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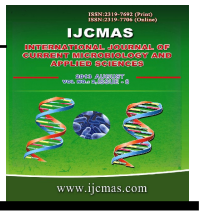

Original Research Article

Characterization and antimicrobial activity of barley grain (*Hordeum vulgare)* extract

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K e y w o r d s

Hordeum vulgare;
OD of broth culture;
Thin layer chromatography

A B S T R A C T

The study aims to characterize and evaluate the antibacterial activity of *Hordeum vulgare* grain with modification using different methods, grain after and before modification was extracted by methanol-water methods then it characterized using Thinlayer chromatography, the phytochemical compound was detected by classical chemical methods. The antibacterial activity of plant extract was detected using three methods (well, disc and OD of broth culture).The results show that extract have 4 phytochemicals compounds before modification and one compound after modification. TLC profile results show that extract contained different polar compound that have different retardation factors. Antibacterial activity results show variations in bacterial sensitivity and different response by different methods. The OD of broth culture show the best results; five of the pathogenic bacteria inhibited by plant extract before modification, and two of these bacteria growth was decreased by plant extract after modification.

Introduction

Hordeum vulgare L. (barley) is one of three species of genus Hordeum belonging to the tribe Triticeae of Poaceae family (Akar *et al.,* 2004). Its Cultivation as domestic food crop started in the beginning of agriculture 10,000 years ago .It has originated in the Fertile Crescent [Israel, Jordan, Syria and southern Turkey to Zagros Mountains in Iran (Molina-Cato *et al.,* 2002), During the early development of agriculture, barley was a staple food in bread-making andin soup and porridge dishes It is adapted to a broad range of ecological conditions but grows best in temperate climates of cool and moderately dry seasons (Poehlman, 1985).Barley is an annual herbaceous monocotyledonous grass. During germination the barley caryopsis develops seedling roots followed by emergence of a coleoptile through the soil surface , the origins of the leaf sheaths and range from 70 cm for dwarf types to >150 cm in length . Multiple stems (tillers) developed per plant depends on stand density and cultivar plant is a spike (head or ear) at the

top of the stem, consisting of either two or six rows offertile spikelet's, in which the mature caryopses develop (Wikipedia, 2012).

Medicinal Uses

On the basis of scientific research barley is considered to be most useful grain. As it is easily digestible compared to wheat, therefore, it is the best diet for patients or those cured persons which are still weak.In Europe pearl barley is considered to be best medicine (Farooqi, 1998). Prophet Muahmmad (Sallallahu Alaiyhi Wallehi Wassallam) liked barley very much and used it in various forms suchas barley bread (Ghaznavi, 1987). The medicinal uses of barley have been summarized in Table 1.

Materials and Methods

Plant modification

To form grain ash grain exposure to 100°C until ash forming.

Plant extract

The plant extract of grain and modification of grain was prepared according to the method of Sato *et al*. (1990) with some modification. The modification procedure is as following, specific weight of the grain and it is mixed with the average of 1g of grain to 3 ml of the dispert solution (20 %methanol: 80 % distilled water volumes).

The mixture is uniformed by electric blender for 30 minutes in room temperature. The solution is filtered by using gauze fabric for getting transudate solution which is concentrated by using rotary evaporator. It is put in the incubator at 50C° for 24 hours for getting the dried dispert. The dispert is kept in a dry place until it is used.

Plant extract aliquot

It prepare in 3 concentration (0.001, 0.01, 0.1 g\ml) then it sterilized using mellipor 0.2 mm.

Biochemical reagent

Classical methods used to detected phytochemichal compounds in plant extract (Saponen, Tanine, Flavonoid, Phenols, Turbine, Steroids, Alkaloid, Resin and Volatile oil).

TLC profile

Plant extract characterized by TLC, by use solvents (chlorophorm: hexane: ethanol) (1:1:1 v\v\v) as mobile phase, then bands exam in visible and UV light in 312 wave length to detect retardation factor.

Pathogenic bacteria

E.coli , S. typhi, S.aureus , Serratia spp. , *Klebsilla pnemoniae, Aeromonas hydrophillia* was isolated from different source in hospital in suitable media, then antibiotic sensitivity was detected using disc methods to (methicillin ME, amikacine AM, azithromycine AZM, fusidic acidFa, imipenenm IPM, naldixic acid NA, piperacillin PRL, ampicilline\cloxacillin APX, doxycycline DO, nitrofurantion F, oxacillin OX, rifampin RA, clindamycin PA and trimethoprim\sulphamethoxazol SXT).

Antibacterial activity of plant extract

This assay performed using three methods (well methods, disc diffusionmethods and

OD of broth culture). Well method performed by add 100 µl of plant extract in every well in 3 concentration (0.1, 0.01,0.001) g\ml. Disc diffusion method performed by immersion blank disk in plant extract for 24 hours then it added to bacterial agar culture. OD of bacterial growth This assay was performed by add 100 µl (0.1 g\ml) of plant extract to 2 ml of nutrient broth before culture.

Result and Discussion

The results show that extract contain saponin, tannin, alkaloid and volatile oil before modification and saponine only after modification as shown in table (2). TLC profile of plant extract contain from different polar compound before and after modification that have different retardation factors clarified in figure (1) and table (3) .

Antibacterial activity

Antibiotic sensitivity of bacteria show variation in sensitivity to antibiotics with different inhibition zone clarified in table (4). Well methods all bacteria spp. Which used in present study show resistant to plant extract using well methods in three concentrations (0.1, 0.01. 0.001)g\ml table (4). Disc methods some bacteria spp. show inhibition zone to plant extract before modification and after modification only one species show resistance in three concentration which use in this study (0.1, 0.01. 0.001)g\ml that show in table (5). OD of broth culture show decreased in OD of broth in all bacterial spp. Before and after modification that show in figure (2).

The Results of present study show that *Hordeum vulgare* grain extract contain of different phytochemical compounds and high percentage of low polar compounds, Mahesh *et al.,* (2010) record that Barley grain is an excellent source of soluble and insoluble dietary fiber (DF) and other bioactive constituents, such as vitamin E (including to co-tri-enols), B-complex vitamins, minerals and phenolic compounds (Mahesh and Abu-Ghannam, 2010). Barley is also a rich source of to cols, including tocophenols and tocotrienols, also it contain amino acids like Arginine ,histidine, lysine, tyrosine, tryptophan, phenylalanine,cystine, methionine, threonine, leucine, isoleucine, valine and glycine have also been reported to be present in it (Abbasi *et al.*, 2009).

For detection the optimize method of antibacterial activity of plant extract; three methods were used at three concentration against 6 species of pathogenic bacteria showed resistance to more than one antibiotics that clarified in table (3). Well method result show that all bacterial spp. resistant to plant extract.

According to review of literature this plant has been used as antibacterial in the world and genetic variation of pathogenic bacteria in Iraq which made it resistants' to antibiotics and antifungal thus other method was experience such as disc method was used, the results of this method show that many species sensitive to plant extract that clarified in table (6).

To perform the optimum methods and detect the definitive growth, OD of growth was detected using spectrophotometer (shemadzo-Germany) on 600 nm and compare with positive control (growth without plant extract using broth as blank) and negative control (broth with plant extract without growth that use as blank) . The results of this method was successful, plant extract show inhibition growth which decreased in OD of growth compare with positive (growth without plant extract) and

Table.1 Medical application of *Hordeum vulgare*

Disease	**Application**
Anti-cough	Decoction of *H. vulgare* seeds with apples, dried figs and pears.
Bladder inflammation,	A decoction of dried seeds is used orally for bladder inflammation in Iran.
Blood glucose level	Seeds of *H. vulgare* 125 gram are roasted and mixed with each of 50 gm of *Cicer arietinum* and *Elettaria cardamomum* and used half teaspoon with water thrice a day to control blood glucose level
Cholera	Powdered flower of Calotropisprocera, fruits of *Piper nigrum*, seed ash of *H. vulgare* and rose water are taken orally for cholera in India.
Dermatitis	Hot water extract of dried seeds is also used externally for Dermatitis in Guatemala.
Diabetes	This remedy is used as dietary supplement to control diabetes.
Inflammations	Hot water extract of dried seeds is also used externally for inflammations in Guatemala.

Table.2 Phytochemicals compounds of *Hordeum vulgare* after and before modification

Phytochemicals	**Before modification**	**After modification**
Saponen	+	+
Tanine	+	-
Flavonoid	-	-
Phenols	-	-
Turbine	-	-
Steroids	-	-
Alkaloid	+	-
Resin	-	-
Volatile oil	+	-

Table.3 R_f value of *Hordeum vulgare* extract before and after modification.

Color	R_f in visible light	R_f in UV light
before modification		
Yellow	0.93	
Green	0.96	
blue		0.89
dark blue		0.91
Brown		0.94
After modification		
Yellow	0.93	
Bowen	0.98	
Blue		0.04
Dark blue		0.84
Blue		0.91
yellow		0.93

Table.4 Inhibition zone (mm) of antibiotic sensitivity of bacterial spp.

Bacteria **AB mcg**	**F 100**	**OX 10**	**APX 25\5**	**PRL 30**	**DO 30**	**IPM 10**	**FA 10**	**SXT 1.25\23.75**	**ME 10**	**RA 5**	**DA 10**	**AK**	**NA 30**	**AZM 15**
E.coli	12	R	10	20	18	26	R	18	R	8	10	12	18	24
S. typhi	10	R	8	14	24	36	10	R	R	R	10	14	26	24
S.aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Serratia spp.	14	8	10	14	28	32	14	R	14	12	26	18	12	26
Klebsilla pneumoniae	R	R	R	6	18	30	R	R	R	R	24	18	26	24
Aeromonas hydrophila	14	R	R	14	18	22	R	16	R	8	20	16	22	18

Table.5 Antibacterial activity of *Hordeum vulgare* plant extract before and after modification by well method.

Bacteria	**0.1g\ ml**		**0.01 g\ml**		**0.001 g\ml**	
	Before	After	Before	After	Before	**After**
E.coli	R	R	R	R	R	**R**
S. typhi	R	R	R	R	R	**R**
S.aureus	R	R	R	R	R	**R**
Serratia Spp.	R	R	R	R	R	**R**
Klebsiella pneumoniae	R	R	R	R	R	**R**
Aeromonas hydrophila	R	R	R	R	R	**R**

Table.6 Antibacterial activity of *Hordeum vulgare* plant extract before and after modification using disc method.

Bacteria	**0.1g\ ml**		**0.01 g\ml**		**0.001 g\ml**	
	Before	After	Before	After	Before	**After**
E.coli	6 mm	R	R	R	R	**R**
S. typhi	R	8 mm	R	R	R	**R**
S.aureus	R	R	R	R	R	**R**
Serratia Spp.	R	R	R	R	R	**R**
Klebsiella pneumoniae	10 mm	R	8 mm	R	R	**R**
Aeromonas hydrophila	R	10 mm	R	R	R	**R**

Figure.1 TLC profile of *Hordeum vulgare* using (chlorophorm: hexane: ethanol) (1\1\1 v\v\v) as mobile phase under visible and UV light.

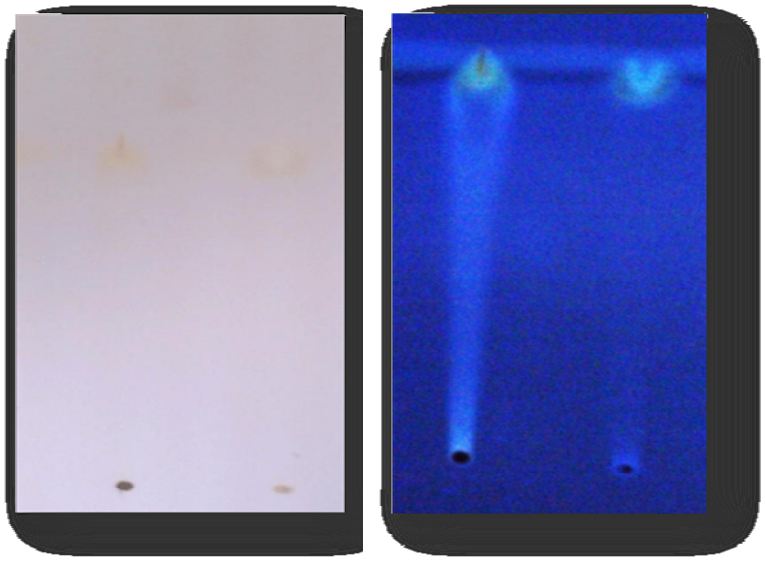
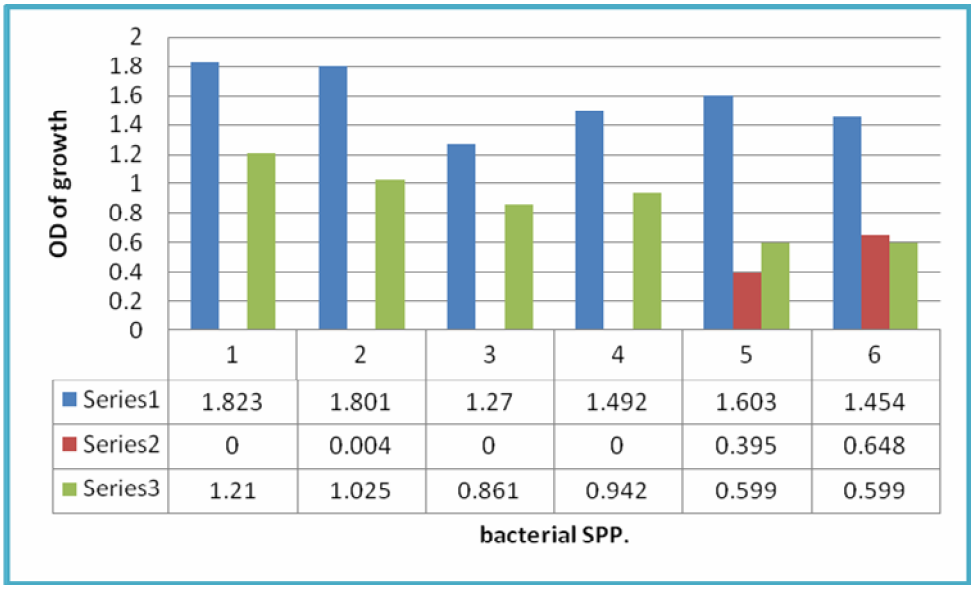


Figure.2 Antibacterial activity of *Hordeum vulgare* grain extract using OD of bacterial growth before and after modification. 1.*E coli,* **2.***Staphylococcus aureus,* **3.***Salmonella typhi,* **4.***Klebsiella pneumonia,* **5.***Seratia Spp. ,* **6.***Aeromonas hydrophila*
*Series 1- positive control, 2- before modification, 3- after modification



	1	2	3	4	5	6
Series1	1.823	1.801	1.27	1.492	1.603	1.454
Series2	0	0.004	0	0	0.395	0.648
Series3	1.21	1.025	0.861	0.942	0.599	0.599

negative control (broth with plant extract without growth).

The inhibition of bacterial spp. by plant extract may because that plant extract contain many compounds such as fibers made up of pentosans, beta glycan and cellulose (Marwat *et al.,* 2012).

Many studies used this plant extract in treated inflammation, in Iran dried seeds used orally to treated bladder inflammation (Ross, 2005). So in India the ash of this plant used for cholera (WebMD, Barley Overview Information, 2012). And hot water extract was used in inflammation (Bussmann *et al.,* 2007).

Also some population used dried fruit for urinary tract infection. Many study clarify the antibacterial study of this plant as aresult of phenolic compound in this plant it is associated with health benefit like phytoestrogen, phyticacid. (Kaneko *et al.,* 2003; Madhujith and Shahidi, 2007).

Sheela and suganya (2010) use symbiotic barley grain extract against some human pathogenic such as *E coli. , staphylococcus aureus, sallmonelaa paratyphi, shigella dysenteriae and vibrio cholera*, the results show that all pathogenic bacterial was inhibited by this extract (Sheela and Suganya, 2012).

Madineni (2012) found that Thaumatin like proteins was extracted from barley have antimicrobial activity against *Candida albicans, Bacillus subtilis, E.coli, Saccharomyces cerevisiae* as they are pathogenic organisms. The results showed that it has maximum inhibitory affect against *Candida albicans* using well method (Madineni, 2012).

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