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PHARMACOGNOSTIC AND ANTIMICROBIAL EVALUATION OF *HORDEUM VULGARE* LEAVES

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ABSTRACT: *Hordeum vulgare* (Barley) grass is the best functional food for providing nutrition and eliminating toxins from human cells; however, its functional ingredients have also played an important role in health benefits. Flavonoids, saponarin, lutonarin, K, Ca, Se, dietary fiber, polysaccharide, alkaloid, glycoside, tannin, metallothionein and polyphenols are all found in barley grass. Microscopical studies showed that stomata, epidermal cells and trichomes were conducted as part of a pharmacognostic investigation of the grass of *H. vulgare*. The qualitative assays were done to predict the presence of phenols, tannin and flavonoid content. The Minimum inhibitory concentration (MIC) method was used to screen *H. vulgare* leaf extract for antimicrobial potential against *S. typhi, S. aureus, B. subtilis* and *E. coli* and chloramphenicol was used as a standard. Amongst all the extracts, the chloroform extract of *H. vulgare* was found to be more effective as its MIC value is almost equal to the standard antibiotic chloramphenicol.

INTRODUCTION: *Hordeum vulgare* Linn, also known as barley, is a member of the Poaceae family. Barley (*Hordeum vulgare* L.) is the world's fourth most important cereal crop, with the highest dietary fiber content. For nutritional peak, barley grass has young green leaves and stems of vegetative growth stage from a seedling at 10 days after sprouting (barley sprout) to elongation stage (barley green). Barley grass grows to be 0.5-1.2 m long and 0.5-0.1 cm thick. Plants contain a variety of constituents, including p-coumaroylagmatine, hordenin, pyrrolidine, luteolin glycoside, flavones glycosidesorientoside and orientin, 2- -Dglucopyranasyloxy, pangamic acid, protein, carbohydrates, calcium, phosphorus-glucan, phenolic acids, flavonoids, lignans, tocols, phytosterols and folate. Beta-sitosterol, stigmasterol, campesterol and brssicastrol are major chemical constituents of barley [1]. Consuming whole grain barley and its hydroalcoholic extract regularly lowers the risk of chronic diseases. In India, barley has traditionally been used to treat atonic dyspepsia and to treat malaria [2]. It is used to treat respiratory and urinary tract infections in Argentina [3].

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It is used to treat diabetes in China [4]. In Pakistan, barley treats rheumatoid arthritis and joint pain [5]. Barley is used to treating dysentery, diarrhoea and gastrointestinal disorders in the United States [6]. Barley has antioxidant activity and has the potential to treat circulatory disorders, cancer, obesity, diabetes, arthritis, cholesterol and inflammation [7].

In the present work, we studied the various pharmacognostic parameter and antimicrobial activity of *H. vulgare.* Traditionally, *H. vulgare* is used in atonic dyspepsia, respiratory and urinary tract infections, diabetes and as anti-malarial.

MATERIAL AND METHODS:

Plants Collection and Authentication: *Hordeum vulgare* (Barley) leaves are cultivated in Rohtak, Haryana, and the same was sent to NIScPR for authentication. Dr. Sunita Garg, Head, Raw Material Herbarium and Museum Division (RHMD) CSIR-National Institute of Science Communication and Policy Research (NIScPR), same was identified as *Hordeum vulgare* L. under reference number NIScPR/RHMD/Consult/2021/3951-52-2, 27/12/2021, Pusa Road, New Delhi.

Solvents and Reagents: Petroleum ether (60-80), Chloroform, ethanol, ascorbic acid, gallic acid, Folin Ciocalteu'sreagent, phosphomolybdenum, *etc* were procured from CDH. All chemicals used in the study were of all analytical grade.

Pharmacognostic Evaluation: Pharmacognostic evaluation provides useful information about crude drugs' macroscopical, microscopical and physical properties.

Macroscopic Evaluation: Macroscopic evaluation includes a complete evaluation of plants based on their external features. The plant material was evaluated in shape, size, color, odour, texture, and fracture characteristics [8].

Microscopic Evaluation: Microscopic evaluation includes the study of the transverse section and powder microscopy of *H. vulgare* leaves. After cutting the transverse section (T.S) of the plant was mounted with safranin to study the various characters. In powder microscopy, the plant powder was treated with glycerine, and chloral hydrate: HCL was used to mount various characteristics of plants like fibres, starch grain, calcium oxalate crystals, sclereid and many more [9].

Physicochemical Evaluation: Standardization parameter includes foaming test, ash values (total ash, water-soluble ash, acid-insoluble ash and sulphated ash) loss on drying, extractive value, swelling index crude fibre content were determined by standard procedures [8, 10-11].

Preparation of Extracts: Extraction of leaves of *H. vulgare* was done by two methods maceration and soxhlation. 500g of coarse powder of leaves of *H. vulgare* was weighed and treated with four solvents on the basis of polarity (low to high) to get petroleum ether, Chloroform, 80% ethanol, and water extract successively by triple maceration and soxhlation. Total 8 extracts were prepared and concentrated by rotary evaporator. These extracts were stored in an airtight container for further use.

Phytochemical Analysis: All extracts were chemically tested for the presence and absence of different phytoconstituents like alkaloids, flavonoids, tannins, glycosides, saponins, proteins and carbohydrates.

Quantitative Analysis: All extracts were tested for the different classes of phytochemicals present in them like total phenolic content, flavonoid content, and tannin content.

Total Phenolic Content: Total phenols present in all extracts *viz*., Petroleum ether, Chloroform, 80%, hydroalcoholic, and aqueous extract of leaves of *H. vulgare* were determined using Folin Ciocalteu's reagent method without any modification [12].

Total Tannin Content: Total tannin present in all extracts *viz*., Petroleum ether, Chloroform, 80%, hydroalcoholic and aqueous extract from leaves of *H. vulgare* was determined using Folin Ciocalteu's reagent method without any modification [13].

Total Flavonoid Content: Total flavonoid present in all extracts *viz*., Petroleum ether, Chloroform, 80%, hydroalcoholic and aqueous extract from leaves of *H. vulgare* was determined using the Aluminium Chloride without any modification [14].

Antimicrobial Activity: Antimicrobial activity of all extracts *viz*., Petroleum ether, Chloroform, 80%, hydroalcoholic and aqueous extract from leaves of *H. vulgare* were determined using 96-well microtitration plate method, two-fold dilutions of the antimicrobial agent in a liquid growth medium are placed in smaller proportions in 96-well microtitration plate. After that, a microbial inoculum is added to each well. After thoroughly mixing, the inoculation in a 96-well microtitration plate is incubated (usually without agitation) under appropriate conditions depending on the test microorganism. After 24 to 27 hours of incubation, plates were stained with the Alamar blue dye (resazurin), which was a useful growth indicator [15].

RESULTS:

Pharmacognostic Evaluation: Pharmacognostic evaluation was screened to identify commercial varieties, substitutes, adulterants and the quality of drugs. It is a simple and dependable tool for obtaining information about raw drugs' biochemical and physical properties. Methods covered include macroscopic and microscopic analysis, organoleptic the character of plant powder and extracts. Phytoconstituents of plant powder were identified qualitatively and quantitatively with various chemical reagents.

Macroscopic Evaluation of *H. vulgare*: Macroscopic evaluation was done by studying the external features of the fruit of *H. vulgare*. *H. vulgare* in green colour, 0.7-1.8 m long and 0.1-0.6 cm thick and smooth texture.

FIG. 1: REPRESENTATIVE PHOTOGRAPH OF *H. VULGARE*

Microscopic Evaluation of *H. vulgare*: Different photographs showed the presence of stomata's, epidermal cells and trichomes (T). **Fig. 2** represents the T. S. of *H. vulgare* leave A) Epidermal Cell (EC) (S), B) Open Guard cell (OGC), C) Stomata, D) Upper epidermal cell (UEC). **Fig. 3** represents the powder microscopic characteristics of *H. vulgare* leave A) Small barb-like trichome (SBT), B) Bundle of fiber (BF), C) Sieve tube (ST), D) Trichome (T), E) Epidermal Cell (EC), F) Sieve cell (SC).

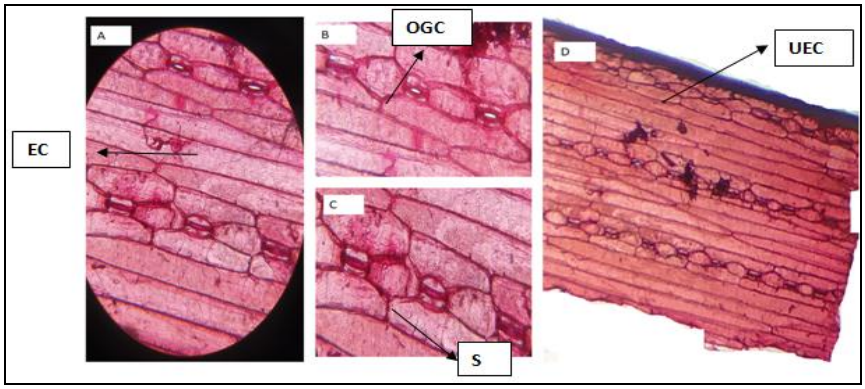

FIG. 2: REPRESENTATIVE PHOTOMICROGRAPH (X400) OF T.S OF *H. VULGARE* LEAVE A) EPIDERMAL CELL (EC) (S), B) OPEN GUARD CELL (OGC), C) STOMATA, D) UPPER EPIDERMAL CELL (UEC)

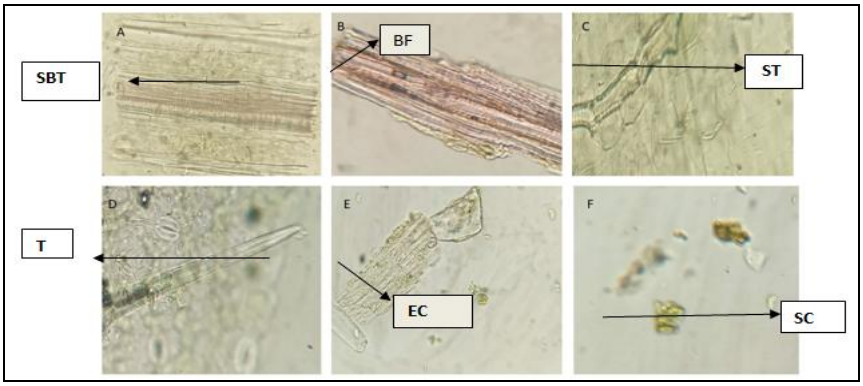

FIG. 3: REPRESENTATIVE PHOTOMICROGRAPH (X400) OF THE POWDER MICROSCOPY OF *H. VULGARE* LEAVE A) SMALL BARB LIKE TRICHOME (SBT), B) BUNDLE OF FIBER (BF), C) SIEVE TUBE (ST), D) TRICHOME (T), E) EPIDERMAL CELL (EC), F) SIEVE CELL (SC)

Standardization of Plant Material: Plants standardization entails confirming their identity and determining their quality, purity, and chemical compounds to establish a minimum standard of quality.

The total ash value denotes the purity of the material. In contrast, acid-insoluble, water-soluble, and sulphated ash values denote the presence of siliceous, earthy and inorganic material in the plant.

The crude fiber content of a material indicates the amount of indigestible cellulose, pentosans, and lignin present. Extractive values indicate the class of phytoconstituents present in plants. The swelling index measures the amount of mucilage in plant material.

The results showed that barley does not contain mucilage. Foaming index is used to identify the presence of saponin in the plant. The results show the presence of saponinin the barley. **Table 1** summarized the results of various standardization parameters of the plant.

TABLE 1: STANDARDIZATION PARAMETER OF *H. VULGARE*

Sr. no.	Parameter	*H. vulgare* (Percentage) w/w
1	Ash value	
	Total ash value	0.67
	Water soluble ash value	0.14
	Acid insoluble ash value	0.10
	Sulphated ash value	0.065
2	Crude fiber content	18
3	Extractive value	
	Petroleum ether soluble	3.2
	Alcohol soluble	13.6
	Chloroform water soluble	32
4	Moisture content	5.4
5	Swelling index	8
6	Foaming index	More than 100

Phytochemical Analysis: Petroleum ether, Chloroform, 80% ethanolic and water extracts of leaves of *H. vulgare* were dissolved in respective solvents and tested for the presence of various chemical groups of compounds. **Table 2** summarises the results of the phytochemical screening.

TABLE 2: PHYTOCHEMICAL SCREENING OF PETROLEUM ETHER, CHLOROFORM, 80% ETHANOLIC AND, WATER EXTRACTS OF LEAVES OF *H. VULGARE*

Classes of Phyto-constituent	Name of tests performed	Petroleum ether Extract	Chloroform Extract	Hydroalcoholic Extract	Aqueous Extract
Carbohydrates	Molisch's Test Benedict's Test Fehling's Test	---	---	+++	+++
Amino acid	Xanthoproteic Test Ninhydrin Test	--	--	--	--
Steroids	Salkowski's Test Libermann Burchard's test	--	+ +	--	--
Cardic glycoside	Legal's Test	-	-	-	-
Coumarin glycoside	Killer killiani's Test	-	-	+	+
Flavonoids	Alkaline Reagent Test Lead acetate Test	+ -	+ +	+ -	+ -
Phenolic Compound	Ferric chloride Test Liebermann's Test Litmus's Test	- - -	- - -	+ + -	+++
Alkaloids	Mayer's Test Wagner's Test Dragendroff's Test	- - -	+ + -	+++	+++
Tannin	Gelatine Test	-	+	+	+

Total Phenolic Content: Phenols can reduce free radicals; the more phenols present in the plant, more its antioxidant potential will be.

All the extracts (8) obtained from H. vulgare were prepared by different extraction techniques (maceration and soxhlation) using different solvents like petroleum ether, Chloroform, 80% ethanolic, and water extracts of leaves of *H. vulgare* were screened for total phenolic content.

Amongst all extracts, hydroalcoholic extract (Soxhlation) of leaves of *H. vulgare* contains maximum phenolic content is shown in **Table 3.**

TABLE 3: TOTAL PHENOLIC CONTENT OF ALL 8 EXTRACTS OF *H. VULGARE*

Sr. no.	Extracts	Total Phenolic content (mg GAE/g)
1	Barley Maceration Petroleum ether extract (BMP) B1	17.2±0.024
2	Barley Soxhlation Petroleum ether extract (BSP) B2	19.7 ±0.036
3	Barley Maceration Chloroform extract (BMC) B3	10.2 ±0.068
4	Barley Soxhlation Chloroform extract (BSC) B4	12.3±0.079
5	Barley Maceration Hydroalcoholic extract (BMHA) B5	83.5 ±0.052
6	Barley Soxhlation Hydroalcoholic extract (BSHA) B6	87.2 ±0.067
7	Barley Maceration Aqueous extract (BMA) B7	61.2±0.056
8	Barley Soxhlation Aqueous extract (BSA) B8	58.2±0.087

Total Tannin Content: All the extracts (8) were prepared by different extraction techniques (maceration and soxhlation) by using different solvents like petroleum ether, Chloroform, 80% ethanolic, and water extracts of leaves of *H. vulgare* were screened for total tannin content. Among all extracts, the aqueous extract (Soxhlation) of leaves of *H. vulgare* contains maximum tannin content, are shown in **Table 4.**

TABLE 4: TOTAL TANNIN CONTENT OF ALL 8 EXTRACTS OF *H. VULGARE*

Sr. no.	Extracts	Total Tannin content (mg tannic acid/g)
1	Barley Maceration Petroleum ether extract (BMP) B1	4.32±0.59
2	Barley Soxhlation Petroleum ether extract (BSP) B2	5.06±0.089
3	Barley Maceration Chloroform extract (BMC) B3	9.67±0.023
4	Barley Soxhlation Chloroform extract (BSC) B4	8.92±0.045
5	Barley Maceration Hydroalcoholic extract (BMHA) B5	25.05±0.098
6	Barley Soxhlation Hydroalcoholic extract (BSHA) B6	26.75±0.034
7	Barley Maceration Aqueous extract (BMA) B7	52.25 ±0.056
8	Barley Soxhlation Aqueous extract (BSA) B8	58.75±0.026

Total Flavonoid Content: All the extracts (8) were prepared by different extraction techniques (maceration and soxhlation) by using different solvents like petroleum ether, Chloroform, 80% ethanolic and water extracts of leaves of *H. vulgare* were screened for total flavonoid content. Amongall extracts, the aqueous extract (Soxhlation) of leaves of *H. vulgare* contains maximum flavonoid content, is shown in **Table 5.**

TABLE 5: TOTAL FLAVONOID CONTENT OF ALL 8 EXTRACTS OF *H. VULGARE*

Sr. no.	Extracts	Total Flavonoid content (mg Quercetin/g)
1	Barley Maceration Petroleum ether extract (BMP) B1	13.4 ±0.057
2	Barley Soxhlation Petroleum ether extract (BSP) B2	12.5 ±0.038
3	Barley Maceration Chloroform extract (BMC) B3	11.87±0.098
4	Barley Soxhlation Chloroform extract (BSC) B4	18.4 ±0.001
5	Barley Maceration Hydroalcoholic extract (BMHA) B5	49.8 ±0.005
6	Barley Soxhlation Hydroalcoholic extract (BSHA) B6	82.08 ±0.023
7	Barley Maceration Aqueous extract (BMA) B7	43.3 ±0.089
8	Barley Soxhlation Aqueous extract (BSA) B8	45 ±0.034

Antimicrobial Activity: All 8 extracts were screened for antimicrobial activity against *S. typhi*, *S. aureus B. subtilis* and *E. coli* using the MIC method, and chloramphenicol was taken as standard. Among all extracts, *H. vulgare* chloroform extract shows maximum antimicrobial potential. Results of antimicrobial activity are reported in **Table 6** and **Fig. 4.**

TABLE 6: ANTIMICROBIAL POTENTIAL OF ALL 8 EXTRACTS OF *H. VULGARE*

Extracts	*S. typhi*	*S. aureus*	*B. subtilis*	*E. coli*
Standard (Chloramphenicol)	62.5	15.6	15.6	31.25
Petroleum ether extract				
Barley Maceration Petroleum ether extract (BMP) B1	500	125	125	250
Barley Soxhlation Petroleum ether extract (BSP) B2	500	125	125	250
Chloroform extract				
Barley Maceration Chloroform extract (BMC) B3	125	62.5	31.25	62.5

Barley Soxhlation Chloroform extract (BSC) B4	125	62.5	31.25	62.5
Hydroalcoholic extract				
Barley Maceration Hydroalcoholic extract (BMHA) B5	500	125	62.5	125
Barley Soxhlation Hydroalcoholic extract (BSHA) B6	500	125	62.5	125
Aqueous extract				
Barley Maceration Aqueous extract (BMA) B7	125	125	500	125
Barley Soxhlation Aqueous extract (BSA) B8	125	125	500	125

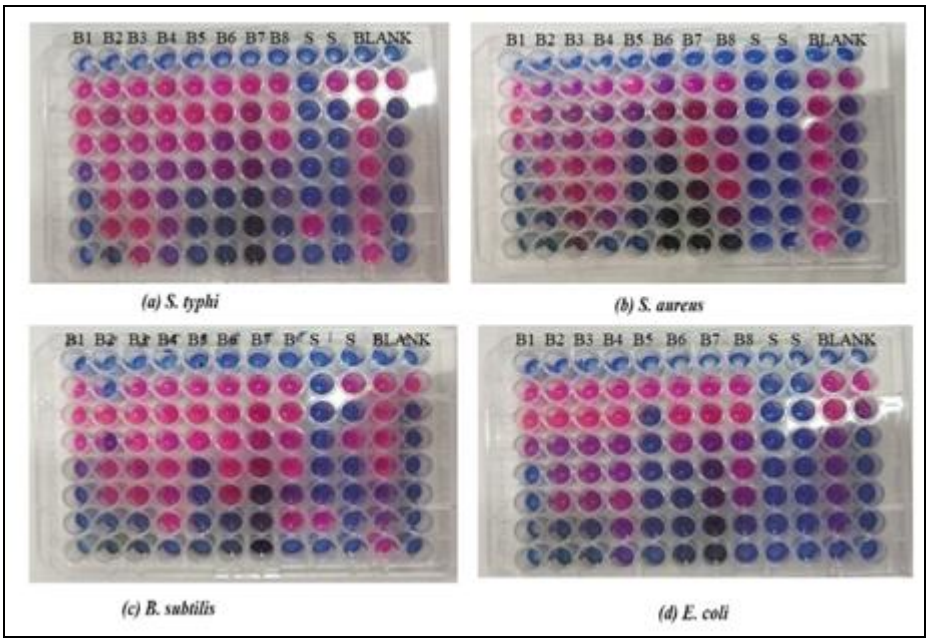

FIG. 4: REPRESENTATIVE PHOTOMICROGRAPH OF ANTIMICROBIAL ACTIVITY OF DIFFERENT EXTRACTS OF *H. VULGARE* AGAINST SELECTED STRAINS (*S. TYPHI, S. AUREUS, B. SUBTILIS, E. COLI*). Barley Maceration Petroleum ether extract (B1), Barley Soxhlation Petroleum ether extract (B2), Barley Maceration Chloroform extract (B3), Barley Soxhlation Chloroform extract (B4), Barley Maceration Hydroalcoholic extract (B5), Barley Soxhlation Hydroalcoholic extract (B6), Barley Maceration Aqueous extract (B7), Barley Soxhlation Aqueous extract (B8).

DISCUSSION: Histological evaluation showed that diacytic stomata is present in the*H. vulgare*. Standization of leaves of *H. vulgare*reveled that plant material is free from erarthy material, silicons, carbonates and inorganic material which assuer the quality and purity of the leaves. Leaves cantain 18% of dietry fiber which is good for consumption as its normalized the bowl movement. Literature reveled the presence of phenolic compounds such as benzoic acid, cinnamic acid, ferulic acid vannlic acid, flavonoids (cyanidin, delphindin, pelargonidin, catchin and myricitin, phytosterols like beta – sitosterol, stigmasterol, canpesterol, brssicastrol are present in leaves of *H. vulgare.* Phytochemicals studies showed the presence of amino acid, carbohydrates, steroides, flavaonids, phenols, alkaolids and tannin in the leaves of *H. vulgare*. Quantity of the phenols, flavonids and tannin content in the leaves of *H. vulgare* were also estminated; Barley Soxhlation Hydroalcoholic **(**BSHA) extract contain the maximum phenols i.e., 87.2 GAE/g, Barley Soxhlation Hydroalcoholic **(**BSHA) extract contain the maximum flavanoids i.e., 82.08 GAE/g**,** Barley Soxhlation Aqueous**(**BSA) extract contain the maximum tannin i.e., 58.75tannin acid/g. Results of quantitative analysis suggested that *H. vulgare* leaves may have good antioxidant potential as its contain a good amount of phenols and flavonoids. Therefore, *H. vulgare* leaves were evaluated for antimicrobial activity. Chloroform extract of *H. vulgare* showed maximum antimicrobial activity amongst all 8 extracts.

CONCLUSION: The data gathered from the current study on taxonomy, macroscopy and microscopy, preliminary phytochemical screening, and phytochemical evaluation of the plant will aid in the correct identification of *H. vulgare*. It is also concluded that *H. vulgare* leaves have

antimicrobial potential against *S. typhi, S. aureus, B. subtilis* and *E. coli*. With MIC values of 62.5, 31.25, and 62.5, chloroform extract of Barley leaves showed the highest antimicrobial activity against *S. aureus, B. subtili and E. coli*. The current study suggested that *H. vulgare* chloroform extract could be a good candidate for a natural antimicrobial agent because it has activity against gram-positive and gram-negative microbes.

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