*

Anti-inflammatory: Germinated barley seeds are known to contain several anti-inflammatory compounds, such as polyphenols, which can help reduce inflammation and pain in the body. Antioxidant: Germinated barley seeds contain an abundance of antioxidants, which can help protect the body from free radical damage and slow down the ageing process[7]. Cardiovascular Health: Germinated barley seeds contain Vitamin E, which is known to help reduce the risk of heart disease by lowering cholesterol levels and reducing inflammation[8]. Digestive Health: Germinated barley seeds contain dietary fibre, which can help improve digestion and reduce constipation. Blood Sugar Control: Barley seeds that have sprouted have beta-glucan, which is a type of soluble fibre that can help control blood sugar levels and help you lose weight[9].

Germination Process for Barley

1 kg of barley seeds were collected from random field. Following the selection of the plant, the following stage was to gather it and identify it botanically authentication of seen of Barley was done[10]. Plant collection was done professionally and the collection location, season, plant state, and other details with proper voucher specimens, pictures, and written notes were recorded. Barley seeds were gathered, cleaned with water from the tap, and added to 3 L of water for 24 hours. The seeds were then taken out and kept in a muslin cloth. In next 3 days, the barley seeds were germinated. For examination, the dried plant components were pulverised and stored[11].

Figure 1: Germinated barley and it's powder

Preparation of Aqueous, Methanolic and Pet. ether extract of *H. vulgare*

Two procedures were employed

Soxhlet method: The dried powdered of Barley seeds (200gm) were extracted with 600 ml of methanol through Soxhlet apparatus for 72 h at a temperature not exceeding the boiling point of the solvent.The dried powder of 200g germinated barley was extracted with 600 ml of petroleum ether by Soxhlet apparatus for 72 h at a temperature not exceeding the boiling point of the solvent[12].

Figure 2: Soxhlet apparatus

Maceration method: To make the aqueous extract, distilled water (600 ml) was utilised as solvents for infusion and maceration, respectively. To prevent fungal development, the infusion took three hours, and the maceration took 48 hours. After 3 days the extract was filtered and dried over rotary evaporator.Dried methanolic extract (LM), Pet.ether extract (LP) and aqueous extract (LA) were weighed and yield calculated[13]. The extracts LM, LP and LA were subjected to TLC separation and phytochemical screening. Further these extracts were sent to CDRI Lucknow for determination of Antibacterial activity[14].

Figure 3: Prepared extracts

Phytochemical analyses of prepared extract (LM, LP and LA)

For phytochemical characterisation of significant photoactive compounds, all the prepared extracts were exposed to a variety of qualitative assays. HI media and Merck in India provided the chemicals utilised in the phytochemical study. Qualitative tests were conducted to determine the existence of flavonoids, polysterols, tannins, saponins, alkaloids, phenolics, and carbohydrates[15]**(Table 1)**.

Table 1: Phytochemical analyses of prepared extract (LM, LP and LA)

S. No	Test	Extraction Process
1	**Benedict's test for Carbohydrates**	The crude extract was filtered after being dissolved in 5ml of distilled water. Benedict's reagents were used to treat the filtrate, which was then gently heated. An orange red precipitate indicates that carbohydrates are present in the sample**.**
2	**Terpenoids test**	The crude extract was diluted in 2 mL chloroform and evaporated until it was completely dried. 2ml concentrated H2SO4 was added to this, and it was heated for roughly 2 minutes. Grey colour indicating the presence of terpenoids.
3	**Salkowski's test for Polysterols**	Separately, a little amount of the crude extract was suspended in 5ml of CHCl3. The Salkowski test was next performed on the chloroform solution. Test of Salkowski: only some drops of concentrated H2SO4 was added up to 1ml of the above-prepared chloroform solution. The presence of phytosterols is shown by the brown colour generated.
4	**Foam test for Saponins**	A little amount of crude extract was mixed with 2ml of H2O and shaken. If the foam formed lasts longer than 10 minutes, it indicates the presence of saponins
5	**Ninhydrin test**	When crude extract was heated with 2millilitre of 0.2 percent Ninhydrin solution, it became violet, indicating the presence of proteins and amino acids.
6	**Test of Ammonium for flavonoids**	Some drops of 1% solution of ammonia were combined in a test tube containing a hydro methanolic extract of each plant sample. Flavonoid presence in the sample was indicated by a yellow blue colour
7	**Steroid Test**	Crude extract was combined with 2 mL concentrated H2SO4 and chloroform was added sideways. The presence of steroids was detected by a red colour in the bottom chloroform layer. Another experiment was carried out by combining crude extract with 2mL chloroform. The mixture was then poured with 2ml of concentrated H2SO4 and CH3COOH. The development of a greenish colour was detected the presence of steroids.

8	**Perform a protein Millon's test**	In this test crude extract is combined with 2ml Millon's reagent, it forms a white precipitate, which becomes red when heated gently, indicating the presence of protein.
9	**Molisch's test**	2 mL of Molisch's reagent was combined with crude extract and well mixed. After that, 2ml of concentrated sulphuric acid was gently poured down test tube's side. The presence of carbohydrate was shown by appearance of a violet ring in the interphase.
10	**Test for Phenolics**	In 5 mL of pure water, the extract (500 mg) was dissolved. Some few drops of neutral 5% FeCl3 were added to this. The presence of phenolic compounds is represented by a dark green colour.
11	**Iodine test**	Take 2ml of iodine solution was combined with crude extract. The presence of carbohydrate was detected by a dark blue or purple colour.
12	**Dragendorff's test for Alkaloids**	A little amount of extract was reacted with a few drops of weak HCl and filtered separately. The Dragendroffs reagent was employed to test the filtrates. The appearance of an orange-brown precipitate indicates the presence of alkaloids.
13	**Perform a carbohydrate Fehling's test**	The two reagents used in this test are Fehling A and B. 2ml from each reagent was taken and added to crude extract and slightly heated. A brick red precipitate indicated the presence of reducing sugars.
14	**Test for tannins using Lead acetate**	A little amount of the crude extract was separately extracted in water, as well as the occurrence of tannins was determined by introducing a solution of lead acetate 10%. The occurrence of tannins is shown by the production of white precipitate.
15	**Liebermann's test for glycosides**	2 mL of acetic acid and chloroform were added to the crude extract. Ice was used to chill the decoction. H2SO4 was added in a concentrated form. A colour shift from violet to blue to green indicating the presence of steroidal nucleus, i.e., glycone part of glycoside.

Thin Layer Chromatography

To prepare the TLC plates, Silica gel 'G' was used. First, thirty grams of silica gel were weighed and then a homogenous suspension was made with sixty milliliters of distilled water for two minutes. This suspension was then distributed over the plate, which was then air dried until the transparency of the layer was no longer visible. After drying in a hot air oven at 110 degrees Celsius for thirty minutes, the plates were kept in an airtight container in a dry environment until they were needed[16]. To prepare the samples, the crude extracts of methanol, water and pet. etherwas first diluted with their respective solvents and then applied to the origins of a TLC plate 2 centimetres above the plate's bottom using capillary tubes. Typical application volumes ranged

from 1 to 10 microliters. For the TLC plates were developed in various solvent systems namely methanol – acetone (1:1), methanol – ethyl acetate (1:1), ethyl acetate – pet. ether (1:1) and methanol –acetone – pet. Ether (2:1:2). the TLC were developed Iodine chambers and number of spots there Rf value reported[17].

Minimum Inhibitory Concentration (MIC) for ESKAPE pathogens

The minimum inhibitory concentration (MIC) of substances against rapidly proliferating bacterial pathogens was calculated using the broth dilution technique in accordance with the European Committee for Antimicrobial Susceptibility Testing (EUCAST)/CLSI. Muller-Hinton Broth II with supplementation was used as the medium. In order for the well to function as a sterility control well, 100 litres were added to it[18]. Additionally, 50 litres were injected into another well that was used as a control well. The appropriate well was then filled with 50 litres of each antibiotic dilution (2:1) before being further infected with bacterial colonies. The injection was given using a method that allowed for 5 105 CFU/mL. The plate was kept for 18–24 hours at 35–370 °C. The MIC was determined to be the level at which no discernible bacterial growth could be seen[19].

Result & Discussion

Dried germinated seeds of *H. Vulgar* were extracted by water, methanol and petroleum ether.

Yield of *Hordeum vulgare* extract: In *Hordeum vulgare*, extracts using water, methanol and petroleum extract, yielded 25.45gm, 26.19gm, and 23.15 gm., respectively of crude extract. Using different solvents, the considerable difference in yields of H. vulgare extracts was shown.

Phytochemical investigation

Table 2: Quantitative investigation of phytochemical components in several extracts of *Hordeum vulgare*

S. No.	Constituents	Aqueous extract	Methanol extract	Petroleum extract
1	Steroids	+	+	+
2	Flavonoids	+	+	+
3	Saponins	+	+	+
4	Glycosides	+	+	-
5	Phenols	+	+	+
6	Tannins	+	+	+
7	Alkaloids	+	+	+
8	Terpenes	+	+	-
9	Anthraquinone	-	+	+
10	Reducing sugar	+	-	-
11	Phytosterol	-	+	-
12	Protein	+	+	-

(+) presence and absence (-) of phytochemical constituents

The phytochemicals found to be present in methanolic extract (LM) are Steroids, Flavonoids, Saponins, Glycosides, phenols, Tannins, Alkaloids, Terpenes, Anthraquinone, Proteins and Phytosterol.The phytochemicals found to be present in pet.ether extract (LP) are Steroids, Flavonoids, Saponins, Phenols, Tannins, Alkaloids and Anthraquinone.The phytochemicals found to be present in aqueous extracts (LA) are Steroids, Flavonoids, Saponins, Glycosides, Phenols, Tannins, Alkaloids, Terpenes, Reducing sugar and Proteins. It was observed that methanolic extract (LM) showed maximum number of Phyto constituents present. Many of the Phyto constituents present in LM, LP, LA were known to exhibit antibacterial activity. Hence, it was concluded that the extracts may be antimicrobial in nature. Thus, they were subjected to antimicrobial screening.

TLC analysis

Far identification of individual components of crude extracts (LA, LM, and LP); TLC analysis was done. TLC plates were developed in various solvents systems (methanol-acetone, methanol –ethyl acetate, ethyl acetate – pet. ether, and methanol – acetone – pet. Ether).But best separation was observed in solvent system methanol – acetone – pet. ether (2:1:2) using iodine vapours as developing agent. The TLC details are mentioned as under

Table 3- R_f value of Hordeum vulgare extract

extracts	TLC analysis
LA	2 spots at R_f -0.2,0.9
LM	3 spots at R_f 0.38,0.62 and 0.96
LP	1 spot atR_f 0.98 major extract showed no movement with solvent

Solvent system: methanol – acetone – pet. Ether (2:1:2), developing agent iodine vapours

TLC analysis shows that maximum numbers of phytochemicals were extracted in methanolic extract. Pet. Ether extract did not separate well in any of the solvent system used and aqueous extract showed a smaller number of phytoconstituents extracted.

Figure 4: Analysis of LA, LM, LP

Antimicrobial activity

Antimicrobial activity was performed from biological assay division (CDRI-Lucknow)

MIC of the extracts: Table 3 displays the results of the minimum inhibitory concentration (MIC) tests performed on the various bacterial strains. Levofloxacin was used as a standard for comparison of antimicrobial activity. The inoculation was performed in such a manner that each well contained 5×10^5 CFU/ml of standard or extracts (LA, LM, LP). Results are shown in table.

Table 4: The MIC values of different bacterial strains against the 3 extracts

S. No.	Bacterial strains	Aqueous extract (mg/ml)	Methanol extract (mg/ml)	Pet. Ether	Levofloxacin
1	E. coli ATCC 25922	>64	>64	>64	0.0078
2	S. aureus ATCC 29213	>64	>64	>64	0.0625
3	*K. pnemoniae BAA 1705*	>64	>64	>64	64
4	A. baumanii BAA 1605	>64	>64	>64	4
5	*P.aeruginosa ATCC 27853*	>64	>64	>64	0.5

Statistical analysis

All data from the antimicrobial activity is presented as mean±SEM.

Discussion

All the extracts showed MIC value >64 mg /ml which was too higher than the MIC value of standard used. Hence it was concluded that the extracts prepared did not show required degree of antimicrobial protection

Conclusion

H. vulgare seeds, locally known as barley seeds, are well known for their various pharmacological activities. A review of the literature reveals few evidences from traditional and scientific sources that *H. vulgare* seeds have antibacterial properties when extracted with methanol. Also, it was determined that the fermentation of barley led to an increase in few phyto-constituents. Hence it that treated *H. vulgarereat H.vulgare* seeds will refermented, dried and extracted with solvents (water and methanolol, pet. ether) to ascertain their antimicrobial activity. As decided, H. vulgare seeds were collected and fermented for 3 days. The fermented seeds were dried. Extraction was methanol, water, ethanol and petroleum ether) all extracts were

subjected to phytochemical investigation and TLC analysis. Phytoconstituents found to be present were LA (steroids, flavonoids, saponins, phenols, glycoosides, tannin, terpenesoids, terpenes and reducing sugar), LM (steroids, saponins, glycosides, phenols, tannin, terpenoids, anthraquinone), anthraquinone) and LP (steroids, flavonoids, saponins, phenalkaloids, iphenols, alkaloids and anthraquinone). Also, TLC analysis showed that crude methanolic extracts (LM) have a greater number of phytoconstituents. Results showed that all extracts have the presence of constituents, generally those that are well known for their antimicrobial activity, like tannins, phenolics, and glycosides. Thus, the extracts were subjected to antimicrobial screening at CDRI Lucknow. Results show that the MIC value of the extracts (LA, LM, and LP) is greater than the MIC value of the standard drug, Levofloxacin. Hence, it is concluded that the extracts LA, LM, and LP do not show a desirable degree of antibacterial activity as compared to standard drugs. Because satisfactory antimicrobial activity was not observed, chemical constituent separation by column chromatography was not done. In future, extract will be tested for anti-diabetic activity as part of current study. Our study aimed at discovering plant-based antimicrobials so that bacterial resistance could be overcome. Further plants will be subjected to antimicrobial research.

Conflict of Interest

There are no conflicts of interests.

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