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Phytochemical Profiling and GCMS Analysis of Two Different Varieties of Barley (*Hordeum vulgare* L.) Under Fluoride Stress

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ABSTRACT

Hordeum vulgare L.(Barley) is an important staple crop in Asia and usually considered more fluoride tolerant than other cereal crops under family Poaceae. In this study, phytochemical and GCMS profiling of two cultivars of *Hordeum vulgare* L. *viz*.., RD2794 and RD2786 have been performed under Fluoride stress. Different concentrations of NaF (Control, 5ppm, 10 ppm, 15ppm, and 20 ppm) were prepared to identify cultivars responsible for tolerance to fluoride stress. Several strategies were identified in response to fluoride stress among the two cultivars. This paper reports the cultivars of fluoride adaptation between the two cultivars. RD2786 contains high alkaloids, carbohydrates, Phytosterols and fixed oil better than RD2794 after imposing fluoride stress. The study showed that total flavonoids and phenolic content is high in RD2786 and reduced significantly in RD2794. This suggests that RD2786 is a desired crop to cultivate under fluoride stress. Through the course of this study on the basis of several important parameters the fluoride tolerant varieties and susceptible varieties will be known. This outcome of the proposed research work may also help the plant breeders to produce resistanace against fluoride stress of barley.

Keywords: *Hordeum vulgare* L., Fluoride stress, GC-MS, Poaceae, Phytochemicals.

INTRODUCTION

Fluorine is a naturally occurring element that belongs to the halogen family. It is highly reactive, pale yellow in color and gaseous in nature. Due to its highly reactive nature, it can exist in combined form with different elements. The elements formed due to the combination of fluorine and other elements are called fluorides. The essential concentration of fluoride present in soil is in soluble form which is present in a very small proportion i.e. about 10-1000 ppm available to the plant (Drury et al., 1980; Sahariya et al., 2021). Fluoride in trace amounts is important for the normal growth of plants. It is easily absorbed in the plant's root, which is then transferred to respiratory organs via xylem flow. It is mainly transferred to the leaves. Various physiological processes are markedly influenced by fluoride, other than those affected by decreased growth of the plant causing chlorosis, leaf tip burn and leaf necrosis (Manoharan et al., 2007). But, the high concentration of this element in arable land is a big problem. In many countries, fluoride (F^-) contamination in soil is a major concern either through irrigation water or excessive fluoride in ground water, which is a big question for the overall economy and environmental sustainability (Chakrabarti and Patra, 2013). Fluoride contamination in groundwater and irrigation water has emerged as a serious health related geo-environmental issue in various portions of the globe. The link between excessive fluoride in the environment and a high risk of fluorosis in people living in contaminated areas is now well accepted. Several field-scale exploratory trials and controlled studies have been done in various contaminated sites across the world to examine fluoride deposition in crops and its consequences on crop productivity. Despite this, the knowledge remains fragmentary. Fluorine has gotten a lot of press because of its environmental and health implications (Margherita et al., 2021). Fluorine pollution induced by regional buildup is a major health and environmental concern in many parts of the world (Lingling et al., 2021).

Barley was formerly utilized as a resource of human sustenance for many years, with archeological evidence dating back to 8000 BC in Iran (Bothmer and Jacobsen, 1986). Barley was discovered to be a good source of insoluble and soluble dietary fibers, particularly the polysaccharide (1/3) (1/4) –β-D- glucan (beta-glucan). Among all the grains, highest concentrations of beta-glucan are found in barley. The beta-glucan amount found in cereals varies from 1% in wheat grains, to 11% in barley as well as 7% in oats (Cui and Wood, 2000). For ages, because of low input requirements and well adaptation to adverse circumstances such as alkalinity, salinity, drought as well as marginal areas, it has been regarded as a poor man's crop. Even though the majority of the crop is used for cattle feed, the modern surge in industrial need for barley as a resource has prompted it to be considered as an industrial crop.

In India, the drought affected states are facing the problem of this contamination, especially the western state of Rajasthan where almost all the 33 districts are seriously fluoride affected and fluorosis is very common (Agarwal et al., 1997). The farmers and consumers both are at the receiving end due to this undesirable toxicity of fluoride. The farmers of this state usually prefer millets over the cereals and Barley is one of the preferred crops due to various environmental constraints such as severe drought and high fluoride.

In recent years, Rajasthan has just replaced Uttar Pradesh as the leading producer of barley, owing to a lack of rainfall and irrigation water throughout the harvest season during the past several years. Barley is the primary balance food crop in tribal regions of this state. Additionally, it is being used as cattle feed. It is also used in the preparation of malt and traditional beverages like *chhang* and *sattu* which are popular in the tribal people. It is utilized as a therapeutic agent in cardiac and urinary disorders (Skendi et al., 2003).

In the present study, two commonly growing cultivars, *viz*., RD2794 and RD2786 of *Hordeum vulgare* have been taken to determine the comparative phytochemicals profiles and GC-MS analysis under graded fluoride stress with the aim to help the local farmers in the better selection of the cultivar to grow with maximum economic and ecological benefits.

DISTRIBUTION AND DESCRIPTION OF *H. VULGARE*

Barley has both nutritional and medicinal properties therefore; it is one of the most popular usable foods in Japan, China and Korea. The conventional physicians have a belief of medicinal use of this crop however, so far, a small number of researches are accessible in the published work carrying the ethno-medical favor of barley-extract as an anti-obesity agent (Nishiyama et al., 1994). Barley contains starch that can be high, normal or waxy amylose starch. Starch is a vital component in hulled barley, accounting for around 60% of dry matter, total dietary fiber for about 20% and for protein *i.e*., about 11% of dry matter.Therefore, this staple crop has great significance in terms of its phyto-constituents (Sullivan et al., 2013). Barley expands widely in its phytochemical composition because of its adaptive interactions with the ambient environment.

PHYTOCHEMISTRY

Phytochemicals are naturally occurring, biologically active chemical compounds present in plants. Phenolic phytochemicals have several useful effects and may also give flavor to the cereal foods. Phenolic acids are hydroxylated byproducts of cinnamic acids and benzoic acid. Various phenolic acids are mainly present in barley, p-coumaric and ferulic acid have appear to be low molecular weight phenolic acids present in barley mostly in the outer layers, but they are also found in the endosperm (Chanput et al., 2012).

Presently, there is rising attention in the utilization of nutritious constituents so as to avoid inflammatory harm and to control immune response production. Barley grains are familiar to have a number of dietary components and several secondary metabolites such as phenols and flavonoids (Li et al., 2012). However, β-glucans obtained from barley are believed to be an anti-inflammatory bioactive element, although their agronomy varies (Braaten et al., 1994).

MATERIALS AND METHODS

Collection of plant material

Seeds of RD2786 and RD2794 varieties of *H. vulgare* L., were collected from the Krishi Vigyan Kendra Banasthali, Vidyapith, Rajasthan. Seeds were grown in a green-house facility located at the Department of Biotechnology, Banasthali Vidyapith, India to evaluate certain physiological responses at vegetative stage of the *Hordeum vulgare* genotypes under fluoride stress. Seed germination took place in petri plates kept in dark for 2 days at 25°C. The germinated seedlings were sown in earthen pots (20L) containing 10 Kg of soil to achieve 5 seeds per pot. After germination, the seedlings were tinned at leaf 4th leaf stage to a stand of one pot. Genotypes were grown under fluoride stress in three replicates and irrigated with 0, 5, 10, 15, 20 ppm of NaF soil under temperature range 18°C to 32°C. Seedlings were watered and allowed to grow for 1 month by exposing the plants to 15th days of fluoride stress.

EXTRACTION PROCESS

The fresh leaves of both barley varieties were taken and washed properly, dried at room temperature for 7 days and then powered with the help of liquid nitrogen. Separately 1 gram of each leaf powder with different concentrations of NaF as well as control of both the varieties were transferred in a thimble and extracted with methanol as a solvent using Soxhlet apparatus for 12 hours. The extracts were concentrated using a rotatory flash evaporator under reduced pressure at temperatures below 35°C (Zawawi et al., 2000). Later, the standard evaluations were carried out to identify and determine secondary metabolites in the extracts. Both qualitative (alkaloids, flavonoids, carbohydrates, fixed oils and fats amino acids) and quantitative (total flavonoid and phenolic contents) calculations were done (Tables 1, 2). According to the Folin-Ciocalteau method (Vats, 2014) total phenolic content was determined and expressed as Gallic acid standards (mean ± S.D). According to the aluminum chloride colorimetric method (Chandra et al., 2004) total flavonoid content was calculated. The stock Quercetin *(Quercetin is a plant flavonol from the flavonoid group of polyphenols. It is found in many fruits, vegetables, leaves, seeds, and grains; red onions and kale are common foods containing appreciable amounts of quercetin. Quercetin has a bitter flavor and is used as an ingredient in dietary supplements, beverages, and foods.* Solution was prepared by dissolving 5.0 mg of quercetin in 1.0 ml of methanol and extract was separately mixed with 0.6 ml of 2% aluminum chloride. Both extract and stock were prepared separately by serial dilutions using methanol. After incubation at room temperature for 60 minute, the absorbance of the reaction mixture was measured against blank at 420 nm. Among all the different concentrations of NaF used for the extract of both the varieties shows best results at concentration of 20 ppm (Figures 1-4). Hence the GC-MS assay was performed to get information about the phyto-constituents.

GC-MS analysis of *Hordeum vulgare* L. under fluoride stress

Hordeum vulgare leaves of methanol extracts were taken for GC-MS analysis using Thermo Scientific triple quadrupole GC-MS (trace 1300 GC, Tsq 8000 tripe quadrupole MS) equipped with TG 5MS (30m × 0.25mm, 0.25µm) column. With a rate of flow 1ml/min and a volume of 1.0µL, helium gas was used as a carrier gas. The ion source temperature was kept at 230°C and the temperature of the injector was kept at 25°C while the oven temperature was kept at 50°C (Mehdi et al., 2021). The total elution was 47.5 min. The relative percent amount of each component was calculated by comparing its average peak area to total areas. MS solution software provided by the supplier was used to control the system and to acquire the data.

Table 1: Qualitative analysis of varieties RD2794 and RD2786 of *Hordeum Vulgare* L. at different concentration of fluoride

Sl. No	Variables	Variety	Concentrations				
			Control	**5ppm**	**10 ppm**	**15 ppm**	**20 ppm**
1	**Alkaloids**						
	Mayer's test	RD 2786	+	+	++	+++	+++
	Wagner's test	RD 2794	+	+	++	++	++
		RD 2786	+	+	++	+++	+++
		RD 2794	+	+	+	++	++
2	**Flavonoids**						
	Alkaline reagent test	RD 2786	+	+	++	+++	+++
		RD 2794	+	+	+	++	++
3	**Carbohydrate test**						
	Benedict' test	RD 2786	+	+	++	+++	+++
		RD 2794	+	+	+	++	++
4	**Phytosterols**						
	Salkowski' test	RD 2786	+	+	++	+++	+++
	Lebermannburchard test	RD 2794	+	+	+	++	++
		RD 2786	+	+	++	++	++
		RD 2794	+	+	+	++	++
5	**Fixed oils and fats**	RD 2786	-	+	+	+	+
		RD 2794	-	-	+	+	+
6	**Saponification test**	RD 2786	-	+	+	+	+
		RD 2794	-	-	+	+	+

a +++ (highly present),b ++ (moderately present), c + (low present), d – (absent)

Table 2: Quantitative analysis of total phenols and flavonoids content of variety 2786 and 2794 of *H. vulgare*

S. no	Variables	Different concentration of fluoride				
		Control	**5ppm**	**10ppm**	**15ppm**	**20ppm**
1.	Total phenolic content of 2786	151.2±0.12 mg/gGAE	213.4±0.26 mg/gGAE	215.6 ± 0.29 mg/gGAE	218±0.31 mg/gGAE	219±0.31 mg/gGAE
2.	Total flavonoid content of 2786	215±0.21 mg/gGAE	236±0.30 mg/gGAE	240±0.30 mg/gGAE	244±0.30 mg/gGAE	246±0.32 mg/gGAE
3.	Total phenolic content of 2794	138.4±0.10mg/gGAE	140±0.12mg/gGAE	146.1±0.14 mg/gGAE	153.2±0.18 mg/gGAE	160.2±0.17 mg/gGAE
4.	Total flavonoid content of 2794	150±0.09 mg/gGAE	168.2±0.13 mg/gGAE	172.0±0.13mg/gGAE	181.4±0.18mg/gGAE	201± 0.20 mg/gGAE

Data are the same means and standard deviation of the mean for n = 3 independent experiments

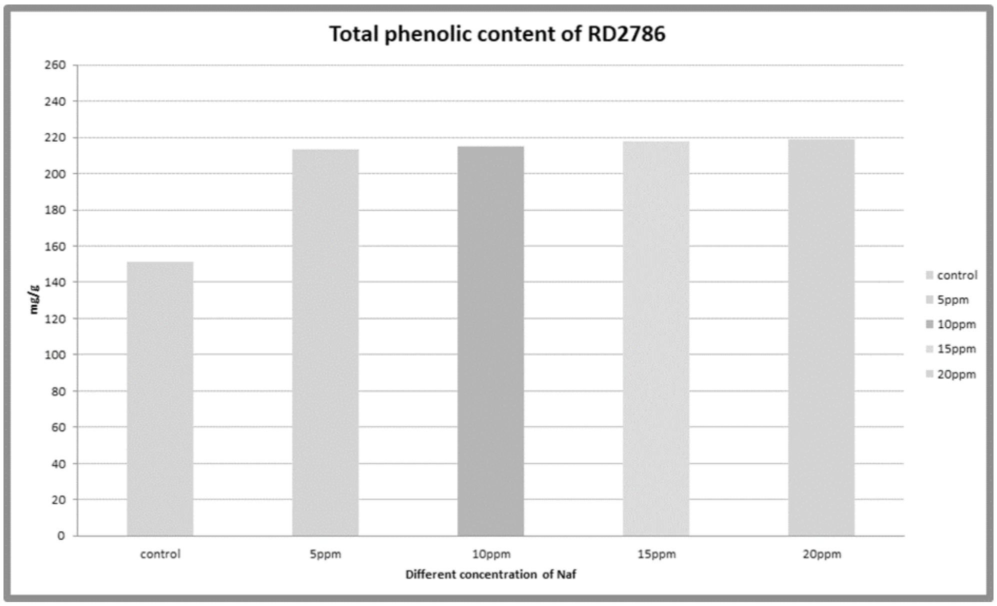

Figure 1: Total phenolic content in *H. vulgare* L. variety RD2786

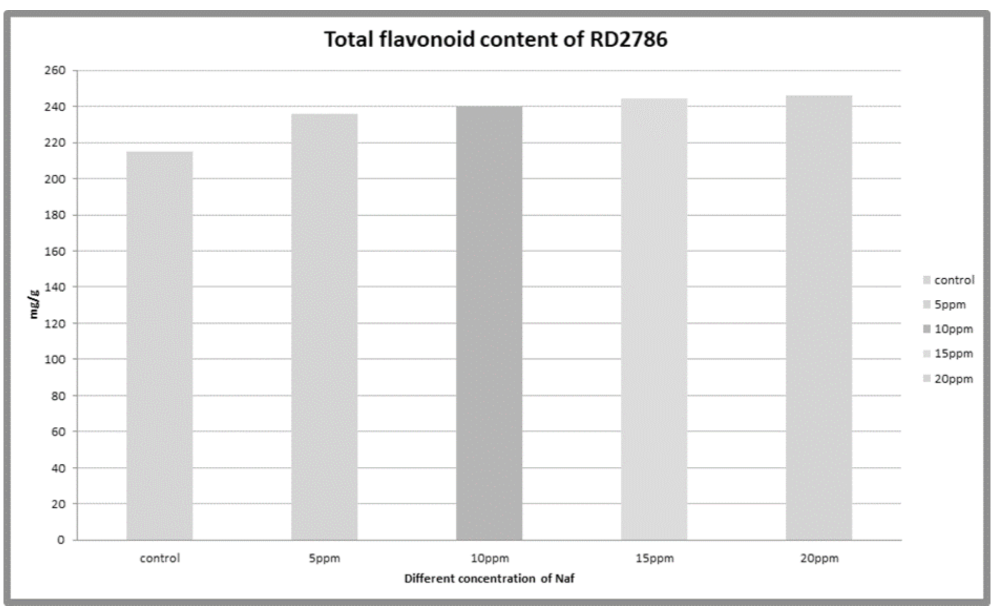

Figure 2: Total flavonoid content in variety RD2786 *H. vulgare* L

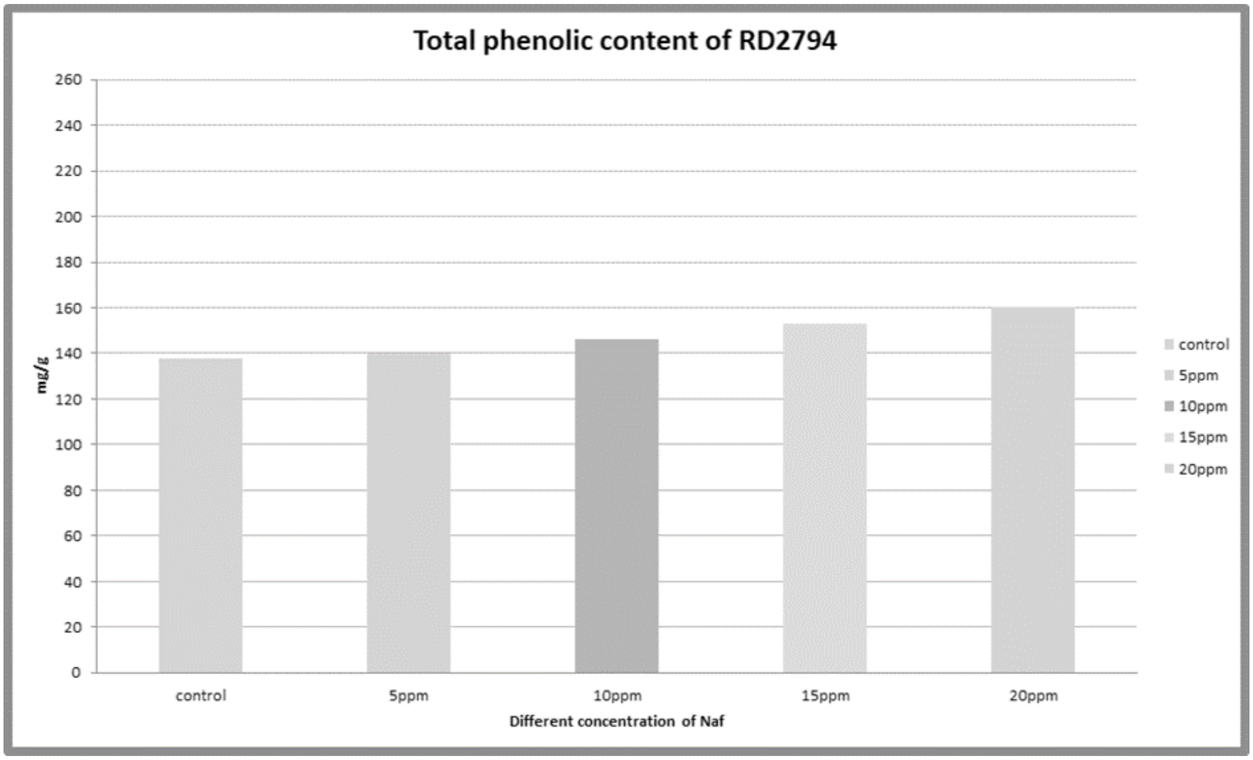

Figure 3: Total phenolic content in variety RD2794 *H. vulgare L*

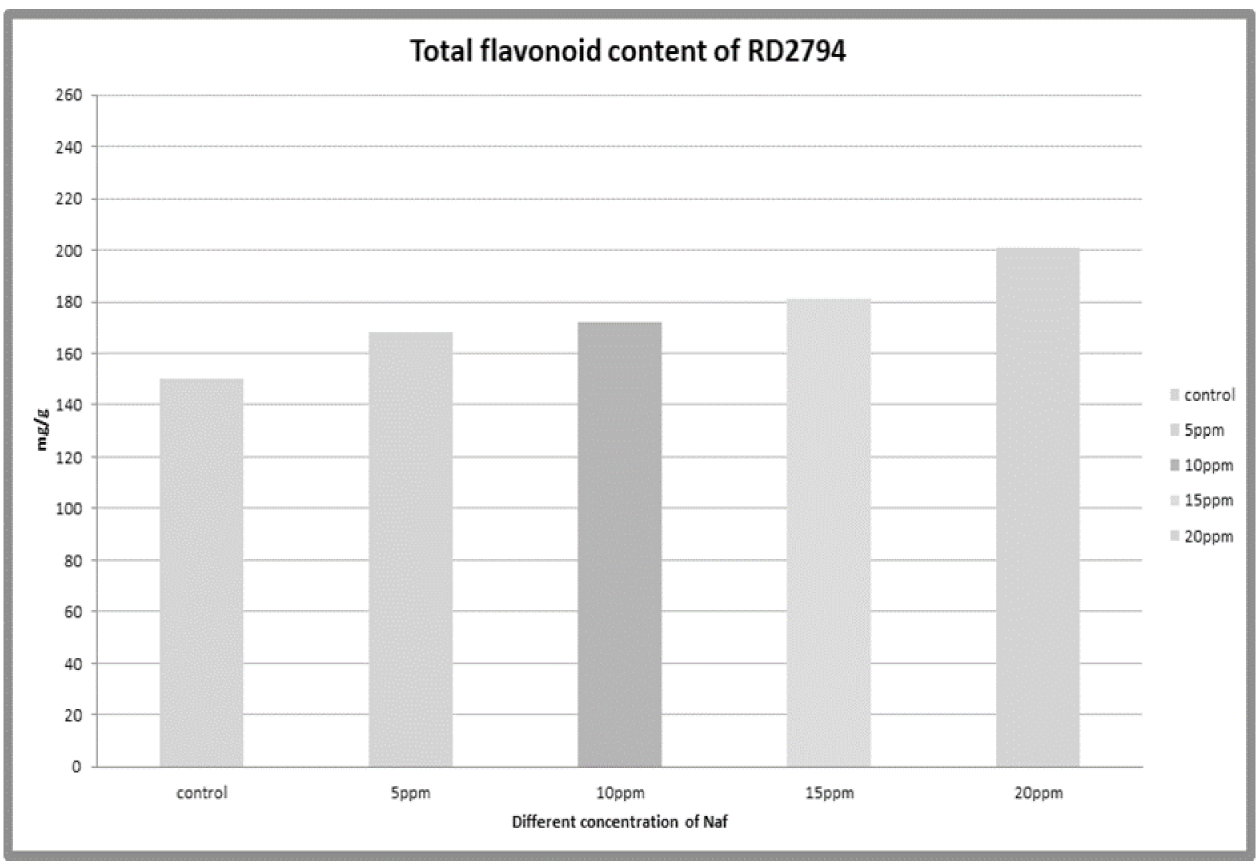

Figure 4: Total flavonoid content in variety RD2794 *H. vulgare* L

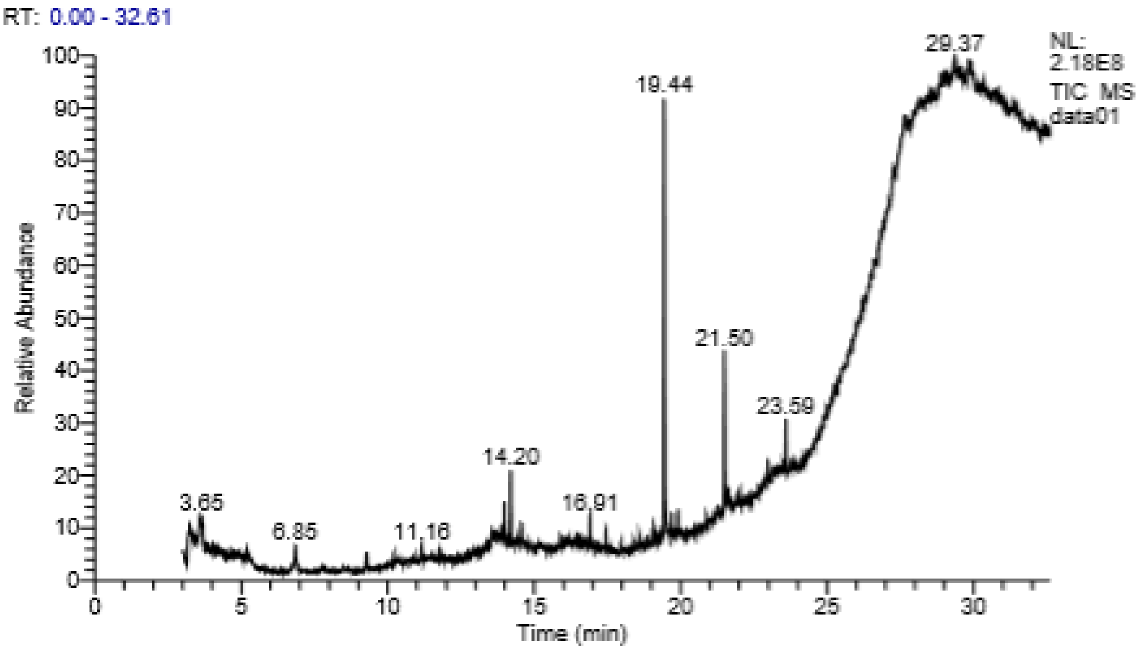

Figure 5: Determination of bioactive compounds through GC-MS

Table 3: GC-MS analysis of *H. Vulgare* variety of RD 2786 in methanol extract

S. No	RT	AREA	COMPOUND NAME	MOLECULAR FORMULA
1.	3.25	7.38 %	Boric acid, trimethyl Ester Phosphinic acid, [methyl(formyl)amino]methyl(2-phenylethyl)-	$C_3H_9BO_3$ $C_{11}H_{16}NO_3P$
2.	3.64	6.68 %	Dibenzene,1,1:4'-bis (1,2-ethanediyl)-2-(1 Hydroxypropyl)-3-trime thylsiloxy- Benzene methanol 2-Phenylethanethiol,TMS derivatives	$C_{22}H_{30}O_2Si$ $C_{11}H_{18}SSi$
3.	6.85	5.17%	1,4-Cyclopentadiene,1(diethylboryl)(trimethylsily1)	
4.	13.99	4.19 %	Heptacosane Octadecane,3-ethyl-5-(2-ethylbutyl)	$C_{27}H_{56}$ $C_{26}H_{54}$
5.	19.44	37.23%	Hexadecanoicacid,methyl ester	$C_{17}H_{34}O_2$
6.	21.50	13.75%	Methyl stearate Heptadecanoic acid, 16- methyethyl Ester	$C_{19}H_{38}O_2$
7.	23.60	4.62%	Dodecanoic acid, undecyl ester	$C_{23}H_{46}0_2$
8.	27.64	6.75 %	DI-n-octyl phthalate	$C_{24}H_{38}O_4$
9.	29.85	8.75 %	Rhodopin	$C_{40}H_{58}O$

STATISTICAL ANALYSIS

All the tests were performed in triplicates (n=3). Data are stated as mean ± SE. The examination of variances among mean were tested by using SPSS software. For every output variable, multiple-comparison Turkey's $p \leq 0.05$ tests were conducted to analyze the variance of the data.

RESULTS

A qualitative analysis of *H. vulgare v*arieties in methanol extract under different concentration of fluoride stress was performed to validate the earlier reports and researches about the occurrence of several phyto compounds. It was found that both selected varieties of *H. vulgare* showed the immoderate presence of carbohydrates (Benedict's test), low amount of saponins, fixed oils and fats were detected, the proximity of the high amount of alkaloids, flavonoids, phenols compounds and phytosterols were also detected. According to qualitative and quantitative analysis, RD2786 has high content of phytochemicals compared to RD2794. The displays the total phenol contents of methanol extract of *H. vulgare* under different fluoride concentrations. The phenolic content increased considerably both in RD2786 and RD2794 varieties of *H. vulgare* with increasing exposure to sodium fluoride. The highest phenol content was found at the concentration of 20 ppm and the lowest phenol content was found at the control and comparing both the varieties, RD2786 has high phenol content.

The result of GC-MS analysis of RD2786 barley in methanol extract contains pharmacologically active substances with anti-inflammatory, antifouling, antioxidant properties.

DISCUSSION

The observed higher level of phenolic content could be responsible for scavenging free radicals formed during fluoride stress. The detection of secondary metabolites under fluoride exposure protects the plant from oxidative damage and improves its bioactive potential and medicinal properties. The increase in the flavonoid level with increased exposure to fluoride concentration may contribute to the metabolism of antioxidants.

The GC-MS analysis of RD 2786 variety of barley in methanol extract exhibited the presence of nine compounds in it. Some of these compounds identified through GC-MS are known to have many important formulation and biological functions, hexadecanoic acid; methyl ester (RT 19.4) acts as an antioxidant and has nematicide, hypocholesterolemic and pesticide properties (Ansorena et al., 2011). Heptacosane acts as alkanes, antimicrobial activity and anti-algal activity (Everall and Lees, 1996). Methyl stearate controls cell-wall porosity and protects from abiotic stress and provides resistance from toxicity (Wu et al., 2018). Dodecanoic acid possesses antifungal properties; it is a saturated fatty acid (Ansorena et al., 2011). The 2, methylbenzene 1,4diol acts as an inflammatory agent. Di-n- octyl phthalate, a plasticizer compound and possesses antifouling and antimicrobial properties (Wu et al., 2018). Because, both varieties were grown under the same environmental conditions and stresses, the differences are due to genetic make-up. The considerable variances found in this study are crucial for getting the optimum utilization of these barleys as food and feed.

CONCLUSIONS

Virtually each part of *H. vulgare*, i.e., fruit, seeds, root, and leaves are utilized. The individual parts of barley produce various phytochemicals and these compounds are used to cure different disorders of human beings and livestocks. On the basis of quantitative analysis, the information attained is that *H. vulgare* is a reservoir of important phyto chemicals such as flavonoids, alkaloids, phytosterols, and phenols. This abundant diversity of botanical compounds can be associated with the various observable biochemical processes of plants. Hence, it can be concluded that *H. vulgare* is a versatile plant with a huge reservoir of various medicinally significant phyto-constituents.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest relevant to this article.

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