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Original article

Chemical composition of *Gastrocotyle hispida* (Forssk.) bunge and *Heliotropium crispum* Desf. and evaluation of their multiple *in vitro* biological potentialsAmir Shahbaz<sup>a,b</sup>, Banzeer Ahsan Abbasi<sup>a</sup>, Javed Iqbal<sup>c,\*</sup>, Iram Fatima<sup>d</sup>, Syeda Anber Zahra<sup>a</sup>, Sobia Kanwal<sup>e</sup>, Hari Prasad Devkota<sup>f</sup>, Raffaele Capasso<sup>g</sup>, Ajaz Ahmad<sup>h</sup>, Tariq Mahmood<sup>a,\*</sup><sup>a</sup> Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan<sup>b</sup> National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA<sup>c</sup> Department of Botany, Bacha Khan University, Charsadda, Khyber Pakhtunkhwa, Pakistan<sup>d</sup> Department of Biotechnology, Fatima Jinnah Women University, Rawalpindi, Pakistan<sup>e</sup> Department of Zoology, Rawalpindi Women University, Rawalpindi, Pakistan<sup>f</sup> Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, chuo-ku, Kumamoto, Japan<sup>g</sup> Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy<sup>h</sup> Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

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## ABSTRACT

Medicinal plants largely serve as a source of bioactive compounds in traditional medicines to cure various diseases. The present study was aimed at chemical composition, antioxidant, antimicrobial, cytotoxic and antihemolytic potential of five different extracts of *G. hispida* and *H. crispum* (Boraginaceae). *G. hispida* methanolic extract displayed highest number (eleven) of polyphenolic compounds by using high performance liquid chromatography (HPLC). Functional groups were identified by Fourier-transformed infrared spectroscopy (FTIR) and elements (Si, Fe, Ba, Mg, Ti, Ca, Mg and Cr) were observed by using laser-induced breakdown spectroscopy (LIBS) which were also highly expressed in *G. hispida* as compared to *H. crispum*. Antioxidant activity was determined via six assays and antibacterial activity was observed in decreasing order of methanol > ethanol > chloroform > ethyl acetate > n-Hexane in both species. Cytotoxic potential was investigated against brine shrimps and then liver (HepG2) and skin (HT144) cancer cell lines which was detected highest in the *G. hispida* ethanolic extract (50.76 % and 72.95 %). However, *H. crispum* chloroform extract revealed highest (31.869 µg/mL) antihemolytic activity and its methanolic extract indicated highest (13.5 %) alpha-amylase inhibitory potential. Altogether, results suggested that both species could be used effectively in food and drug industries owing to the presence of vital bioactive compounds and elements. In future, we recommend to isolate active compounds and to perform *in vivo* biological assays to further validate their potential biological applications.

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## 1. Introduction

Different research and floristic studies have indicated the existence of ~ 500,000 plants species on earth and about 120,000 spe-

cies are used in medicines to treat different diseases (Kallassy, 2019). Plants are the richest source of medicine in both developed and developing countries (Iqbal et al., 2018a, Iqbal et al., 2018b). Natural products derived from these plants serve as valuable starting points for drug discovery (Rodrigues et al., 2016). Plant-derived compounds exhibit remarkable biological potential as compared to synthetic chemical products (Espinosa-Leal et al., 2018). Up till now, scientific community is trying to isolate natural products from medicinal plants for treating different human diseases (Panthi et al., 2020, Zahra et al., 2021a). Plants and their active compounds are also rich source of natural antioxidants. The phytochemical constituents present inside the plants like flavonoids, polyphenols, tannins, carotenoids and terpenes exhibit antioxidant

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activities by quenching free radicals produced in the body (Zahra et al., 2021b, Ali et al., 2021a, Ali et al., 2021b).

Similarly, plant-derived antimicrobials compounds are also one of the most promising sources considered biosafe and biocompatible when compared with synthetic compounds (Casciaro et al., 2019). Use of crude extract of medicinal plants is also gaining attention to treat infectious diseases caused by microorganism (Phan et al., 2019). Plants generally produce many bioactive compounds which act as antimicrobial agents against different pathogenic organisms (Toiu et al., 2019). Due to the antimicrobial property of medicinal plants, these can be utilized as pharmacological agents and could be favorable approach for treating infectious diseases (Mickymaray, 2019).

Natural healing agents are largely used to overcome unwanted side effects of synthetic drugs worldwide. Pakistan comprises of ~ 6000 species of higher plants, of which about 700 species are commonly used for medicinal purposes (Shinwari, 2010). The current study is focusing on the medicinal flora of district Dera Ghazi Khan (DG Khan) which lies in the sub-tropical continental plain of Pakistan, which has sandy soil and harsh climatic conditions. People living in the DG Khan mainly rely on medicinal plants to treat different diseases (Imran et al., 2013). Species belonging to family Boraginaceae exhibit great economic and medicinal values (Dresler et al., 2017). *Heliotropium crispum* aqueous paste is used against infectious and malarial agents while *Gastrocotyle hispida* is used as a refreshing drink like tea and leaves decoction is used as diuretic and in the treatment of rheumatism (Shahat et al., 2019, Khan et al., 2016).

Keeping in view the ethno-pharmacological importance and phytochemical values of *G. hispida* and *H. crispum*, present study was aimed at exploring the biochemical and elemental composition and biological potential of two species collected specifically from Dera Ghazi Khan, Pakistan. It is located at 70.38° longitude, 30.03° latitude, 699 m altitude and is very hot in summers (42 °C) with very little rainfall. Dominant species of this region mainly belongs to family Fabaceae, Solanaceae, Asteraceae, Poaceae and Brassicaceae (Gulshan et al., 2012). Hence, plant extracts were prepared using various polarity-based solvents followed by the compound identification which was done by performing HPLC, functional groups were ascertained via FTIR spectroscopy and elemental analysis was carried out using LIBS technique. Furthermore, biological potential was determined by conducting antioxidant, antimicrobial, cytotoxicity, antihemolytic and  $\alpha$ -amylase inhibitory assays.

## 2. Materials and methods

### 2.1. Plant collection and extracts preparation

*G. hispida* (Acc. No.130864) and *H. crispum* (Acc. No. 130865) were collected from DG Khan, Pakistan during March 2019. Plants were washed, air-dried, powdered and extracted with ethanol, methanol, ethyl acetate, chloroform and n-Hexane (30 g/300 mL each) for five days. Subsequently, extracts were filtered and dried at reduce pressure using rotary evaporator and were stored at 4 °C for future use (Harborne, 1973).

### 2.2. Qualitative phytochemical analysis

Initially some phytochemical tests were carried out using standard protocols to determine the presence and/or absence of terpenoids and steroids (Libermann's test), flavonoids (Alkaline reagent test), saponins (Foam test), phenols (Ferric chloride test), glycosides (Salkowski test), alkaloids (Mayer's reagent), quinones (2 mL extract + 2 mL sulphuric acid – red colour) and coumarins

(2 mL extract + 3 mL NaOH – yellow colour) (Raman, 2006, Harborne, 1973).

### 2.3. Total phenolic and flavonoid contents

For TPC, gallic acid was added in Folin-ciocalteu reagent (90  $\mu$ L) and NaCO<sub>3</sub> solution (90  $\mu$ L) to prepare the calibration curve. Then, 20  $\mu$ L of each extract was added in the same reagent and the absorbance was recorded. Data obtained was expressed as mg GAE/g sample (Chlopicka et al., 2012). Similarly, Aluminum Chloride Colorimetric Method was used to measure Flavonoid contents and the results were recorded as mg QE/g sample (Chang et al., 2002).

### 2.4. High performance liquid chromatography (HPLC) analysis

Polyphenol detection was performed by HPLC analysis of *G. hispida* and *H. crispum* extracts using previously optimized protocol of Qasim et al. (2017). A Zorbex-C8 analytical column (4.6  $\times$  250 nm) and a diode array detector were fitted with the HPLC system (DAD, Agilent technologies, Germany). Two mobile phases including acetonitrile: methanol: water: acetic acid (5:10:85:1) and acetonitrile: methanol: acetic acid (40:60:1) were used with a flow rate of 1 mL/min. Samples were filtered using membrane filter (0.45  $\mu$ m) and then 20  $\mu$ g/mL sample was injected via an injection port into the column. Vanillic acid (257 nm), Thymoquinone (257), Plumbagin (257) Gallic acid (279), Syringic (279), Coumaric acid (279), Catechin (279 nm), Emodin (279), Caffeic acid (325 nm), Gentisic (325 nm), Cinnamic acid (325), Luteolin (325), Apigenin (325) Quercetin (368 nm), Myricetin (368 nm) and Kaempferol (368 nm) were used as standards and detected at respective wavelengths. Compound identification was done by comparing the UV absorption spectra and retention time of samples with the standards.

### 2.5. Fourier-Transform infrared (FTIR) spectroscopy

FTIR spectrometer Perkin Elmer- Spectrum 65 (FTIR model) was used for the identification of the characteristic functional groups in plants. This technique provides information from the obtained absorption spectrum. Plant material was powdered and subjected to a pressure of about 5  $\times$  106 Pa in an evacuated dye. Spectras were recorded at 4000–400 cm<sup>-1</sup> frequency and peak values were recorded (Meenambal et al., 2012).

### 2.6. Laser-induced breakdown spectroscopy (LIBS)

LIBS analysis was done using standard protocol as suggested by Zafar et al. (2016). Q-switched Nd: YAG laser (Brilliant-B Quantel, France) was used at 532 nm wavelength and repetition rate of 10 Hz was carried out for ablation. Laser energy was measured using energy meter and the sample was kept on the rotating stage in order to obtain the homogenous plasma. Four spectrometers (wavelength 250–870 nm) were used to detect radiations and the emission spectra was assessed with the help of laser that was synchronized with the spectrometer.

### 2.7. Determination of antioxidant assays

#### 2.7.1. Reducing power assay

Protocol of Oyaizu (1986) was followed to observe the reducing potential of selected plant extracts. Each extract (200  $\mu$ L) was added in the phosphate buffer (500  $\mu$ L) and potassium ferricyanide (500  $\mu$ L) and then incubated (20 min) at 50 °C. About 500  $\mu$ L of TCA was added in each sample using micropipettes followed by

centrifugation for 10 min. Almost 100  $\mu\text{L}$  of 0.1 %  $\text{FeCl}_3$  was added in the supernatant and absorbance was recorded at 630 nm.

### 2.7.2. Cupric ions reducing antioxidant capacity

About 0.01 M  $\text{CuCl}_2$  solution (10  $\mu\text{L}$ ), 7.5 mM ethanol neocuproine solution (10  $\mu\text{L}$ ) and ammonium acetate buffer solution (1.0 M) of 10  $\mu\text{L}$  were added in gallic acid and plant extracts (20  $\mu\text{L}$ ). Volume was adjusted upto 1 mL using  $\text{dH}_2\text{O}$  and absorbance was recorded at 515 nm (Apak et al., 2004).

### 2.7.3. Phosphomolybdate assay

Reaction mixture was prepared by dissolving 28 mM sodium phosphate (1.68 g), 4 mM ammonium molybdate (0.25 g) and sulphuric acid (1.63 mL) in  $\text{dH}_2\text{O}$  (50 mL). Each extract (50  $\mu\text{L}$ ) was dissolved in the reaction mixture and then incubated at 95 °C. Finally, absorbance was taken at 630 nm (Prieto et al., 1999).

### 2.7.4. DPPH radical scavenging assay

Initially, 3.9 mg of DPPH (3.9 mg) was mixed in methanol (100 mL) followed by the incubation (30 min) in dark. Then, DPPH solution (180  $\mu\text{L}$ ) was mixed with the sample solution (20  $\mu\text{L}$ ) and ascorbic acid was used as a standard. The absorbance was measured at 517 nm using UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). Percentage inhibition was calculated using formula provided below and then  $\text{IC}_{50}$  values were determined (Tepe et al., 2005).

$$\% \text{ Scavenging activity} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

### 2.7.5. ABTS radical scavenging assay

ABTS stock solution (7 mM) and potassium persulphate (2.45 mM) were added together and after 12–16 h, solution was diluted (i.e.  $0.70 \pm 0.02$  absorbance). Then  $\text{ABTS}^+$  solution (160  $\mu\text{L}$ ) was mixed with different plant concentrations (10  $\mu\text{L}$ ) and absorbance was determined at 734 nm (Loziene et al., 2007). Percentage inhibition and  $\text{IC}_{50}$  values were expressed using same formula which was used for DPPH assay.

### 2.7.6. NBT (or superoxide radical scavenging) assay

In this assay,  $\text{Na}_2\text{CO}_3$  (1 mL), 24 mM NBT (0.4 mL), plant extract (1 mL), EDTA solution (0.2 mL) and hydroxylamine hydrochloride (0.4 mL) were added in eppendorf tubes and placed at 25 °C for incubation (15 min). Then, the absorbance (540 nm) was measured and % inhibition and  $\text{IC}_{50}$  value were calculated (Munir and Sarfraz, 2014).

## 2.8. Antimicrobial assay

Antibacterial potential was evaluated against *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 90271), *Bacillus subtilis* (ATCC19659), *Escherichia coli* (ATCC 33456) and *Klebsiella pneumoniae* (ATCC 1705) by using agar-disc diffusion assay (Jo et al., 2004). Bacterial strains were cultured in nutrient broth medium, incubated (37 °C) for 18 h (hr) and then standardized by regulating OD to 0.5 ( $1 \times 10^8$  CFU/mL). Plant extracts of different concentration (3.7, 11.11, 33.33, and 100  $\mu\text{g}/\text{mL}$ ) was used for this method. Zones <8 mm were not considered significant and MIC values were calculated. Similarly, *Fusarium solani* (FCBP 0291), *Aspergillus niger* (FCBP 0918), *Aspergillus flavus* (FCBP0064), *Candida albicans* (FCBP 478) and *Mucor racemosus* (FCBP 0300) were treated with varying doses (3.7, 11.11, 33.33 and 100  $\mu\text{g}/\text{mL}$ ) of plant extracts to assess antifungal activity. Strains were sub-cultured for 24 hr in SDA media at 25 °C. Fungal plates were placed in incubator for 24 hr after treatment with plant extracts and then inhibition zones were measured. Clotrimazole was used as a standard drug and MIC values were measured.

## 2.9. Brine shrimp cytotoxicity assay

Cytotoxicity potential was investigated at 62, 125, 250, 500 and 1000  $\mu\text{g}/\text{mL}$  using standard protocol. Each extract was added in a separate vial and the final volume was brought to 5 mL using saline solution. Afterwards, ten (10) shrimps were taken in each vial (after 24 hr) and placed in incubator at 32 °C. Number of survivors were counted after 24 hr and then,  $\text{LC}_{50}$  values and % mortality was determined (Mehwish et al., 2019).

## 2.10. Anti-cancer assay

HepG2 (Liver cancer), HT144 (Skin cancer) cell lines were grown in DMEM supplemented with 10 percent FBS along with 1 percent antimycotic (ABAM) and maintained in a tissue culture cabinet under 37 °C conditions with 95 percent air and 5 percent  $\text{CO}_2$  (Manassas, VA, United States). MTT assay was carried out to detect the inhibitory effect of *G. hispida* and *H. crispum* extract on HepG2 (Liver cancer) and HT144 (Skin cancer) cell line. The reactant cell lines were held for 24 hr at a density of ( $5 \times 10^4$ ) cells/well in 96-well plates, followed by the addition of plant extracts. Media were subsequently extracted and 5 mg/mL of MTT reagents were loaded along with sterile PBS, followed by 4 h incubation. In addition, the MTT solution was extracted and DMSO was used to dissolve precipitate formazan. Finally, absorbance was measured at 570 nm.

## 2.11. Antihemolytic assay

Six milliliters of human blood were taken in EDTA vials and centrifuged (5 min) at  $1000 \times g$ . The obtained pellet was washed (3x) with 0.2 M PBS (pH 7.4) and then 0.5 mL of each extract (100–1000  $\mu\text{g}/\text{mL}$  in PBS) was added to 1 mL of erythrocyte suspension followed by the incubation (20 min). Then  $\text{H}_2\text{O}_2$  solution (0.5 mL) was added in the samples and then centrifuged for 10 min. Absorbance of supernatant was measured at 540 nm using  $\text{H}_2\text{O}_2$  as a negative control and quercetin as a standard with PBS. Study approval (#BEC-FBS-QAU2019-143) was taken from the Bioethical Committee of Quaid-i-Azam University, Islamabad (Yang et al., 2005).

## 2.12. Alpha-amylase inhibition assay

Initially, a reaction mixture of 15  $\mu\text{L}$  phosphate buffer, 25  $\mu\text{L}$  alpha-amylase enzyme (0.14 U/mL), 10  $\mu\text{L}$  extract sample (4 mg/mL DMSO) and 40  $\mu\text{L}$  starch solution were incubated (50 °C) for 30 min. Subsequently, 20  $\mu\text{L}$  of 1 M HCl and 90  $\mu\text{L}$  of the iodine reagent (5 mM phosphate buffer potassium iodide) were applied to each well. Acarbose was used as a positive control and absorbance was noted at 540 nm (Khalil et al., 2018). The inhibition of alpha-amylase was measured using following formula:

$$\% \alpha\text{-amylase inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

## 2.13. Statistical analyses

All experiments were performed thrice and mean  $\pm$  standard error was calculated. ANOVA was performed using statistix version 8.1 and mean values were compared by Tukey LSD (Steel et al., 1996).  $\text{IC}_{50}$  values were calculated using Graphpad prism while  $\text{LC}_{50}$  values were determined via probit analysis program (Finney, 1952). Moreover, origin pro 7 was used for the interpretation of spectras obtained from LIBS.

### 3. Results

#### 3.1. Phytochemical analysis

##### 3.1.1. Qualitative tests

Results revealed that secondary metabolites are present in most of the methanolic and ethanolic extracts as compared to the chloroform, ethyl acetate and n-Hexane extracts which revealed the presence of some compounds. Among these, flavonoids, glycosides, phenols, hormones, terpenoids, saponins and tannins were observed in most of the extracts, while alkaloids, quinone and coumarins were present in only few extracts (Table 1).

##### 3.1.2. Total phenolic and flavonoid contents

In case of *H. crispum*, phenolic contents were found highest in the ethyl acetate extract ( $79.889 \pm 1.97$  mg GAE/g) and lowest in n-Hexane extract ( $16.286 \pm 2.69$  mg GAE/g) while among *G. hispida* highest TPC was detected in the chloroform extract ( $78.272 \pm 4.4$  mg GAE/g) while lowest in n-Hexane extract ( $52.708 \pm 5.53$  mg GAE/g) respectively. Similarly, *H. crispum* also showed highest TFC in ethyl acetate extract ( $22.685 \pm 2.05$  mg QE/g) and lowest in methanolic extract ( $15.934 \pm 1.13$  mg QE/g). However, *G. hispida* showed highest TFC in ethanolic extract ( $25.338 \pm 1.44$  mg QE/g) and lowest in methanolic extract ( $14.183 \pm 1.04$  mg QE/g) as shown in Fig. 1.

##### 3.1.3. Hplc analysis

HPLC analysis of methanolic, ethanolic and ethyl acetate extracts of *H. crispum* and *G. hispida* was performed and their chromatographs were compared with the standards. Presence or absence of sixteen (16) compounds in *G. hispida* and *H. crispum* extracts is presented in table 2. Highest number of compounds were expressed in *G. hispida* methanolic extract namely Plumbagin, Thymoquinone, Gallic acid, Vanillic acid, Coumaric acid, Syringic acid, Gentisic, Caffeic acid, Luteolin, Myrcetin, Quercetin followed by *H. crispum* ethanolic extract (Plumbagin, Thymoquinone, Vanillic acid, Gallic acid, Syringic acid, Cinnamic acid, Coumaric acid and Caffeic acid). Chromatogram of standards and *G. hispida* methanolic extract derived at different wavelengths is presented in Fig. 2a and 2b.

#### 3.2. FTIR analysis

FTIR spectroscopy was performed to determine the bioactive functional groups present in plant extracts. In both species, total seven prominent peaks were observed indicating the presence of seven different kinds of bonds i.e. OH-stretch, C-H stretch, C-C

stretch, N-H bend, C-H wag ( $-\text{CH}_2\text{X}$ ), C-O stretch, C-N stretch and C-Br stretch (Fig. 3a and b).

#### 3.3. LIBS analysis

In present study, the elemental analysis of selected plant samples was observed using LIBS spectra and the spectral lines were identified using NIST database. Emission spectrum revealed that Si, Fe and Ba were present in both plants, but their concentration was much higher in *G. hispida* as compared to *H. crispum*. Emission spectrum presented in Fig. 4 clearly shows the signature of Mg, Si and Ti. It seems that the concentration of Mg was almost comparable in both plants, but the concentration of Si and Ti was higher in *G. hispida* as compared to *H. crispum*. A very small concentration of heavy metal chromium (Cr) was also detected in *G. hispida*. Moreover, Ca and Mg were also detected in both plants with comparable concentration (Fig. 4).

#### 3.4. Biological assays

##### 3.4.1. Antioxidant assays

Highest reducing potential was observed in *H. crispum* chloroform extracts ( $33.379 \pm 1.66$  mg/g) and lowest in the methanolic extract of *H. crispum* ( $14.243 \pm 0.50$  mg/g) while in *G. hispida* highest reducing potential was observed in methanolic extract and lowest in n-Hexane extract. In case of CUPRAC assay, highest activity was recorded in the ethyl acetate extract of *H. crispum* ( $40.943 \pm 1.50$  mg/g) and lowest in the *H. crispum* ethanolic extract ( $09.620 \pm 0.91$  mg/g) while in *G. hispida* highest activity was observed in n-Hexane and lowest in ethanolic extracts. However, TAC was found highest in *H. crispum* ethanolic extract ( $78.128 \pm 6.79$  mg/g) and lowest in *H. crispum* n-Hexane extract ( $16.877 \pm 1.64$  mg/g) respectively. Similarly, TAC was revealed highest in ethanolic extract of *G. hispida* and lowest in n-hexane extract. Additionally, free radicals (DPPH, ABTS and SOR) scavenging activities of the methanolic and chloroform extracts of *H. crispum* and *G. hispida* was determined and the results were compared with ascorbic acid which is used as a standard. Among both plants, highest DPPH scavenging activity was observed in methanolic extract of *G. hispida* ( $12.636 \pm 2.07$   $\mu\text{g/mL}$ ) and lowest in the chloroform extract of *G. hispida* ( $34.480 \pm 3.62$   $\mu\text{g/mL}$ ). Similarly, highest NBT activity was determined in the methanolic extract of *H. crispum* ( $38.556 \pm 5.34$   $\mu\text{g/mL}$ ) and lowest in the chloroform extract of *G. hispida* ( $119.23 \pm 5.80$   $\mu\text{g/mL}$ ). Moreover, highest ABTS scavenging activity was found in the methanolic extract of *H. crispum* ( $7.980 \pm 2.94$   $\mu\text{g/mL}$ ) and lowest in the methanolic extract of *G. hispida* ( $29.083 \pm 0.37$   $\mu\text{g/mL}$ ) (Table 3).

**Table 1**

Qualitative phytochemical analysis of methanol, ethanol, ethyl acetate, chloroform and n-Hexane extracts of *G. hispida* and *H. crispum*.

Phytochemicals	<i>H. crispum</i>					<i>G. hispida</i>				
	HCM	HCE	HCEA	HCCHL	HCHEX	GHM	GHE	GHEA	GHCHL	GHHEX
Alkaloids	+	+	+	-	+	+	+	+	-	+
Flavonoid	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+
Quinone	+	+	-	-	-	+	+	-	-	-
Coumarin	+	+	-	+	+	+	+	-	+	+

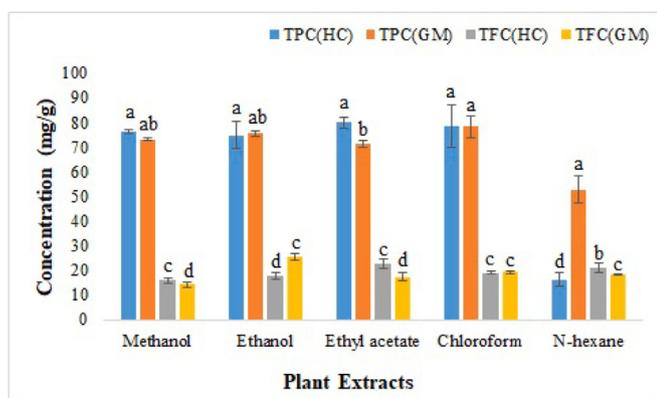
'+' and '-' indicates the presence and absence of secondary metabolites in selected plant extracts. (HCM: *H. crispum* methanolic extract; HCE: *H. crispum* ethanolic extract; HCEA: *H. crispum* ethyl acetate extract; HCCHL: *H. crispum* chloroform extract; HCHEX: *H. crispum* n-Hexane extract; GHM: *G. hispida* methanolic extract; GHE: *G. hispida* ethanolic extract; GHEA: *G. hispida* ethyl acetate extract; GHCHL: *G. hispida* chloroform extract; GHHEX: *G. hispida* n-Hexane extract).

**Table 2**

Polyphenolic compounds detected at different retention time in the methanolic, ethanolic and ethyl acetate extracts of *G. hispida* and *H. crispum* by using HPLC technique.

Polyphenols	Retention time	GHM	GHE	GHEA	HCM	HCE	HCEA
Gallic acid	3.72	+	+	+	-	+	-
Catechin	7.33	-	+	-	-	-	-
Gentisic acid	8.08	+	+	-	-	-	-
Vanillic acid	9.54	+	-	+	+	+	-
Caffeic acid	9.59	+	-	+	+	+	-
Syringic acid	10.00	+	-	-	-	+	+
Cinnamic acid	13.08	-	-	-	+	+	-
Coumaric acid	14.76	+	-	+	+	+	-
Myrcetin	15.51	+	+	-	-	-	-
Quercetin	18.51	+	-	-	+	-	-
Luteolin	19.58	+	-	+	-	-	-
Kaempferol	21.20	-	-	-	-	-	-
Apigenin	22.00	-	-	-	-	-	-
Plumbagin	22.39	+	+	-	+	+	+
Thymoquinone	22.65	+	-	+	+	+	+
Emodin	29.05	-	-	-	-	-	+

\*Data is representing compounds obtained from minimum to maximum retention time. '+' and '-' indicates the presence and absence of polyphenolic compounds in plant extracts (GHM: *G. hispida* methanolic extract; GHE: *G. hispida* ethanolic extract; GHEA: *G. hispida* ethyl acetate extract HCM: *H. crispum* methanolic extract; HCE: *H. crispum* ethanolic extract; HCEA: *H. crispum* ethyl acetate extract).

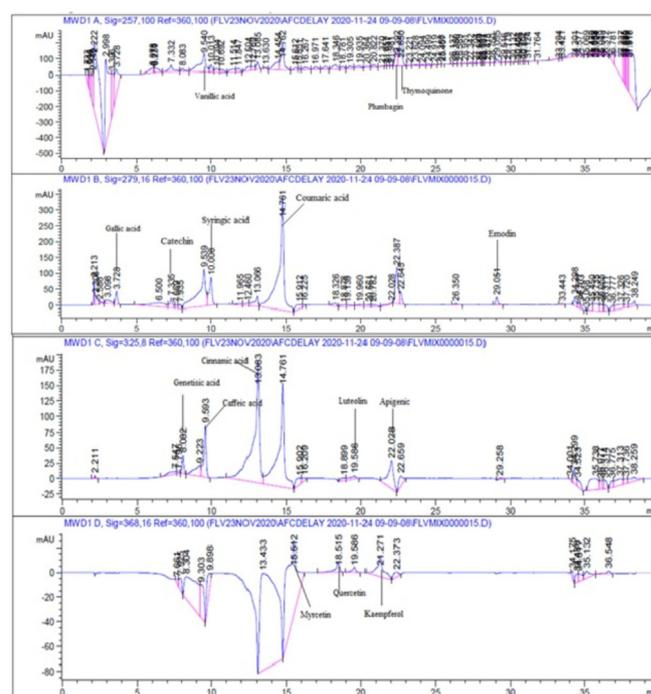


**Fig. 1.** Total phenolic (mg GAE/g) and flavonoid (mg QE/g) contents observed in different extracts of *H. crispum* and *G. hispida*. Data represents the mean of three replicates, error bars indicates standard deviation and each letter (a-d) indicates significance at  $P < 0.05$  by using ANOVA.

The data is expressed as mean  $\pm$  SD (n = 3) and the alphabetical letters (a-d) indicates significant difference ( $P < 0.05$ ) from each other (DPPH = 2,2-diphenyl-1-picrylhydrazyl; NBT = Nitro Blue Tetrazolium; ABTS = 2, 2'-azino-bis -3- ethylbenzthiazoline-6-sulphonic acid; HCM: *H. crispum* methanolic extract; HCCHL: *H. crispum* chloroform extract; GHM: *G. hispida* methanolic extract; GHCHL: *G. hispida* chloroform extract). Altogether, *H. crispum* and *G. hispida* extracts made in polar solvents showed more antioxidant potential than in non-polar extracts (Fig. 5).

**3.4.2. Antimicrobial activity**

Antibacterial activities of the plant extracts were evaluated against five bacterial strains including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia*. *H. crispum* revealed highest activity in its ethyl acetate (10.66  $\pm$  0.57 mm), chloroform (10.33  $\pm$  0.72 mm) and n-Hexane (15.33  $\pm$  0.57 mm) extracts against *E. coli*. While *G. hispida* extracts showed highest activity in the methanolic (11.3  $\pm$  0.57 mm) and chloroform (19.0  $\pm$  1.01 mm) extracts against *B. subtilis*. Overall, among all extracts n-Hexane extracts showed minimum activity against most of the bacterial strains. Furthermore, results revealed that selected plant extracts did not possess significant antifungal potential and thus, considered as the most resistant strains (Table 4).



**Fig. 2.** RP-HPLC chromatograms indicating the existence of polyphenolic compounds at different wavelengths (a) Chromatograms of standards (b) Chromatograms of *G. hispida* methanolic extract.

**3.4.3. Cytotoxic activity**

In case of *H. crispum*, highest brine shrimp cytotoxicity was recorded in its methanolic (LC<sub>50</sub> – 8.4980 ppm), ethanolic (LC<sub>50</sub> – 8.3640 ppm) and chloroform (LC<sub>50</sub> – 10.817 ppm) extract while among different extracts of *G. hispida* highest activity was revealed in n-Hexane (LC<sub>50</sub> – 42.380 ppm) and methanolic (LC<sub>50</sub> – 75.883 ppm) extracts. However, chloroform extracts showed lowest activity in both species and vincristine sulfate (control) displayed 0.839 ppm LC<sub>50</sub> value (Table 5).

Results of anticancer assay revealed that *G. hispida* ethanolic extract exhibited highest cell viability against HepG2 (50.76 %) and HT144 (72.95 %) cell lines. Similarly, ethyl acetate extract of *H. crispum* also showed significant viability (16.14 % cell viability) against HepG2 cell lines. However, other *H. crispum* extracts were

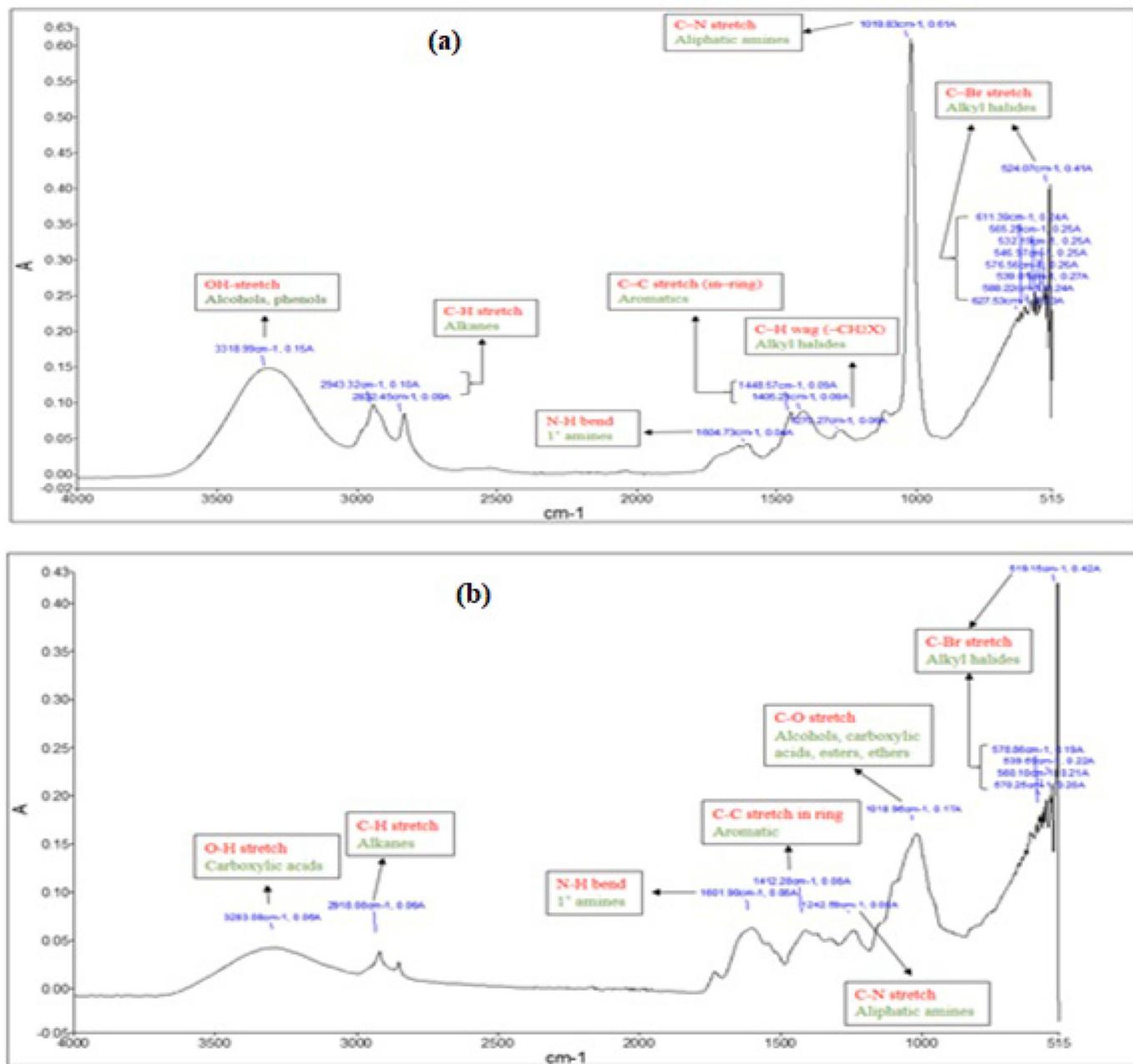


Fig. 3. FTIR spectra of two species of family Boraginaceae showing the presence of functional groups at different peaks (a) FTIR spectrum of *G. hispida* (b) FTIR spectrum of *H. crispum*.

determined as ineffective against cancer cell lines while *G. hispida* extracts showed moderate anticancer activity (Fig. 6).

#### 3.4.4. Antihemolytic activity

Results revealed highest antihemolytic activity in the *H. crispum* chloroform extract (31.869 µg/mL) followed by *H. crispum* ethanolic extract (18.634 µg/mL) and *G. hispida* methanolic extract (14.991 µg/mL) respectively. However, extracts of both species prepared in ethyl acetate indicated lowest antihemolytic potential (8.227 µg/mL and 9.560 µg/mL) as shown in Table 6.

#### 3.4.5. Alpha-amylase inhibition assay

In this assay, acarbose was used as a standard which showed 11.513 % inhibition. Among all extracts, highest % inhibition was observed for the methanolic extract of *H. crispum* (13.5 %) followed

by the n-Hexane extract of *G. hispida* (14.5 %) and ethyl acetate extract of *G. hispida* (14.5 %) respectively. However, all other extracts showed moderate alpha-amylase inhibitory potential (Fig. 7).

## 4. Discussion

Plant natural products are gaining interest now-a-days for their biological activities due to the presence of phenolics, alkaloids and other compounds (Iqbal et al., 2018c, Abbasi et al., 2019, Abbasi et al., 2020). Ethno-medicinal and biological application of different species belonging to genus *Heliotropium* and *Gastrocotyle* (family Boraginaceae) have been reported extensively (Aïssaoui et al., 2019, Aslam et al., 2017, Bazzaz and Haririzadeh, 2003) but the two species namely *H. crispum* and *G. hispida* have not been

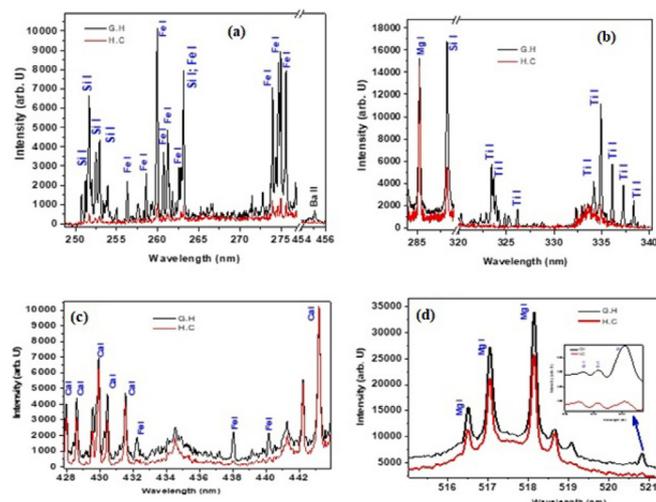


Fig. 4. The emission spectrum of *H. crispum* and *G. hispida* covering the wavelength region from (a) 250–280 nm (b) 285–340 nm (c) 428–442 nm (d) 516–521 nm.

Table 3

IC<sub>50</sub> values of radical scavenging activities observed in the methanolic and chloroform extracts of *G. hispida* and *H. crispum* in comparison with standard.

Plant Extracts	DPPH scavenging activity	NBT scavenging activity	ABTS scavenging activity
HCM	20.256 ± 0.35 <sup>bc</sup>	38.556 ± 5.34 <sup>bc</sup>	7.980 ± 2.94 <sup>c</sup>
HCCHL	26.720 ± 3.07 <sup>b</sup>	99.323 ± 9.33 <sup>a</sup>	12.520 ± 0.18 <sup>b</sup>
GHM	12.636 ± 2.07 <sup>d</sup>	49.610 ± 2.15 <sup>b</sup>	29.083 ± 0.37 <sup>a</sup>
GHCCL	34.480 ± 3.62 <sup>a</sup>	119.23 ± 5.80 <sup>a</sup>	13.443 ± 0.11 <sup>b</sup>
Ascorbic acid	16.913 ± 2.57 <sup>cd</sup>	32.250 ± 4.67 <sup>c</sup>	2.804 ± 0.29 <sup>d</sup>

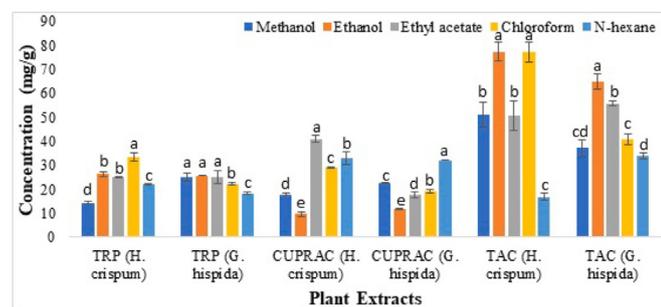


Fig. 5. Total reducing power (TRP), cupric ions reducing antioxidant capacity (CUPRAC) and total antioxidant capacity (TAC) of the selected plant extracts. The data is the mean of three replicates (SD ± 3) and each letter (a-e) indicates significance at  $P < 0.05$  [Total reducing power and total antioxidant capacity expressed as ascorbic acid equivalent (mg AE/g extract); Cupric ions reducing assay expressed as gallic acid equivalent (mg GA/g extract)].

explored so far. As a result, recent experiments have been planned to investigate the biological ability of the two species.

Various compounds occurring naturally in the plant kingdom are part of our daily diet and are used in pharmaceutical and cosmetic industries as well (Iqbal et al., 2017, Batool et al., 2019, Batool et al., 2020). Among these, flavonoids and polyphenols exhibit significant values and are detected in Boraginaceae family (Iqbal et al., 2005). Secondary metabolites derived from plants are also involved in regulating the mechanism of enzyme action for detoxification (Baidez et al., 2007, Abbasi et al., 2021). Flavonoids provides defense to the living systems by stabilizing the free radicals and act as anti-inflammatory agents (Iqbal et al., 2019, Karmakar and Halder, 2019, Iqbal et al., 2020). Results revealed

that both species showed the presence of significant phenolic contents (greater than 50 mg GAE/g) in all extracts except n-Hexane extracts which exhibited < 50 mg GAE/g phenolic contents. However, flavonoid contents were detected in the range of  $25.338 \pm 1.44$  mg QE/g (*G. hispida* ethanolic extract) to  $14.183 \pm 1.04$  mg QE/g (*G. hispida* methanolic extract) respectively (Table 1).

Different solvents are used to separate compounds and their extraction efficiency is determined by the solvent type and the extraction process (Goli et al., 2005). In present study, HPLC analysis revealed the presence of highest number of compounds in the *G. hispida* methanolic extract (eleven compounds) and *H. crispum* ethanolic extract (eight compounds) as compared to the other extracts (Table 2). It can be concluded that increase in the number of compounds depends on polarity of extraction solvents. These phenolic compounds detected in selected extracts have various pharmacological uses. The medicinal value of selected plant species is further potentiated by the identification of all these polyphenols. Quercetin and coumaric acid were found to be dose-dependent inhibitors of multiple cancer cell lines (Zhang et al., 2008). In particular, Gallic acid has various therapeutic uses as an antiangiogenic, anticancer, anti-inflammatory and antimicrobial agent (Choubey et al., 2015). Antioxidant properties of caffeic acid are extensively tested, exceeding numerous other antioxidants in curbing the production of aflatoxin by more than 95 percent in one such research (Dai and Mumper, 2010). These polyphenols have ability to hinder the proliferation of cancer cells, activate pro-carcinogens and suppress growth signaling pathways (Hussain et al., 2016, Amin et al., 2015). Hence, this study confirmed the potential involvement of polyphenols in *G. hispida* and *H. crispum* for pharmacological operation.

In proposed study, the plant extracts were passed through the FTIR and functional groups of the components get separated depending on peaks ratio. The obtained spectra confirmed the presence of alkanes, carboxylic acids, aldehydes, alcohols, ketones and amides in selected species. As both species belong to the same family, so they displayed the presence of almost similar kind of functional groups. Our study correlate with the earlier findings of Ahmad et al. (2016) who reported the presence of similar functional groups in the relative member of genus *Heliotropium* i.e. *H. bacciferum*. This is the first report on the FTIR analysis of selected species and is initial step towards biological research to explore *H. crispum* and *G. hispida*. Moreover, LIBS technique was used to determine the elements existing in any material including plants. Previously, many studies have been conducted on different medicinal plants reporting the presence of elements using LIBS (Zafar et al., 2019, Rai et al., 2013). Our studies confirmed the presence of eight elements (Si, Fe, Ba, Mg, Ti, Ca, Mg and Cr) indicating that the selected species can be effectively used in industries as a major source of elements.

As a rich source of antioxidants, medicinal plants can reduce oxidative stress, thereby helping to cure various diseases (Ulewicz-Magulska and Wesolowski, 2019, Thatoi et al., 2014). The medicinal values of plants were assessed by its antioxidant potentials using different antioxidant assays as these are widely used assays owing to the benefits such as ease of use, inexpensive reagents used in these experiments, non-laboratory, and the ability to easily evaluate the antioxidant properties of large numbers of samples at a time (Gülçin et al., 2005). Our results revealed that the *H. crispum* and *G. hispida* extracts made in polar solvents showed more antioxidant potential than in non-polar extracts. Previously, Shahat et al. (2019) revealed that bioactive compounds (Rosmarinic acid and  $\beta$ -sitosterol) are present in *G. hispida* that are responsible for its antioxidant activity. However, antioxidant activity of *H. crispum* have been evaluated for the first time. It can be inferred that the phytochemicals such as phenolics, flavonoids, anthocyanin and coumarin present in these species are pre-

**Table 4**  
Antibacterial and antifungal activity of five different extracts of *G. hispida* and *H. crispum*.

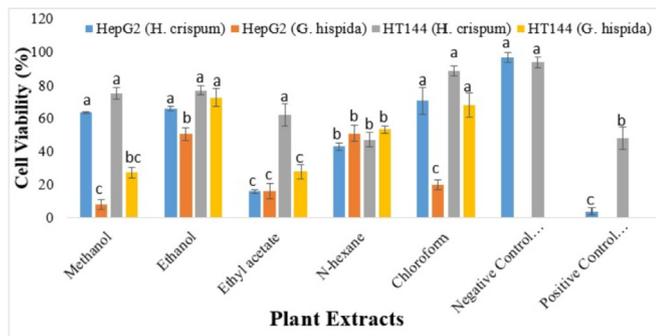
Microbial strains	<i>G. hispida</i>					<i>H. crispum</i>					Control	
	Methanol	Ethanol	Ethyl acetate	Chloroform	n-Hexane	Methanol	Ethanol	Ethyl acetate	Chloroform	n-Hexane		
<b>Bacterial strains</b>												
<i>S. aureus</i>	ZOI	7.6 ± 1.5	7.6 ± 2.0	8.0 ± 1.0	8.0 ± 1.0	NI	10.6 ± 0.1	9.1 ± 0.4	NI	8.6 ± 0.2	NI	32.0 ± 2.8
	MIC	625	625	625	312.5	–	150	312.5	–	312.5	–	–
<i>P. aeruginosa</i>	ZOI	8.6 ± 1.1	8.6 ± 0.5	NI	7.6 ± 0.5	NI	NI	7.0 ± 1.0	7.3 ± 1.1	9.3 ± 0.5	NI	30.0 ± 1.5
	MIC	312.5	312.5	–	625	–	–	312	625	150	–	–
<i>B. subtilis</i>	ZOI	11.3 ± 0.5	NI	NI	19.0 ± 1.0	NI	8.3 ± 0.5	NI	NI	NI	NI	31.0 ± 2.0
	MIC	150	–	–	75	–	312.5	–	–	–	–	–
<i>E. coli</i>	ZOI	8.1 ± 0.5	NI	7.1 ± 1.1	NI	NI	9.3 ± 0.5	NI	10.6 ± 0.5	10.3 ± 0.7	15.33 ± 0.57	29.0 ± 1.5
	MIC	312.5	–	625	–	–	312.5	–	150	150	75	–
<i>K. pneumonia</i>	ZOI	NI	7.4 ± 1.0	NI	NI	7.0 ± 1.1	NI	NI	NI	7.3 ± 0.5	NI	28.0 ± 1.8
	MIC	–	625	–	–	625	–	–	–	625	–	–
<b>Fungal strain</b>												
<i>F. solani</i>	ZOI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	32.0 ± 1.8
	MIC	–	–	–	–	–	–	–	–	–	–	–
<i>A. niger</i>	ZOI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	33.0 ± 1.8
	MIC	–	–	–	–	–	–	–	–	–	–	–
<i>A. fumigatus</i>	ZOI	NI	NI	NI	NI	NI	NI	NI	NI	8.0 ± 1.2	NI	30.0 ± 2.6
	MIC	–	–	–	–	–	–	–	–	312.5	–	–
<i>Mucor racemosus</i>	ZOI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	28.0 ± 2.0
	MIC	–	–	–	–	–	–	–	–	–	–	–

ZOI: Zone of inhibition (mm ± SD); MIC: Minimum inhibitory concentration (µg/mL); NI: No inhibition; Positive control: Oxytetracycline (Antibacterial standard) and Chloramphenicol (Antifungal standard).

**Table 5**  
Cytotoxicity of brine shrimps observed at different concentrations of selected plant extracts and their LC<sub>50</sub> values.

Plants	Solvents used for extracts	Percentage mortality at different doses					LC <sub>50</sub> (ppm)	95 % Confidence Interval
		62	125	250	500	1000		
<i>H. crispum</i>	Methanol	86.66	93.33	100.0	100.0	100.0	8.4980	2.7070 – 26.683
	Ethanol	73.33	86.66	86.66	100.0	100.0	8.3640	2.2820 – 30.654
	Ethyl acetate	43.33	50.00	80.00	96.66	100.0	93.162	59.329 – 146.28
	Chloroform	46.66	63.33	80.00	96.66	100.0	78.107	47.551 – 128.29
	N. hexane	80.00	86.66	90.00	96.66	100.0	10.817	3.5780 – 32.703
<i>G. hispida</i>	Methanol	40.00	70.00	80.00	90.00	100.0	75.883	42.196 – 136.46
	Ethanol	23.33	50.00	76.66	86.66	100.0	129.86	82.146 – 205.30
	Ethyl acetate	23.33	33.33	63.33	70.00	96.66	178.16	112.97 – 280.97
	Chloroform	30.00	50.00	76.66	83.33	100.0	119.71	70.435 – 203.47
	N. hexane	63.33	83.33	100.0	100.0	100.0	42.380	23.657 – 75.923

LC<sub>50</sub>: Lethal concentratin which causes death of fifty percent (one half) of group of tested animals.



**Fig. 6.** Percentage viabilities of HepG2 and HT144 cells in five different extracts of *H. crispum* and *G. hispida* relative to untreated control (mean ± SD). Each letter (a-c) indicates significance at *P* < 0.05 using ANOVA.

dominately responsible for radical scavenging potential (Aqil et al., 2006).

The continuous increase in resistance of microbial agents resulted in mortality worldwide. Bacteria have genetic capability to gain resistance against therapeutically active drugs and thus natural products with less toxicity and more antibacterial activity

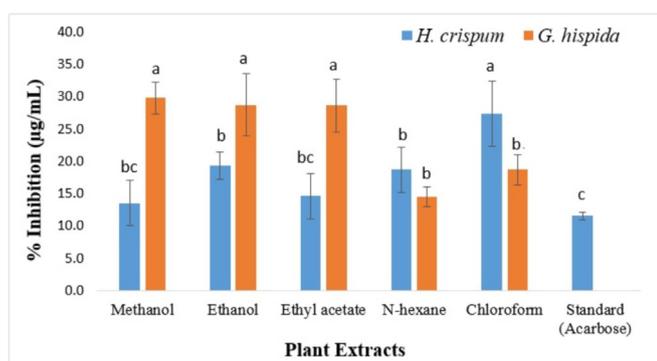
are usually explored (Maleki et al., 2008). Results revealed that selected species exhibit significant antibacterial potential, however, these extracts were found to be ineffective against the selected fungal strains. In case of antibacterial activity, *K. pneumonia* and *B. subtilis* were found to be highly resistant as most of the extracts formed no zones against them. Conversely, *S. aureus*, *P. aeruginosa* and *E. coli* were considered as the most sensitive bacterial strains. Present data also correlate with the earlier findings of Ihtesham et al. (2019) who reported that *H. crispum* showed highest activity against *S. aureus* (i.e. 30 ± 0.09 mm). Previously, Khan et al. (2016) also reported strong antibacterial activity of synthesized silver nanoparticles using *H. crispum*. Moreover, present study revealed that the extracts prepared in non-polar solvent (n-Hexane) exhibited lowest antibacterial potential. It can be suggested that the crude extracts of *H. crispum* and *G. hispida* prepared in polar solvents can be effectively utilized to treat different diseases caused by resistant microbes.

Selected plant extracts showed significant toxicity potential against shrimp's. LC<sub>50</sub> in *H. crispum* extracts ranged from 8.3640 ppm to 93.162 ppm while among *G. hispida* extracts ranged from 42.380 ppm to 178.16 ppm respectively (Table 5). Additionally, selected plant extracts were screened using the MTT reduction assay for anti-proliferative activity against HepG2 (liver cancer) and HT144 (skin cancer) cells lines. Our findings showed that all

**Table 6**  
Antihemolytic activity of different extracts of *H. crispum* and *G. hispida* observed against H<sub>2</sub>O<sub>2</sub> induced hemolysis.

Plants	Solvents used to prepare extracts	Absorbance at 560 nm concentration (µg/mL)					% Inhibition of hemolysis at 1000 (µg/mL)
		62.5	125	250	500	1000	
<i>H. crispum</i>	Methanol	0.049 ± 0.004	0.052 ± 0.001	0.076 ± 0.015	0.099 ± 0.019	0.113 ± 0.024	11.056 <sup>def</sup>
	Ethanol	0.051 ± 0.007	0.053 ± 0.004	0.063 ± 0.004	0.117 ± 0.011	0.191 ± 0.004	18.634 <sup>c</sup>
	Chloroform	0.050 ± 0.005	0.083 ± 0.019	0.150 ± 0.063	0.159 ± 0.014	0.326 ± 0.072	31.869 <sup>b</sup>
	Ethyl acetate	0.040 ± 0.037	0.049 ± 0.001	0.050 ± 0.001	0.068 ± 0.017	0.084 ± 0.026	8.227 <sup>f</sup>
	n-Hexane	0.064 ± 0.015	0.068 ± 0.020	0.071 ± 0.002	0.081 ± 0.026	0.107 ± 0.010	10.439 <sup>ef</sup>
<i>G. hispida</i>	Methanol	0.047 ± 0.001	0.059 ± 0.001	0.077 ± 0.001	0.122 ± 0.010	0.153 ± 0.018	14.991 <sup>cd</sup>
	Ethanol	0.047 ± 0.005	0.050 ± 0.005	0.068 ± 0.005	0.080 ± 0.004	0.126 ± 0.003	12.292 <sup>def</sup>
	Chloroform	0.051 ± 0.003	0.056 ± 0.001	0.069 ± 0.009	0.077 ± 0.002	0.108 ± 0.004	10.601 <sup>def</sup>
	Ethyl acetate	0.049 ± 0.003	0.049 ± 0.001	0.060 ± 0.007	0.080 ± 0.021	0.098 ± 0.018	9.560 <sup>ef</sup>
	n-Hexane	0.059 ± 0.015	0.060 ± 0.007	0.085 ± 0.003	0.123 ± 0.003	0.134 ± 0.004	13.138 <sup>de</sup>
Quercetin		0.570 ± 0.020	0.620 ± 0.040	0.790 ± 0.090	0.891 ± 0.010	1.012 ± 0.025	98.700 <sup>a</sup>

Values in same column followed by different letter (a–f) are significantly different ( $P < 0.05$ ) as detected by LSD - all pair-wise comparison test.



**Fig. 7.** Alpha-amylase inhibitory potential of *H. crispum* and *G. hispida* extracts prepared in five different solvents. Data is expressed as mean of three replicates, error bars indicates standard deviation and each letter (a–c) indicates significance at  $P < 0.05$  by using ANOVA.

extracts suppressed HepG2 (liver cancer) and HT144 (skin cancer) cancer cell line proliferation, and the relative viability of HepG2 (liver cancer) and HT144 (skin cancer) cells in various solvents increased significantly. All-inclusive, *G. hispida* revealed lowest viability percentage in methanolic extract (8.08 % and 27.43 %) against HepG2 and HT144 cancer cell lines (Fig. 6). It is a known fact that plants contain a wide variety of bioactive compounds that are reported helpful in the treatment of cancer (Saddiqi et al., 2017). Hence, it can be suggested that the phenolic and flavonoid compounds present in these extracts might be responsible for the biological activities of selected plants.

Hydrogen peroxide and many other free radicals also damage the erythrocyte membrane causing hemolysis. Thus, it is important to eliminate H<sub>2</sub>O<sub>2</sub> from our food systems. In plant extracts, organic compounds are present which scavenge H<sub>2</sub>O<sub>2</sub> by neutralizing H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O (Escher et al., 2018, Ebrahimzadeh et al., 2010). Similarly, the cornerstone of diabetes regulation is blood glucose level management, which can be accomplished by the use of oral hypoglycaemic agents and inhibitors of carbohydrate hydrolysis enzymes (Patel et al., 2012). The tested extracts had lower mechanical hemolysis when compared with the control, indicating that these extracts exhibit minimum erythrocyte toxicity. The increase in antihemolytic activity was proportional to the extracts concentrations. Present results of alpha-amylase assay indicated highest activity in the *H. crispum* methanolic extracts (13.5 %). Present study is coherent with the earlier findings of Di Sotto et al. (2019) and Ahmed et al. (2017) who revealed alpha-amylase inhibitory potential in medicinal plants such as *Punica granatum* and *Quercus dilatata*. It can be suggested that the polyphenols present

in plant extracts provide protection to the erythrocytes from hemolysis and can also be used as antidiabetic agent.

## 5. Conclusion and future prospective

The goal of the present study was to investigate the phytochemicals, elements and the biological profiling of *G. hispida* and *H. crispum* extracts. For this purpose, the use of a wide-ranging polarity of the solvent system is proved as an effective and reliable source. Identifying essential polyphenols and evaluating the antimicrobial activity of selected plants in different crude extracts helps to clarify some of their common uses. The plant extracts also displayed strong anticancer activity against HepG2 and HT144 cancer cell line. Biochemical profiling and elemental analysis revealed that *H. crispum* and *G. hispida* might be a potential source of bioactive compounds and essential elements which needs to be isolated and characterized so that they could be further exploited in drug development. From the present study, it can be recommended to conduct a systematic *in vivo* study targeting the anti-cancer and anti-diabetic aspects of the selected extracts. The existence of significant secondary metabolites including phenolics and flavonoids, which causes antioxidant and antimicrobial properties in plants, further encourages to undertake research in detail.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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