doi.org/10.36721/PJPS.2026.39.8.211.1

Chemical composition, antioxidant, antifungal, antibacterial and anticancer activities of essential oils of *Berberis balochistanica* Ahrendt

Rukhsana Taj[1]*, Samar Ali[1], Shafiullah Khan[2] and Naila Taj[1]
[1]Department of Chemistry, University of Balochistan, Quetta, Pakistan
[2]Institute of Chemical Sciences, Gomal University, Dera Ismail Khan, Pakistan

Abstract: **Background**: In Balochistan, the biotechnological potential of medicinal plants has not been widely investigated. Thus, searching medicinal plants for novel compounds is vital source for developing emerging and new medications. **Objectives**: The present study aims to describe the chemical profile and evaluate the antimicrobial, antioxidant and anticancer effects of the essential oil (EO) of *Berberis balochistanica*. Gas Chhromatography-Mass Spectrometry (GC-MS) analyzed the chemical constituents. **Methods**: Antioxidant potential was evaluated by 2-2-diphenyl-1-picylhydrazyl (DPPH) assay. Antifungal action was evaluated using agar well and disc diffusion methods. The antibacterial screening was evaluated against Gram-positive and Gram-negative bacterial strains through the disc diffusion technique and by determining minimal inhibitory concentration (MIC). To investigate anticancer activity, an 3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay was used. **Results**: GC-MS analysis revealed 19 components accounting for 99.9% of the total EO. The EO exhibited a substantial effect against the fungal strain *Alternaria alternate* and remarkable antibacterial activity against *Staphylococcus aureus,* while showing no cytotoxic activity. **Conclusion**: These results show that *B. balochastanica* based EO has the potential to be evaluated in clinical applications and pharmaceutical industries as an antimicrobial agent for the treatment of various ailments. It may serve as a preservative in food stuffs, serving as a natural replacement for harmful synthetic antioxidants.

Keywords: Antimicrobial activity; Antioxidant activity; *Berberis balochistanica*; Essential oil composition; GC-MS; MTT

Submitted on 18-12-2024 – Revised on 14-02-2025 – Accepted on 23-07-2025

INTRODUCTION

Medicinal, aromatic plants have been consumed by civilizations in Asia and Africa in traditional prescriptions for thousands of years. During the last few eras in both emergent and urbanized countries, interest and acceptance of natural remedies have increased. Up to 85 percent of the world's inhabitants use herbal medical items as a substitute for obsolete medications. Furthermore, medicinal plants, directly or indirectly, are found in about 25% of all modern medications (WHO, 2019).

High concentrations of EOs derived from aromatic flora can be used as healing alternatives to treat different illnesses. EOs are chemical mixtures with a distinctive aroma composed of aromatic components separate from aromatic plants (El-Said *et al*., 2021). EOs are complex, volatile compounds that are naturally found in plants as secondary metabolites. They are important components of defense system, protecting plants from worms, micobes, mildew and vermin (Mohamed and Alotaibi, 2023). (Mohamed and Alotaibi, 2023). EO has a distinctive odor, possesses high optical activity and refractive index, is usually colorless and exists in the molten state at room temperature. They are widely used in clinical microbiology, food preservation, scent, the cosmetics industry, pharmacology and aromatherapy (Jugreet *et al.,* 2020).

The role of EO in aromatherapy is envisioned as a soothing technique comprising baths, kneading or gasps. EOs in research areas and industry has become an attention-grabbing topic due to its wide variety of solicitations. GC–MS is the most widely used chromatographic system to separate and identify the different ingredients of EOs, (Mohamed and Alotaibi, 2023). Due to the volatile nature of EOs the Clevenger method is the most commonly used technique in the laboratory scale, to refine EOs, hydrodistillation (Sadeh *et al*., 2019). EOs are blends of organic composites of diverse chemical classes comprising 20–100 diverse volatile composites; merely 2-3 molecules initiated with 20–70% higher concentration are accountable for distinguishing descriptions of EOs (Chouhan *et al*., 2017). These compounds include mostly terpenes in addition to amines, phenylpropanoid components, esters, phenols, alcohols, ethers, heterocycles, sulfur-containing ingredients, oxides, ketones, amides and aldehydes (El-Said *et al*., 2021).

Various factors such as plant maturity, harvest period, seasonal deviations, topographical origin, isolation method, plant organ and heredities affect the composition and % yield of EOs (Arumugam *et al*., 2016; Megawati *et al*., 2019). The enormous range of bioactive components in

**Corresponding author:* e-mail: rukhsana.taj88@yahoo.com

plant-extracted EOs has piqued interest of researchers worldwide over the past few decades. Additionaliy, EO has a variety of biological activities, such as antifungal, anti-inflammatory, antiprotozoal, anticancer, antiseptic, antioxidant and antimutagenic effects (Mohamed and Alotaibi, 2023).

EOs in different foodstuffs are used as natural antioxidants and beverage products (Hellali *et al*., 2016) as they improve the lifespan life of food. Antioxidant activity is due to the existence of phenolic constituents of EOs. The DPPH assay and other recognized methods are used to measure antioxidant activity (Aebisher *et al*., 2021). The aniceptic qualities against oral pathogens have been documented several researchers in recent years (Sousa *et al*., 2015). Sesquiterpenes, monoterpenes and phenylpropanoids are EOs' sources of antibacterial activity (Raut and Karuppayil, 2014). Several useful compounds isolated from plant sources, i.e., demethylpodophylotoxin, taxol, paclitaxel, vinoselbine, deacetylbaccatin III, podophyllotoxin, vinblastine, α-peltatin, β-peltatin, vincristine, topotecan, vindesine and docetaxel, have played a vital role in anticancer therapy (Pervez *et al*., 2019).

The genus Berberis belongs to the family Berberidaceae, which has about 15 genera and 650 species distributed around the globe (Behrad *et al*., 2023). *B. balochistanica* is a Shrub about 1-3m tall; the foliage is copious and stiff and the long yellow flower blossoms from March to May (Muddassir *et al*., 2022). It exists in Ziarat, Harboi, Hanna Urak and Kalat (Uddin *et al*., 2021). The Pashto local name in Pakistan for this plant is Zralga and Zarch in Brahivi (Gul *et al*., 2022). It is used in traditional remedies to heal the discomfort in the body. Root powder and latex are mixed with milk to make molasses, then used as a tonic for internal injuries, such as rheumatism, torso infections, wound pain, coughing, joint aching, jaundice and ophthalmic issues Berberine, which is known to be beneficial in the treatment of numerous ailments, may be responsible for its ability kidney gravel (Baloch *et al*., 2013; Pervez *et al*., 2019).

Previous studies on the phytochemical exploration of different species of *B. balochistanica* exposed the occurrence of flavonoids, carotenoids, terpenoids, lignans, proteins, steroids, vitamins, lipids and alkaloids (Jahan *et al*., 2022).

The phytochemical composition of *B.balochistanica* has been reported by (Gul *et al*., 2023), showing the existence of phenols, cardiac glycosides, saponins, flavonoids, alkaloids, sugar, diterpenes, steroids and tannins The chemical analysis of some Berberis species of this shrub EO received little attention from earlies researchers (Hosseinihashemi *et al*., 2015; Tavakoli *et al*., 2017). The previous experimental studies supported the bioactive potential of the plant, which includes antioxidant (Abbasi *et al*., 2013), anti-diabetic, antifungal, cytotoxic, antileishmanial, antibacterial, insecticidal (Pervez *et al*., 2019; Baloch *et al*., 2013), antiseptic (Kakar *et al*., 2012), anti-inflammatory (Xu *et al*., 2021) and antimicrobial (Rehman *et al*., 2017) activity.

According to the investigation of accessible literature, to our knowledge, there is still no report on the volatile constituents of *B. balochistanica* Ahrendt. This study assessed the chemical composition, antioxidant, antimicrobial and anticancer activities of the EO extracted from *B. balochistanica* Ahrendt (Berberidaceae) as a new potential source of natural antibiotics and antioxidant components.

MATERIALS AND METHODS

Chemicals

Butylated hydroxytoluene (BHT), Dimethylsulfoxide (DMSO), 2, 2′- diphenyl-1-picrylhydrazyl (DPPH), n-hexane, (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) (MTT), Distilled water, anhydrous sodium sulphate, chloroform, Methanol and acetone were procured from Merck KGaA (Darmstadt, Germany). All culture media, Sabarose Dextrose Broth, Nutrient agar (NA), Potato dextrose agar (PDA), Nutrient Broth, Mueller Hinton agar (MHA) and standard antibiotic discs were purchased from Oxoid Ltd. (Hampshire, UK). All chemicals used in the experiments were analytical grade and the water was ultra-pure.

Plant materials

Prof. Dr. Rasool Bakhsh Tareen of the University of Balochistan identified the planned sample of *B. balochistanica* Ahrendt and collected the sample from the Ziarat District of Balochistan, Pakistan, during March to May.

Drying process of the plant material

The influence of the desiccating process on the composition and content of EO from *B. balochistanica* was evaluated before the extraction of EO; it was submitted to a natural drying process at room temperature. In this process, Fresh plant samples were first washed through distilled water to remove dirt and soil. Afterward, it was spread on paper sheets in a well-ventilated room and air-dried at room temperature for 7 days, sheltered from solar radiation. Dried plant materials were cut into small parts and stored in firmly packed glass jars.

Extraction of EO

EO extracted from air-dried parts of *B.balochistanica* using a Clevenger-type apparatus for 3-hour hydrodistillation. The process was repeated several times to get sufficient oil. EO thus isolated and dehydrated over anhydrous sodium sulfate, after filtration, stored in hermetically sealed glass vessels in the refrigerator at 4 °C

until analyzed (Chand *et al*., 2017). Based on the weight of the fresh plant material (*v/w*), the % yield of the obtained EO was calculated.

GC-MS analysis of EO

GC-MS scrutiny of EO was done on Agilent Technologies 7890A GC and MS 7000 coupled with split-splitless injector using OPTIMA-5MS (5 % diphenyl – 95 % dimethylpolysiloxane) column (30 m x 250µm x 0.25 µm in thickness). Carrier gas (Helium) with a pressure of 9.66 Psi and flow rate of 3 mL/min. The split ratio 1:30 was applied, 260 °C injector temperature and injected volume was 1000 µL. At the same time, the oven temperature was initially set at 60 °C for 5 min and linearly programmed from 180 (Hold time: 10 min) to 310 °C (Hold time: 25 min) at an 8°C/min rate. At 260°C, the transfer line was heated. In EI mode, the GC-MS system operated at 70 eV with a scan time of 3.50 scans/min in an *m/z* range of 40–750amu. Identification of individual constituents was made by comparison of their retention times with computer databank in the Replib and Mainlib libraries of mass spectra and with published data (Admas, 2007).

Free radical (DPPH) scavenging assay

Free radical scavenging action of isolated EO from the plant was estimated by the DPPH technique (Bouyahya *et al*., 2016) with slight alteration. After adding samples of methanolic EO solution (10µl / 2ml mL) at various concentrations (15, 30, 60, 125 and 250 µg/ml) to 0.002g / 50ml of DPPH methanol solution and left mixture for 30 minute in dark. The mixture's absorbance was observed at 517 nm wavelength via a UV-Vis digital spectrophotometer (Shimadzu Spectrophotometer model UV-1601). Different dilutions of BHT (15, 30, 60, 125, 250 µg/ml) were standard. DPPH/MeOH mixture was used as a blank. The % of DPPH scavenging radical potential of the tested samples was measured by using the formula:

$$\text{DPPH scavenging activity (\%)} = \frac{Ac - At}{Ac} \times 100 \quad (1)$$

Where *Ac* is the absorbance of control (without EOs) and *At* is the absorbance of the test sample (with EOs).

IC_{50} (50% inhibition) values were calculated by plotting the graph of inhibition percentage of DPPH radicals against EOs concentration (µg/ml). The assay was performed in triplet while results were described in means ± SEM.

Antimicrobial screening

Fungal and bacterial strains were assimilated from the culture collection of the Applied Biotechnology Laboratory, BUITEMS, Quetta. *Fusarium oxysporum* (NRRL13305) and *Alternaria alternate* (NRRL54028) were used to test antifungal bioactivity via agar well diffusion and disc diffusion methods. After preliminary screening, Potato Dextrose Agar (PDA) was used as a culture medium for fungal strains, followed by the incubation of samples at 24-28ºC for 24 hours. Standard microbial strains comprising Gram +ve *(Bacillus subtilis)* and Gram -ve *(Escherichia coli)* were used to examine the antibacterial potential of EO of *B. balochistanica* by disc diffusion assay and determining the MIC. Macchonkey agar (MA), Eosin methylene blue (EMB) (for Gram -ve bacteria) and Mannitol Salt Agar (MSA) (for Gram +ve bacteria) were used as selective media to ratify the presence of bacteria. All bacterial species were cultured on Mueller-Hinton Agar (MHA), used as nutrient agar to sustain all the tested bacterial strains after preliminary screening and samples were incubated for 24 hours at 24-28ºC.

Agar well diffusion method

The agar well diffusion method was applied for a quantitative study of EO against fungal strains to assess antifungal action. In sterilized petri plates, potato dextrose agar was poured and kept at room temperature to solidify it and it was incubated for about 24 hours at 24 °C. 10µl of fungal inoculum was spread on petri plates containing Potato Dextrose agar. The well depth was 8mm in diameter and cut from agar with a sterilized cork borer. In DMSO, EO was dissolved and prepared in different concentrations ranging from 20-100µL. It was followed by pouring each concentration into the well and keeping it upturned for 3-4 hours to dry. Now incubated at 37°C for 72 hours and observed for growth of inhibition zones.

The inhibition zone was determined via the following formula after incubation;

$$\text{\%age mycelial Inhibition} = \frac{dc - dt}{dc} \times 100 \quad (2)$$

Where

dc = Diameter of fungal growth in control plates

dt = Diameter of fungal growth in the tested sample plates.

The samples were studied in triplicate in order to attain reproducible results.

Disc diffusion method

The disc diffusion method was used to determine the qualitative investigation of the antifungal potential of EO. Nutrient agar (PDA media) was poured into the sterilized Petri plates and left for solidification at room temperature. Sterile~6 mm diameter filter paper discs were immersed in EO (10µl) and spread on the nutrient agar surface. The sample and tested fungal strain were cited at the edge and midpoint of the petri plate. The petri dishes were incubated at 37°C in the dark for 24 hours. After retro incubation, the inhibition zone diameter around each disc was measured in millimeters (mm) with Vernier caliper (Rajendran *et al*., 2014). All measurements were done in triplicate.

Agar disc diffusion method

The agar disc diffusion method was used to evaluate zone inhibition diameters of EO against tested bacterial strains.

MHA was prepared as an NA for the growth of bacterial strains. Bacterial strains were streaked on the NA plate through cotton swabs to get fresh colonies and incubated for about 36 hours. Takings a fresh colony of tested bacterial strain was taken from NA plate, suspended in a saline solution and the bacterial inoculum was adjusted to 0.05 McFarland standard. Bacterial saline suspension was spread through cotton swabs on petri plates containing MHA medium. Under aseptic conditions, Whatman sterile 4 mm filter paper discs impregnated with 10 µL DMSO solution of the tested EO were placed on the inoculated petri plates agar surface using purified forceps. The plates have been refrigerated for 2-3 hours at 4 °C to allow EOs diffusion and incubated at 37°C for 24 hours to attain maximum microbial growth. DMSO and Doxycyclin D-30 were used as -ve and +ve controls, respectively. Antibacterial activity was estimated by measuring the inhibition zone diameter (mm) around each disc after the incubation period to check the effectiveness of the samples and compared it with the inhibitory zone of the antibiotic and MIC was measured. All measurements were done in triplicates (Abdelli *et al*., 2017).

Determination of minimum inhibitory concentration (MIC)

With minor modifications, the reported method was used to determine MIC of EO (Sadiq *et al*., 2017). Nutrient broth (NB) and petri plates were prepared under aseptic conditions. A fresh colony of the tested bacterial strain was dissolved in saline water and the bacterial suspension was adjusted to a 0.05 McFarland standard. A series of various dilutions (0.1, 0.12, 0.14, 0.16 and 0.18 g/ µL) of EO were prepared in DMSO, used as the positive control. Poured 100 µL of the tested oil sample in the first row of 96-well microplates and 50 µL of growth control (nutrient broth + DMSO) was added in the second wells, leaving it to cool till it jellified. In each test sample and growth control well, 10 µL of bacterial inoculum dispensed. Negative control (DMSO) was run in parallel to confirm the viability of the fungal strains. Microplates were left for incubation at 30 ºC for 24 hours. The minimum oil concentration that endorsed inhibition of bacterial growth was taken as the MIC value. All tests were performed in triplicate.

Anticancer activity

The cytotoxic potential of *B. Balochistanica* EO was assessed in bottomed flat 96-well microplates using the standard MTT colorimetric method according to (Mosmann 1983). For this purpose, penicillin (100 IU/ml), streptomycin (100 µg/ml) and 10% of FBS (Fetal Bovine Serum), appended with Least Essential Medium Eagle in 75 cm^2 flasks, used for culturing Prostate Cancer (PC_3) cells and incubated at 37ºC in 5% CO_2. The precise medium was used to dilute rapidly growing harvested cells, which were counted with a haemocytometer. Cell culture was prepared in the $7x10^4$ cells/ml concentration and transferred to 96-well plates (100 µL/well). After removing the medium, different concentrations (1-30µM) of EO were added with fresh medium (200 µL) after overnight incubation. After 48 hours, in each well, 200 µL MTT (0.5 mg/ml) was incubated at 37 ◦C for 4 hours. Consequently, DMSO (100µL) was added to every well. The magnitude of MTT decreased within cells to purple colored formazan, which was calculated through a microplate reader (Spectra Max plus, Molecular Devices, CA, USA) and at 570 nm absorbance was measured. A standard drug, doxorubicin, was used in this assay. The cytotoxicity was reported as IC_{50} (50% inhibition growth), which causes a concentration of PC_3.

The inhibition percent (%) was calculated through the subsequent formula:

$$\% \text{ inhibition } = \frac{100-|(\text{mean of O.D of tested EOs} - \text{mean of O.D of negative control})}{(\textit{mean of O.D of positive control} - \textit{mean of O.D of negative control})} \times 100 \quad (3)$$

Soft- Max Pro software (Molecular Device, USA) was used to evaluate % inhibition results.

Statistical analysis

Experiments were performed in trios for every assay and the final results were displayed in the arithmetic mean ± SEM. At the same time, the standard curve, magnitude of the means and SD were evaluated using MS Excel software. IC_{50} values were calculated via the linear regression technique. A substantial statistical difference amongst means of $p<0.05$ was employed using Turkey's SPSS software.

RESULTS

Chemical composition of EO by GC-MS

The dried whole plant of B. balochistanica yielded 10 ml of EO after hydrodistillation, characterized by a colorless and pungent odor. GC/MS analysis resulted in the characterization of 19 components, which constituted 99.98% of the entire oil. EO of *B. balochistanica* was characterized with 2-Pantanone, 4-hydroxy-4-methyl-, Cyclohexanone, Dodecane, Octane, 3,5-dimethyl-, 1-Dodecanol, 3,7,11-trimethyl-, Undecane, Dodecane, 2,6,11-trimethyl, 2-Isopropyl-5-methyl-1-heptanol, 1-Octanol, 2-butyl-, Heptadecane, 2,6,10,15-tetramethyl-, 1-Iodo-2-methylundecane, Dodecane, 2,6,10-trimethyl, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Diisooctyl phthalate, Octadecenoic acid,2- hydroxy-1- (hydroxymethyl) ethyl ester, 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol, Dimethyl[bis(tridecyloxy)]silane, Eicosanoic acid, octadecyl ester, 9- Octadecenoic acid (Z)-, hexyl ester. The chemical composition of EO of *B. balochistanica,* i.e., molecular formula, molecular mass, structure and R.T of *B. balochistanica* EO, is listed in table 1. The chromatograms of *B. balochistanica* EO are shown in Fig. 1.

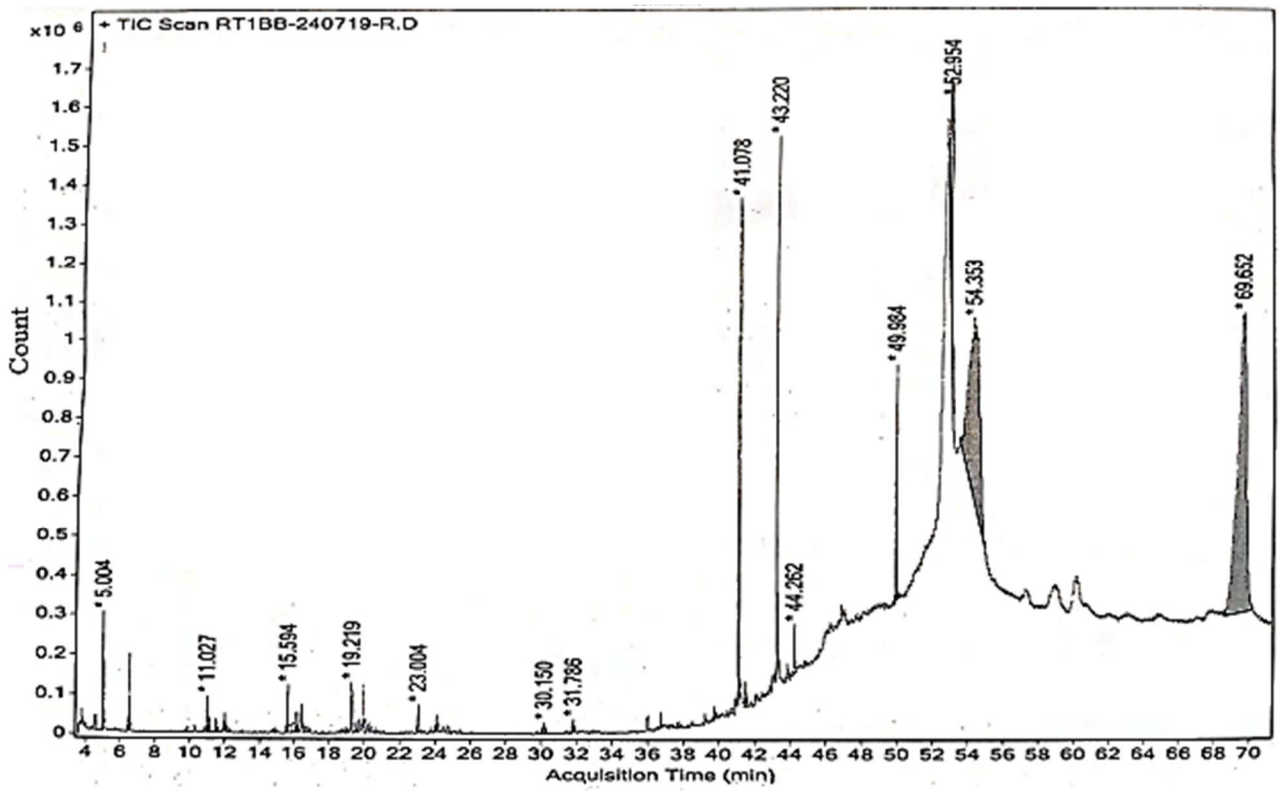


Fig. 1: GC-MS Chromatogram of *B. Balochistanica* EO.

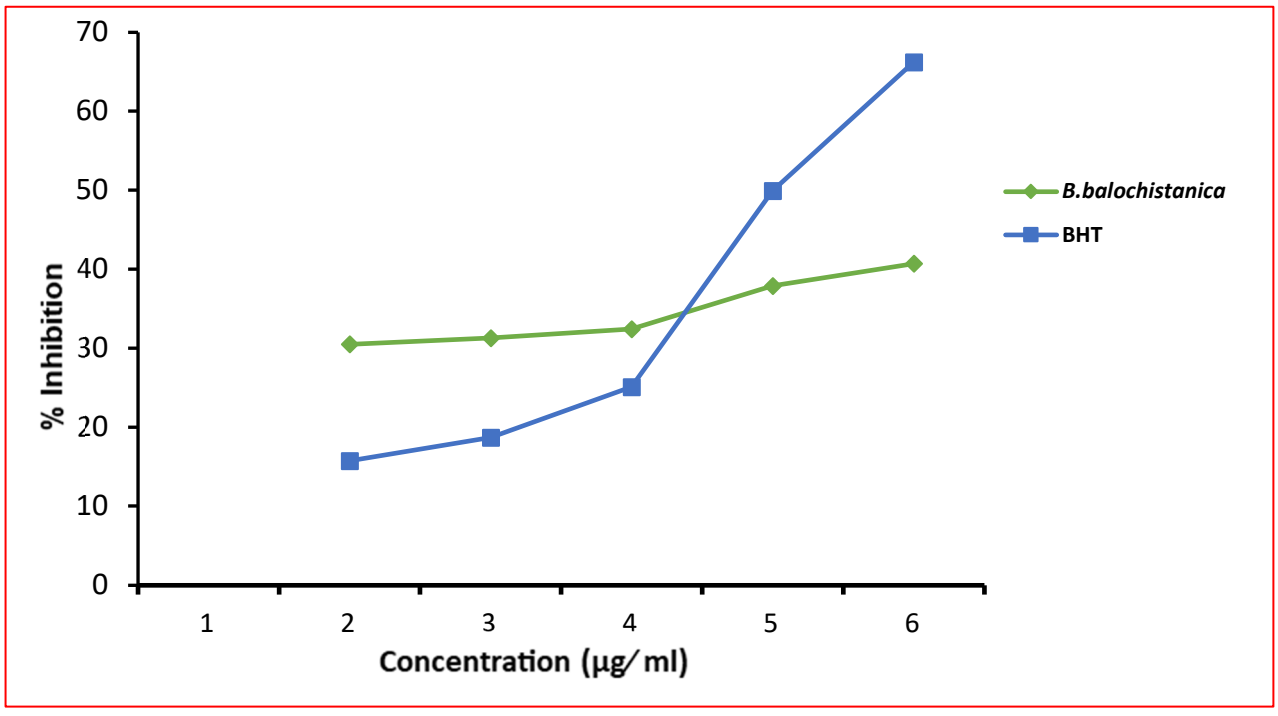


Fig. 2: Comparison of free radical scavenging activity of *B. balochistanica* EOs with standard BHT

Antioxidant activity

The DPPH method was used to evaluate the antioxidant activity of the EO of *B. balochistanica* for measuring its ability to scavenge free radicals. Table 2 summarizes the results, while figs. 2 and 3 describes the graphical illustration of the antioxidant action of EO and signify that tested samples exhibit a concentration-dependent activity profile as EO concentration (µg/mL) increases, causing an increase in DPPH percentage inhibition. The lower the IC_{50} value, the greater the antioxidant potential of the sample. The results illustrated that B. balochistanica EO indicated the highest scavenging activity (40.7±0.05%) at 250 µg/mL concentration. The lowest scavenging value was shown by BHT (15.73±0.28 %) at 15µg/mL. As can be seen from table 2, the EO indicated lesser antioxidant potential with IC_{50} = 436±0.40 µg/mL than the synthetic antioxidant used as a reference, BHT. The DPPH scavenging radical effect increases in the following order: EO < BHT.

Antifungal activity

Antifungal activity of *B. balochistanica* EO was examined against *A. alternate* and *F. oxysporum* fungoid strains via agar well and disc diffusion assay. Results are presented in tables 3-4 and figs. 4-5. *B. balochistanica* EO exhibited varying antifungal potential against tested fungal strains, though an equivalent oil concentration was applied for various strains. In the agar well and disc diffusion method, plant isolated EO were active against *A. alternata* and exhibited no activity against *F. oxysporum*. In the agar well method, *B. balochistanica* EO inhibited fungal growth by 13.7±0.66% against *A. alternata*, while it was not active against *F. oxysporum*.

Antibacterial activity

The antibacterial action of *B. Balochistanica* EO oil was examined against Gram -ve (*E. coli*) and Gram +ve (*B. Subtilus*) bacterial strains via disc diffusion. Results are shown in tables 5-6 and fig. 6. *B. balochistanica* EO exhibited varying antibacterial potential against the tested bacterial strains. In this method, plant-isolated EO was active against *B. Subtilis* (12±0.18 mm) and at 0.18 g/µL it showed an MIC, while *E. coli* not to be susceptible to the inhibitory effects of *B. balochistanica* EO.

Anticancer activity

Using the colorimetric MTT method, the anticancer potential of the EO of *B. balochistanica* was assessed for its impact on the cancer cell proliferation. Results shown in table 7 showed that dead cells cannot cleave the MTT ring. An increase in % inhibition will increase the cytotoxicity potential of EO. For a formazan product, no reduction of MTT appears to be carried out by the cell types we have examined. PC_3 cell line results have shown that EO extracted from the whole plant of *B. balochistanica* (BB) was inactive against the anticancer (PC_3) cell line. Simultaneously, doxorubicin (standard drug) exhibited anticancer potential against the PC3 cell line with an IC_{50} value of 1.9± 0.08.

DISCUSSION

Chemical composition of B.balochistanica EO

The principal constituents of the *B.balochistanica* EO were Hexyl oleate (74.6 %) and Stearylarachidate (20.1 %). Some studies have reported the chemical composition of other *Berberies* species' essential oils. (Hosseinihashemi *et al*., 2015) Scrutinized the inner stem bark of *B. vulgaris* for chemical analysis through the technique of GC-MS and identified phthalic acid, 1, 2-benzendicarboxylic acid, tetracosanoic acid, 1, 2-bis (trimethylsiloxy) ethane, methyl ester and diisooctyl ester as chief components. Another group of researchers studied the volatile components of *B. integerrima* seeds by using the GC-FID technique and identified principal constituents were Erosic acid, Arachidic acid, Lauric acid, Linolenic acid, Behenic acid, Oleic acid, Palmitic acid, Myristic acid, Stearic acid, Palmitoleic acid, Capric acid and Linoleic acid (Tavakoli *et al*. 2017). The difference in climatic and geographical conditions plays a vital role in EO's chemical composition variation. Research has displayed that EO's anticancer, antioxidant and antimicrobial potential are owed to bioactive components (Hussain *et al*., 2013).

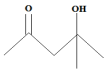
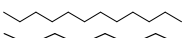
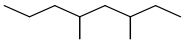
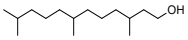
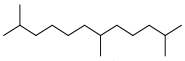
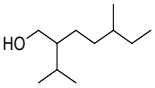
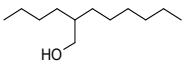
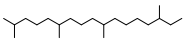
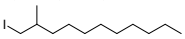
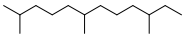
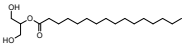
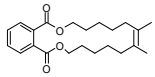
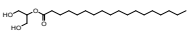
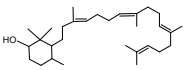
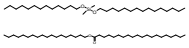
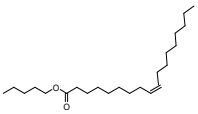
Antioxidant activity

Studies show that oxidative stress amongst antioxidants and free radicals leads to the cause of various pathological diseases in the human body. The scavenging of reactive species of oxygen can reduce this oxidative stress. The reduction of purple colored stable radical DPPH to colorless diphenylpicryl hydrazine was due to the existence of antioxidants in the medium (Ma *et al*., 2012). Our results are in accord with (Victoria *et al*., 2012), who revealed that EO of *E.uniflora* at the highest dose exhibited maximum % inhibition of DPPH (free radicals). Obtained results of *B. balochistanica* EO seem to agree with already reported results of Algerian EO of *M.pulegium* (IC_{50} = 157 µg/mL) (Ouakouak *et al*., 2015) and Iranian (IC_{50} = 14736±156 µg/mL) (Kamkar *et al*., 2010). EO and extract's antioxidant capacity was due to their various antioxidants.

Upon removal of oxidizing molecules, Flavonoids are highly active and contain a lot of free radicals, which are linked with number of illnesses. Phenolic content in plants provides Oxidative stress. Sages, fruits, vegetables and other plant resources are rich in flavonoids and phenols, which are used in the nutrition industry because of their antioxidant capacity and health advantages. (Ghafoor *et al*., 2020).

The effectiveness of EO's antioxidant capacity was due to the presence of phenols (Fatiha *et al*., 2015). Differences among the results obtained in this research study and reported by previous authors may be due to the duration of extraction, isolation technique, season and origin (Gul *et al*., 2022). Greater antioxidant capacity was linked to a higher polyphenolic content (Belwal *et al*., 2020).

Table 1: Chemical Constituents of EO of *B. balochistanica*

Compd.	Name of Components	Molecular formula	Molecular mass (g/mol)	Structure	R.T (min.)
1	2-Pantanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116		5.004
2	Cyclohexanone	$C_6H_{10}O$	98		6.536
3	Dodecane	$C_{12}H_{26}$	170		11.027
4	Octane, 3,5-dimethyl-	$C_{10}H_{22}$	142		11.157
5	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	228		11.529
6	Undecane	$C_{11}H_{24}$	156		12.039
7	Dodecane, 2,6,11-trimethyl	$C_{15}H_{32}$	212		15.594
8	2-Isopropyl-5-methyl-1-heptanol	$C_{11}H_{24}O$	172		16.065
9	1-Octanol, 2-butyl-	$C_{12}H_{26}O$	186		16.219
10	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296		19.219
11	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296		24.054
12	Dodecane, 2,6,10-trimethyl	$C_{15}H_{32}$	212		30.15
13	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330		41.078
14	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390		41.5
15	Octadecenoic acid,2- hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{42}O_4$	358		43.220
16	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	428		44.262
17	Dimethyl[bis(tridecyloxy)]silane	$C_{28}H_{60}O_2Si$	456		49.984
18	Eicosanoic acid, octadecyl ester	$C_{38}H_{76}O_2$	564		52.954
19	9- Octadecenoic acid (Z)-, hexyl ester	$C_{24}H_{46}O_2$	366		54.353
	Total quantified components				99.98%

R.T: Retention Time

Table 2: DPPH free radical scavenging capacity (%) and IC_{50} value of *B.balochistanica* EO.

Sample	Concentration (µg/mL)	%age of DPPH radical Scavenging ±SEM	IC_{50} of DPPH (µg/mL) ±SEM
Berberies balochistanica	250	40.7±0.05	436±0.40
	125	37.9±0.38	
	60	32.4±0.11	
	30	31.3±0.16	
	15	30.5±0.14	
BHT[a)]	250	66.2±0.20	160±0.69
	125	49.9±0.30	
	60	25.1±0.129	
	30	1.7±0.16	
	15	15.73±0.28	

All results are represented as mean ± SEM, [a)] Standard antioxidant.

Table 3: Antifungal activity by agar well diffusion method

Type of EO	% Inhibition of Fungal Growth[(dc – dt/ dc) × 100] Fungal strains	
B.balochistanica	*A. alternata*	*F. oxysporum*
	13.7±0.66	NA

Data represented as Mean ± SEM; NA= Not Active.

Table 4: Antifungal activity by Disc diffusion method

Type of EO	Fungal strains	
B.balochistanica	*A .alternata*	*F. oxysporum*
	+	–

+ = indicates presence of fungal activity; – = indicates absence of fungal activity.

Table 5: Antibacterial activity of *B.balochistanica* EO by Disc diffusion

Bacterial strains	Type of EO *B. balochistanica (mm)*	Deoxycycline D-30* (mm)
E. coli	NA	18+0.18
B. subtilis	12+0.18	27+0.11

DI= Diameter of inhibition zone (mm); NA= Not Active;* Standard antibiotics for bacteria.

Table 6: MIC* determination of *B.balochistanica* EO for antibacterial activity

Type of EO *B. balochistanica*	Concentration (g/ µL)	Bacterial strains *E.coli*	*B. subtilis*
	0.10	-	0
	0.12	-	4
	0.14	-	6
	0.16	-	7
	0.18	-	8

*MIC= Minimum inhibitory concentration.

Table 7: Anticancer Activity (PC3) of *B.balochistanica* EO

S. No.	Sample Code	Concentration (µg/ml)	%Inhibition	IC_{50} ± S.D.
1	BB	30	7.5	Inactive
2	Doxorubicin*	30	89.9	1.9 ± 0.08

*Doxorubicin=standard drug

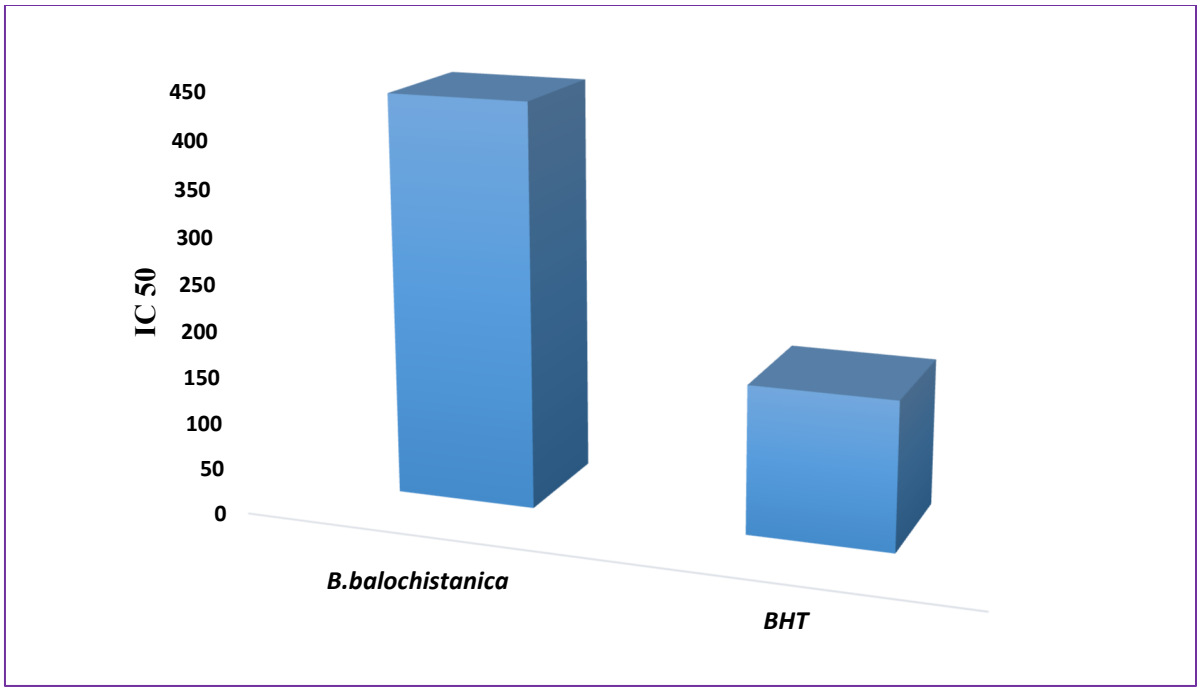
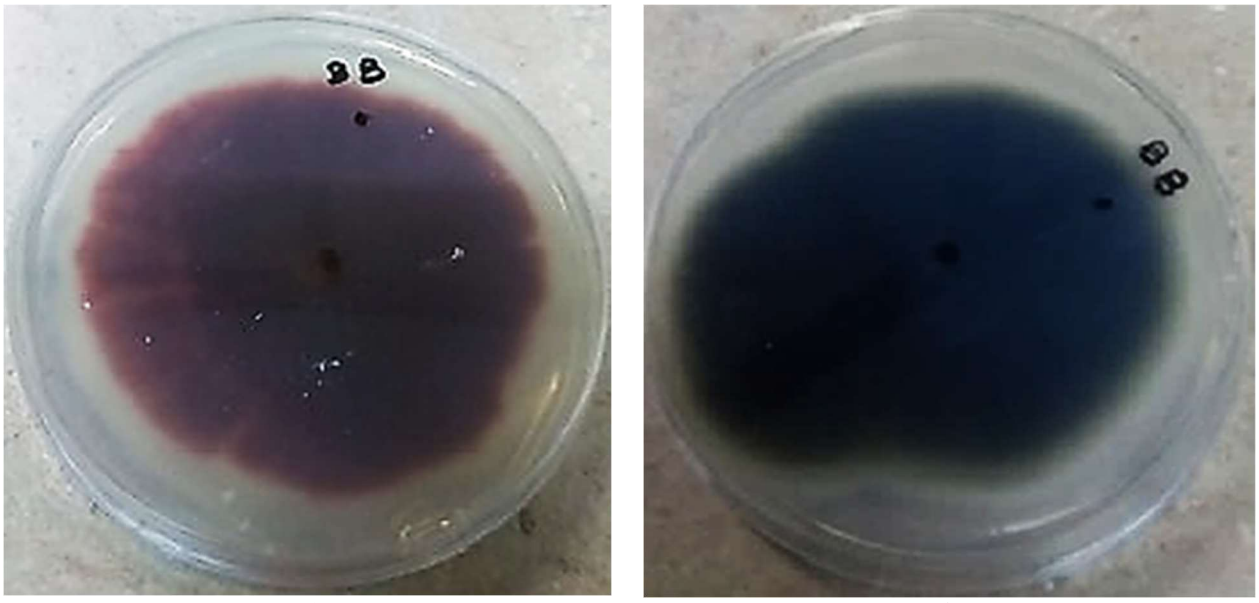


Fig. 3: Comparison of IC_{50} values of sample and BHT



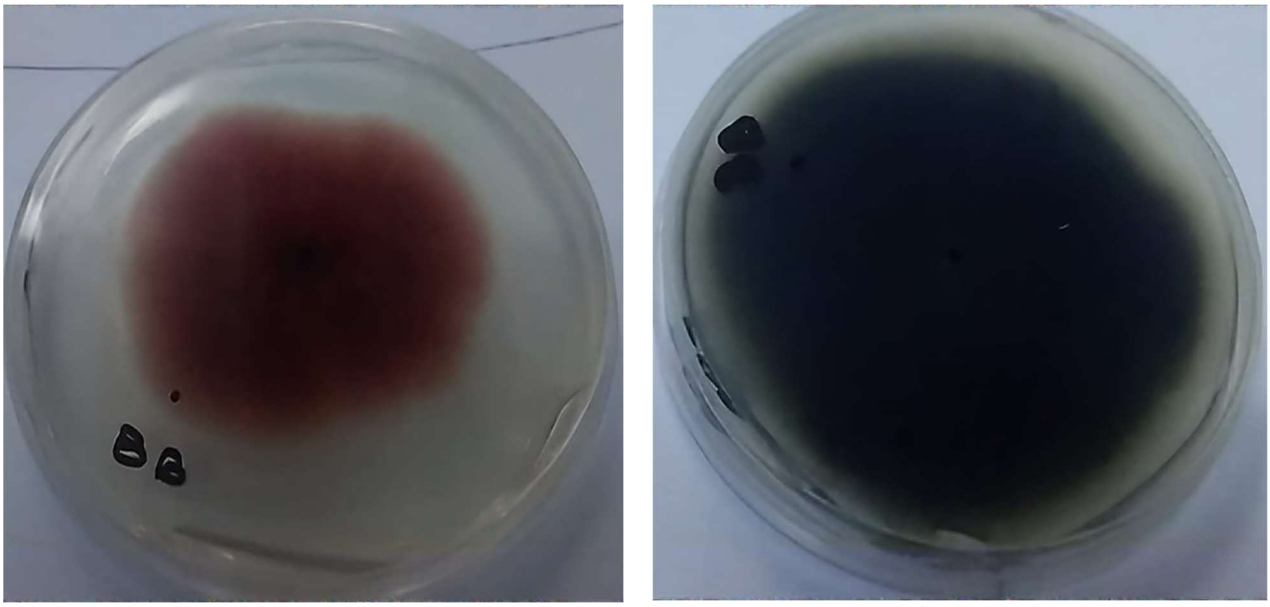
(a) (b)

Fig. 4: Antifungal activity of *B.balochistanica* EO against (a) *F. oxysporum* and (b) *A. alternate* by agar well diffusion method.

Numerous studies have shown that the antioxidant capacity of EO is lower than that of extracts from the same plant. Among the various fractions of *B. balochistanica* roots, antioxidant investigation of crude Methanolic extract exhibited maximum antioxidant potential with IC_{50} = 7.03 µg/ml using ascorbic acid as reference (Baloch *et al*., 2013). Another study showed that soluble ethyl acetate fraction displayed higher radical scavenging capacity (IC_{50} = 15.96±1.50 µg / mL) relative to BHT as reference standard (IC_{50} = 12.33±0.87 µg/mL) (Abbasi *et al*., 2013).

Antimicrobial activity

Antimicrobial action would be narrated to the composition of the chemical profile of EO and possible synergistic relations among constituents (Habbab *et al*., 2016). EO's low antifungal potential in this study due to the lack of strong antifungal components such as eugenol, phenolic components and linalool reported their occurrence in numerous plant extracts (Campaniello *et al*., 2010).



(a) (b)

Fig. 5: Antifungal activity of *B.balochistanica* EO against (a) *F. oxysporum* and (b) *A. alternate* by Disc diffusion method.

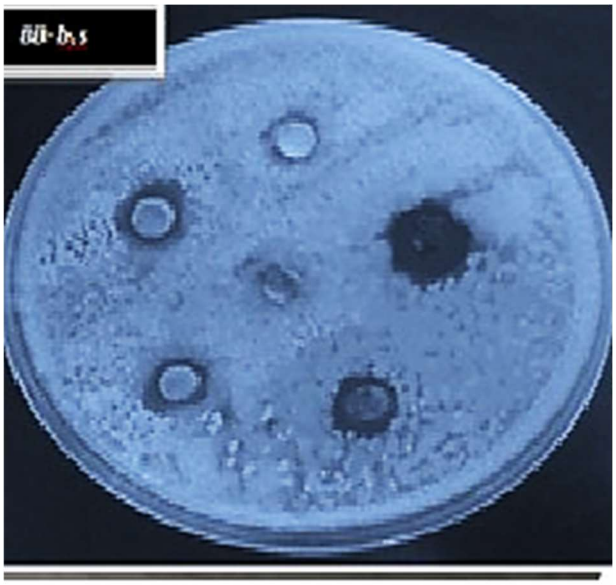


Fig. 6: MIC against *B.subtilus*

Inhibitory potential of an ethanolic fraction of *B.balochistanica* against various fungi. Mild to adequate antifungal potential was displayed against *M. canis* (50 %), *T. schoenleinii* (23.6%), *F. solani* (15.4%), *M. phaseolina* (28.5%) and *P. boydii* (50%) (Chand *et al*., 2017). The BBS-NiONP antifungal reaction in contradiction of *A. niger*, *F. oxysporum* and *A. alternate* (fungal strains) was reported in 2021 by Uddin *et al*. with the inhibition in the following order: *A. alternata* > *A. niger* > *F. oxysporum*. While in 2019, Pervez *et al*., described berberisinol compound antifungal potential against *F. solani*, *M. canis*, *A. niger*, *C. albicans*, *C. glabrata* and *A. flavus* different fungal species, although no substantial antifungal potential against the tested fungal species was shown.

It was demonstrated that the EO was inactive against Gram -ve bacteria but had inhibitory potential against Gram +ve bacteria. Given the nature of the exterior membrane, which provides intrinsic resistance, this tolerance is caused by wide variety of biocides. The cell wall in Gram +ve bacteria has no outer covering to prevent the attack of foreign substances and in the outer membrane, the presence of hydrophobic lipopolysaccharides protects against different agents. Because teichoic acid containing outer membrane polysaccharides interact with other species and provide less resistance to antibiotics, Gram +ve bacteria showed greater resistance.

This resistance is attributed to the double layer complexity of the cell membrane in comparison to the gram +ve bacteria cell membrane (Abdelli *et al*. 2016). The inhibition zone under 10 mm is considered weak, moderate with 10-16 mm and active at greater than 16 mm (Irshad *et al*., 2018).

EO extracted from medicinal and aromatic plants showed antimicrobial potential, attributed to their chemical profile, especially terpene alcohol. Numerous studies have been reported on the antibacterial capacity of different extracts of the same plant.

Antibacterial potential of crude methanol extracts of *B. balochistanica* was reported against *S. aureus, S.pyogenes*(gram +ve bacteria), *P. aeruginosa, K. pneumoniae, S. typhimurium, S. pneumoniae, E .coli* (gram -ve bacteria) via disc diffusion method. In contrast, MIC was verified using agar well diffusion and agar dilution methods. Methanolic extract of *B. balochistanica* exhibited greater inhibition zones with the lowest MIC values (Kakar *et al*., 2012). Research reported the inhibitory potential of methanolic extract of *B. baluchistanica* with eight medicinal plants through agar well diffusion method against human pathogenic microbes, i.e., *Klebsiella spp, Shigella spp, E. coli, Clostridium spp* and *Salmonella spp.* Results revealed that all plants showed good inhibitory potential against tested bacterial strains. The antibacterial action of the ethyl acetate fraction of *B.balochistanica* was tested through agar well diffusion assay and displayed dynamic inhibitory action against *C. diphtheriae* (59.2%), *B. cereus* (58.31%), *S. typhi* (39.9%) and *E. coli* (30%) (Chand *et al*., 2017). The result agreed with the formally documented results of other Berberis species (Ilyas *et al*., 2021). The results showed that the extract had broad-spectrum antimicrobial activity. The biosynthesis of nucleic acid hindrance and other metabolic acid processes occurred due to higher concentrations of flavonoids and phenolic compounds. At the same time, secondary metabolites i.e. coumarin, alkaloids, terpenoids and saponins, are probably responsible for strong antibacterial effects (Iqbal *et al*., 2020).

Whereas berberisinol antibacterial action demonstrated in 2019 by Pervez *et al*. revealed substantial potential against *S.pyogenes*, *E.coli* and *S. ureus*, against *K. pneumonia* and *B.subtilis* and *P. aeruginosa,* exhibited modest and weak activity, respectively. These results follow the antiseptic potential of methanol extract isolated from B. balochistanica roots against *E.coli, P.aeruginosa, S. aureus* and *B.subtilus* (Idress *et al.,* 2012*).*

The maximum bacteriostatic potential of foliage was exhibited against *B.licheniformis*, While extract of roots was detected against *B.licheniformis*, *B.subtilis* and *R. erythropolis*. Other *Berberies* species have exhibited remarkable antibacterial action (Rafique and Salman, 2021). Alcholic extracts of stem and roots of *B. lyceum*, *B. chitria* and *B. aristata* revealed substantial activity against *S. pneumoniae, S aureus, P. aeruginosa, K. pneumoniae, E. coli*.

Anticancer activity

The colorimetric MTT assay scrutinised cancer cell proliferation by measuring the mitochondrial dehydrogenase enzyme activity that reduces pale yellow tetrazolium compound MTT to insoluble purple color formazan dye crystals. The reduction of MTT is mainly due to the glycolytic activity of metabolically active cells occurring inside the cell, which relies upon NADH and NADPH. Activated cells produce more formazan than resting cells, which could allow activation measurement even in the absence of proliferation. In this technique, the mitochondrial dehydrogenases cleave the tetrazolium ring of MTT by viable cells and yield formazan form but not by erythrocytes or dead cells (Sajid *et al*., 2018).

Since the EO of *B. balochistanica* (BB) did not exhibit any activity against the cancerous cell line, our r findings are consistent with the previous study, which used the brine shrimp method to assess cytotoxic activity and found no cytotoxic activity in the various extracts and fractions of *B. balochistanica* plant (Chand *et al*., 2017). The Brine Shrimp Cytotoxicity Assay revealed that the roots of this plan had a significant cytotoxic potential, with an ED50 of 0.92 µg/ml (Baloch *et al*., 2013). To date, other investigators have not published any report on the anticancer potential of *B. balochistanica* EO, whereas *in vitro* anticancer potential against various disparaging cell lines of numerous *Berberis* species is reported (El Fakir *et al*., 2021; Ilyas *et al*., 2021). The cytotoxic potential of roots, foliage and bark ethanolic extract of *B. balochistanica* was evaluated against the HeLa cell line via MTT cell techniques. Ethanolic bark extract, in comparison to standard (doxorubicin), displayed the greatest anticancer potential with the lowest IC_{50} value (12 ± 0.15 µg/ml) tracked by roots and leaves (Gul *et al*., 2022).

The anticancer potential of *B. aristata* methanolic extract was inspected against human breast cancer cell lines (MCF-7) by Soft agar colony formation assay, Scratch assay and Live / Dead assay. The IC_{50} value for methanolic extracts was observed at 220µg and deliberated as a prophetic indicator of anticancer activity (Serasanambati *et al*., 2015).

CONCLUSION

The data obtained from the present investigation revealed novel findings in aspects of drug discovery from medicinal plants. This paper reported chemical analysis in addition to the antifungal, antibacterial, antioxidant and anticancer potential of EO extracted from *B. balochistanica*. The

investigation of GC-MS chemical composition analysis showed the existence of various chief constituents in the EO. The presence of these chemical components is responsible for the biological activities of the EO. However, it was found that the EO possessed a weak radical scavenging activity and moderate antifungal activity. Scavenging free radicals' antioxidant potential might be owed to sterols, phenolic components, esters and the synergistic impact of various minor compounds. The interactivity among them was also taken into account. The antimicrobial action of EO is due to terpene alcohols. The biological potential of medicinal plants is due to the diversity of phyto-constituents. It can be considered an effective antibiotic and plays a significant role in the infection antidote, preventing or reducing the senescent process and other deteriorating maladies. The variance of biological characteristics in the EO could be credited to the extraction method and isolated chemical components. The substantial antimicrobial and antioxidant aptitude of the EO of B. balochistanica may assist in the preparation and development of novel and beneficial drugs with few side effects that are used to treat a variety of illness, such as oxidative stress, aging and infectious diseases. The anticancer activity was assessed by MTT assay and no activity was observed in the *B. balochistanica* EO. Thus. Researched EOs represent a potential replacement for eliminating microorganisms that may be harmful to human health, food and agricultural products and utilized as natural additives in a variety of food side effects. However, further studies are needed to search for the effectiveness of the chemical identity of the bioactive components in appropriate concentrations, to examine how the combined product demonstrated potential and to evaluate the safety and poisonousness of EO to human intake prior to considering their usage for preservation of food products and medicinal tenacities.

Acknowledgments
The authors greatly appreciate the support of the Higher Education Commission, Islamabad, Pakistan, the Department of Chemistry, University of Balochistan, Quetta and BUITMS for providing laboratory facilities. Thanks to Hussain Ebrahim Jamal (HEJ), Research Institute of Chemistry, University of Karachi, Pakistan, for providing anticancer PC_3 cell lines and GC-MS analysis.

Authors' contributions
R.T: Methodology and writing original draft; S.A: Supervision and conceptualization; S.K: Project administration; N.T: Review and editing manuscript.

Funding
The Higher Education Commission, Islamabad, Pakistan, provided financial support for the study under the Indigenous PhD scholarship program Batch II (PIN:213-56263-2PS2-084(50024286)).

Ethical approval
Not applicable.

Data availability statement
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest
It has been verified that the authors declare no conflict of interest.

REFERENCES

Abbasi MA, Naqvi SSH, Rehman A and Tareen RB (2013). *Berberies balochistanica*: Assessment of natural antioxidants reprieve from oxidative stress. *Int. Res. J. Pharm*., **49**(5): 101-105.

Abdelli M, Moghrani H, Aboun A and Maachi R (2016). Algerian *Menthapulegium* L. leaves essential oil: Chemical composition, antimicrobial, insecticidal and antioxidant activities. *Indust. Crop. Produc.*, **94**: 197-205.

Adams RP (2007). Identification of essential oil components by gas chromatography / Quadrupole mass spectroscopy, 4thed.; Allured publishing corporation, Carol Stream, Illinois, USA. Pp: 61-285.

Aebisher D, Cichonski J, Szpyrka E, Masjonis S and Chrzanowski G (2021). Essential oils of seven lamiaceae plants and their antioxidant capacity. *Molecules*, **26**(13): 3793.

Arumugam G, Swamy MK and Sinniah UR (2016). *Plectranthus amboinicus* (Lour.) Spreng: Botanical, phytochemical, pharmacological and nutritional significance. *Molecules*, **21**(4):369.

Baloch N, Nabi S and Al-Kahraman YM (2013). *In vitro* antileishmanial, cytotoxic, antioxidant activities and phytochemical analysis of *Berberis baluchistanica* roots extracts and its fractions. *Int. J. Phy*., **4**(2): 82-87.

Behrad Z, Sefidkon F, Ghasemzadeh H, Rezadoost H and Balandary A (2023). Determination of phenolic compounds and antioxidant activities of 55 iranian berberis genotypes. *J. Med. Plan. and By-prod*., **12**(2): 181-189.

Belwal T, Bisht A, Devkota HP, Ullah H, Khan A, Pandey A, Bhatt ID and Echeverria J (2020). Phytopharmacology and clinical updates of berberis species against diabetes and other metabolic diseases. *Front. Pharmacol*., **11**: 41.

Bouyahya A, El Moussaoui N, Abrini J, Bakri Y and Dakka N (2016). Determination of Phenolic contents, antioxidant and antibacterial activities of strawberry tree (*Arbutus unedo* L.) leaf extracts. *British Biotec. J*., **14**(3): 1-10.

Campaniello D, Corbo MR and Sinigaglia M (2010). Antifungal activity of eugenol against *Penicillium*,

Aspergillus and Fusarium species. *J. Food Protect*., **73**(6): 1124-1128.

Chand RR, Jokhan AD, Gopalan RD and Osborne T (2017). Antibacterial and antifungal activities of essential oils from medicinal Plants found in the South Pacific. *The South Paci. J. Nat. Appl. Sci*., **35**(1): 10-19.

Chouhan S, Sharma K and Guleria S (2017) Antimicrobial activity of some essential oils-present status and future perspectives. *Medicines*, **4**(3): 58.

El Fakir L, Bouothmany K, Alotaibi A, Bourhia M, Ullah R and Zahoor S (2021). Antioxidant and understanding the anticancer properties in human prostate and breast cancer cell lines of chemically characterized methanol extract from *Berberis hispanica* Boiss. *Reut. Applied Sci*., **11**(8): 3510.

El-Said H, Ashgar SS, Bader A, AlQathama A, Halwani M, Ascrizzi R and Flamini G (2021). Essential oil analysis and antimicrobial evaluation of three aromatic plant species growing in Saudi Arabia. *Molecules*, **26**(4): 959.

Fatiha B, Didier H, Naima G, Khodir M, Martin K, Leocadie K, Caroline S, Mohamed C and Pierre D (2015). Phenolic composition, *in-vitro* antioxidant effects and tyrosinase inhibitory activity of three Algerian Mentha species *M. Spicata* (L.), *M. pulegium* (L.) and *M. rotundifolia* (L.) Huds (Lamiaceae). *Ind. Crop. Prod*., **74**: 722-730.

Ghafoor K, Al Juhaimi F, Ozcan MM, Uslu N, Babiker EE and Ahmed IAM (2020). Total phenolics, total carotenoids, individual phenolics and antioxidant activity of ginger (*Zingiber officinale*) rhizome as affected by drying methods. *Lwt*., **126**(4): 109354.

Gul Z, Akbar A and Leghari SK (2022). Elucidating therapeutic and biological potential of *Berberis baluchistanica* Ahrendt Bark, leaf and root extracts. *Front. Microbiol.* **13**: 823673.

Gul Z, Akbar A, Naseem M, Achakzai JK, Rehman Z and Khan NM (2023). Phytonutrient and antinutrient components profiling of *Berberis baluchistanica* Ahrendt bark and leaves. *J King Saud. Uni. Sci*., **35**(5): 102517.

Habbab A, Sekkoum K, Belboukhari N, Cheriti A and Aboul-Enein HY (2016). Essential oil chemical composition of *Vitexagnus-castus* L. from southern-west Algeria and its antimicrobial activity. *Current Bioact. Comp*., **12**(1): 51-60.

Hellali N, Mahammed MH, Ramdane F and Talli A (2016). Antimicrobial and antioxidant activities of *Cybopogon schoenanthus* (L.) spreng. essential oil, growing in lllizi. Algeria. *J. Med. Plant. Res*., **10**(14): 188-194.

Hosseinihashemi SK, Anooshei H, Aghajani H and Salem MZM (2015). Chemical composition and antioxidant activity of extracts from the inner bark of *Berberis vulgaris* Stem. *Bio. Re*., **10**(4): 7958-7969.

Hussain AI, Anwar F, Chatha SAS, Latif S, Sherazi STH, Ahmad A, Worthington J and Sarker SD (2013). Chemical Composition and bioactivity studies of the essential oils from two Thymus species from Pakistan flora. *LWT- Food sci. Tech*., **50**(1): 185-192.

Idrees M, Saeed-Ur-Rehman SKL, Ur Rehman M, Rehman KU, Achakzai AKK and Zareen B (2021). Antimicrobial activity of three medicinally important plants (*Berberis balochistanica*, *Thymus serpyllum* and *Salvia bucharica*) of Shudden, Mazhoo Tehsil Nana Sab area, District Pishin, Balochistan-Pakistan. *Pure and Appli. Bio*., **11**(2): 570-576.

Ilyas S, Tabasum R, Iftikhar A, Nazir M, Hussain A and Hussain A (2021). Effect of *Berberis vulgaris* L. root extract on ifosfamide-induced *in-vivo* toxicity and in vitro cytotoxicity. *Scienti. Rep*., **11**(1): 1708.

Iqbal J, Abbasi BA, Ahmad R, Mahmoodi M, Munir A and Zahra SA (2020). Phytogenic synthesis of nickel oxide nanoparticles (NiO) using fresh leaves extract of *Rhamnus triquetra* (wall.) and investigation of its multiple in vitro biological potentials. *Biomed*., **8**(5): 117.

Irshad A, Khan N, Farina Y, Baloch N, Ullah S, Ali A, Mun LK, Murtaza G and Rehman A (2018). Synthesis, spectroscopic characterization, X-ray diffraction studies and in-vitro antibacterial activities of diorganotin (IV) derivatives with N-methyl-4-bromobenzohydroxamic acid. *Inorg. Chim. Act*., **469**(1): 280-287.

Jahan F, Alvi SS and Islam MH (2022). Pharmacological research - modern chinese Medicine. *Front. Pharmacol*., **11**: 41.

Jugreet BS, Suroowan S, Rengasamy RK and Mahomoodally MF (2020). Chemistry, bioactivities, mode of action and industrial applications of essential oils. *Trends Food Sci. Technol*., **101**: 89-105.

Kakar SA, Tareen RB, Kakar MA, Jabeen H, Kakar SU, Al-Kahraman YM and Shafee M (2012). Screening of antibacterial activity of four medicinal plants of Balochistan-Pakistan. *Pak. J. Bot*., **44**: 245-50.

Kamkar A, Javan AJ, Asadi F and Kamalinejad M (2010). The antioxidative effect of Iranian *Mentha pulegium* extracts and essential oil in sunflower oil. *Food Chem. Toxicol*., **48**(7): 1796-1800.

Ma C, Yang L, Zu Y and Liu T (2012). Optimization of conditions of solvent- free microwwave extraction and study on antioxidant capacity of essential oil from Schisandra chinensis (Turcz.) Baill. *Food Chem*., **134**(4): 2532-2539.

Megawati FD, Sediawan WB and Hisyam A (2019). Kinetics of mace (*Myristicae arillus*) essential oil extraction using microwave assisted hydrodistillation: Effect of microwave power. *Ind. Crops. Prod*., **131**: 315-322.

Mohamed AA and Alotaibi BM (2023). Essential oils of some medicinal plants and their biological activities: A mini review. *J. Umm Al-Qura Univ. Appll. Sci*., **9**: 40-49.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and

cytotoxicity assays. *J. lmmuno. Methods*., **65**(1-2): 55-63.

Muddassir M, Batool A, Alam M, Miana GA, Altaf R, Alghamdi S, Almehmadi M, Abdulaziz O, Alsaiari AA and Sahibzada MUK (2022). Evaluation of *in-vitro*, in silico antidiabetic and antioxidant potential of bioactivity based isolated "pakistanine" from *Berberis baluchistanica*. *Arabian J. Chem*., **15**(11): 104221.

Ouakouak H, Chohra, M and Denane M (2015). Chemical composition, antioxidant activities of the essential oil of Menthapulegium L. South east of Algeria. *Int. Letter Nat. Sci*., **39**: 49-55.

Pervez S, Saeed M, Ali MS, Fatima I, Khan H and Ullah I (2019). Antimicrobial and antioxidant potential of berberisinol, a new flavone from *Berberis baluchistanica*. *Chem. Nat. Comp*., **55**: 247-251.

Rafique M and Salman M (2021). Exploration of antibacterial activities of *Berberis royleana* fractions extracts. *BioRx*., 1-22.

Rajendran MP, Beautlin PB and Selvaraj N (2014). Chemical composition, antibacterial and antioxidant profile of essential oil from Murrayakoenigii (L.) leaves. *Avicenna . J. Phytomed*., **4**(3): 200-214.

Rehman F, Sajjad A, Mengal MA, Taj MK, Mengal MA, Mengal MH and Azam S (2017). Antimicrobial activity of selected indigenous medicinal herbs against human pathogenic bacteria. *Pure App. Biol*., **6**(2): 740-747.

Sadeh D, Nitzan N, Chaimovitsh D and Shachter A (2019). Interactive effects of genotype, seasonality and extraction method on chemical compositions and yield of essential oil from rosemary (*Rosmarinus officinalis* L.). *Ind Crops Prod*., **138**(5): 111419.

Sadiq MB, Tarning J, Aye Cho TZ and Anal AK (2017). Antibacterial activities and possible modes of action of Acacia nilotica (L.) Del. against multidrug-resistant Escherichia oli and Salmonella. *Molecules*, **22**(1): 47.

Sajid A, Manzoor Q, Iqbal M, Tyagi AK, Sarfraz RA and Sajid A (2018). *Pinus roxburghii* essential oil anticancer activity and chemical composition evaluation. *Experi. Clin. Sci. J*., **17**: 233-245.

Serasanambati M, Chilakapati SR, Manikonda PK and Kanala JR (2015). Anticancer activity of methanolic extract of *Berberis aristata* in MCF-7 human breast cancer cell lines. *Int. J. Life sci. Biotec. Pharma Res*., **4**(1): 31-35.

Sousa RMF, de Morais SAL, Vieira RBK, Napolitano DR, Guzman VB Moraes TS, Cunha LCS, Martins CHG, Chang R, de Aquino FJT, do Nascimento EA and de Oliveira A (2015). Chemical composition, cytotoxic and antibacterial activity of the essential oil from *Eugenia calycina* Cambess. leaves against oral bacteria. *Ind. Crop. Prod*., **65**: 71-78.

Tavakoli A, Sahari MA and Barzegar M (2017). Antioxidant activity of *Berberis integerrima* seed oil as a natural antioxidant on the oxidative stability of soybean oil. *Int. J. Food Proper*., **20**(3): S2914-S2925.

Uddin S, Safdar LB, Anwar S, Iqbal J, Laila S and Abbasi BA (2021). Green synthesis of nickel oxide nanoparticles from *Berberis balochistanica* Stem for investigating bioactivities. *Molecules*, **26**(6): 1548.

Victoria FN, Lenardao EJ, Savegnago L, Perin G, Jacob RG, Alves D, da Silva WP, da Motta AS and Nascente PS (2012). Essential oi of leaves of *Eugenia uniflora* L. Antioxidant and antimicrobial activities. *Food Chem. Toxicol*., **50**(8): 2668-2674.

World Health Organization (2019). WHO global report on traditional and complementary medicine. World Health Organization.

Xu T, Ge Y, Du H, Li Q, Xu X, Yi H, Wu X, Kuang T, Fan G and Zhang Y (2021). *Berberis kansuensis* extract alleviates type 2 diabetes in rats by regulating gut microbiota composition. *J. Ethnopharmacol*., **273**: 113995.