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STUDIES ON ANTI-BACTERIAL AND ANTIOXIDANT ACTIVITIES OF *BERBERIS BALUCHISTANICA*, *EPHEDRA INTERMEDIA* AND *ACHILLEA SANTOLINA* METHANOLIC EXTRACTS

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Abstract

The Balochistan province is rich in medicinal herbs, yet remains largely unexplored from a scientific perspective. Various plants from this region are traditionally used to treat both human and animal diseases. However, there has been limited scientific evaluation of their efficacy and safety. This study aimed to compare the antibacterial and antioxidant activities of extracts obtained from three plants native to Quetta, Balochistan: Berberis baluchistanica, Ephedra intermedia, and Achillea santolina. The antibacterial activity was assessed using agar well diffusion assays against selected bacterial strains, while antioxidant activity was evaluated using DPPH radical scavenging assays.

Results showed that Berberis baluchistanica extract demonstrated the highest antibacterial activity, with zones of inhibition ranging from 13.0 to 19.5 mm against the tested bacterial strains. Achillea santolina extract exhibited moderate antibacterial activity, with zones of inhibition ranging from 12.6 to 16.71 mm, while Ephedra intermedia extract displayed the lowest activity, with zones of inhibition ranging from 7.19 to 12.2 mm. The B. baluchistanica extract showed good antibacterial activity against all tested pathogens, with the highest activity observed against Bacillus salitus (19.50 mm). Achillea santolina showed good activity against Staphylococcus aureus (16.71 mm), while Ephedra intermedia exhibited the highest activity against Pseudomonas aeruginosa (12.20 mm).

In terms of antioxidant activity, Achillea santolina demonstrated substantial activity with an IC50 value of 0.378 ± 0.67 mg/ml, which was higher than that of Berberis baluchistanica (IC50: 0.482 ± 0.10 mg/ml) and Ephedra intermedia (IC50: 0.915 ± 0.12 mg/ml). Notably, these IC50 values were lower than that of ascorbic acid (IC50: 0.271 ± 0.08 mg/ml), indicating potent antioxidant properties.

The observed antibacterial and antioxidant potentials of Achillea santolina, Berberis baluchistanica, and Ephedra intermedia suggest that further research is needed to isolate and characterize the active compounds responsible for their pharmacological effects.

Keywords: *Anti-bacterial, Anti-oxidant, A. santolina, Balochistan, B. baluchistanica, E. intermedia*

INTRODUCTION

Medicinal plants are a precious natural resource that provides humans with the opportunity to lead a healthy, disease-free life. With over 35,000 species identified for their medicinal properties, these botanical treasures have been used by more than 75% of the global population (1). Notably, two-thirds of the newly discovered chemical compounds each year come from higher plants. Even in the United States, where the pharmaceutical industry largely relies on chemical synthesis, 25% of pharmaceutical products are derived from plant-based chemicals (2). In recent years, there has been a growing trend toward traditional and complementary medicine, driven by the belief that herbal remedies are free from harmful effects (3).

Pakistan is home to a diverse range of plant species, with approximately 20,000 different species thriving in its rich landscapes (4). The country has a wealth of wild plants used not only for their medicinal properties but also for their pleasant fragrances. While some of these plants are well-known for their therapeutic benefits among local communities, many others have yet to be thoroughly studied (5).

Ephedra (*Ephedra intermedia*), a genus of non-flowering plants belonging to the family Ephedraceae, is closely related to the Gnetales, which are near relatives of angiosperms (6). Of the 50 Ephedra species worldwide, most are shrubs adapted to both moist and arid conditions (7, 8). In Pakistan, three species of Ephedra are found. The perennial green shrub *E. intermedia*, locally known as Ma-Huang or Oman in Balochistan, has been used for centuries for its medicinal properties as a diaphoretic, antiasthmatic, and stimulant. These properties are derived from the aerial parts of several species, including *E. distachya, E. equisetina, E. sinica,* and *E. intermedia* (9). Various species of Ephedra contain alkaloids such as ephedrine and pseudoephedrine, which are essential components in several commercially available ephedra extract medications. *E. intermedia* is notably found in Pakistan, Iran, and Northwest India, particularly in the region of Balochistan (5). Additionally, these shrub species have demonstrated antibacterial and antioxidant properties (10). The main constituents of Ephedra are phenols and the alkaloids ephedrine and pseudoephedrine (11).

The family Berberidaceae, comprising 15 genera and 650 species, includes the genus Berberis, one of the earliest angiosperms with significant commercial and therapeutic importance (12). Berberis baluchistanica, known locally as Korae in Balochi, Archin in Brahvi, and Zralag in Pashto, is a native therapeutic herb exclusive to Balochistan and a member of the Berberidaceae family. This plant is commonly found in the regions of Ziarat, Harboi, and the Zarghun mountains of Quetta and Kalat (13). Berberis species are known to contain various phytochemicals, including oleanolic acid, palmatine, stigmasterol, glycosides, anthraquinones, reducing sugars, saponins, alkaloids, phlobatannins, steroids, tannins, terpenoids, and flavonoids (14). Due to the therapeutic compound berberine, this medicinal herb is commonly used locally to treat a range of conditions, including kidney stones, wounds, internal injuries, fever, and disorders of the eyes, nose, and throat (13).

The genus *Achillea* L. (Asteraceae) comprises approximately 115 species, primarily found in the Northern Hemisphere, with most species located in Europe and Asia. Achillea is well-known for its historical use as a wound healer, particularly in military settings, earning it several common names such as nosebleed, soldiers' bloodwort, knight's milfoil, herba milifaris, and staunch weed (15). Various Achillea species have demonstrated numerous therapeutic effects, including hepatoprotective, antioxidant, anticancer, antiulcer, spasmolytic, antidiabetic, choleretic, antimicrobial, anti-inflammatory, and cytotoxic activities (16).

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS

The plant materials of *Berberis baluchistanica* roots, *Ephedra intermedia* and *Achillea santolina* aerial parts were collected from the mountainous area of Hana Urak Valley District Quetta, Balochistan.

The plants were washed three times with tap water to make it impurities and dust free and then let to dry in a shaded area before being chopped into small pieces using a knife and scissors. The plant material was identified by Chairperson Pharmacognosy Department University, of Balochistan, Quetta. Plant was allotted a voucher specimen number 1688/FOPHS/18 to *B. baluchistanica*, 1689/FOPHS/18 to *E. intermedia* and 1690/FOPHS/18 to *A. santolina*.

PREPARATION OF METHANOLIC PLANT EXTRACTS

Grinded powder of *B. baluchistanica* (01 kg), *Achillea santolina* (01 kg), and *Ephedra intermedia* (01 kg) were put in different percolators and soaked in 5.0 liters of methanol. During the maceration process, precautions were taken to prevent exposure to light by conducting the experiments in a dark room. To facilitate the extraction of compounds, the mixture underwent shaking at a specific frequency. The methanolic mixture was filtered using Whatman filter No. 4 after two weeks. The filtered mixture was then subjected to drying using a rotary vacuum evaporator resulting in the formation of concentrated extracts. These extracts were utilized for further analyses to determine biological and functional activities. A. *santolina, E. intermedia,* and *B. baluchistanica* had yield percentages of 38%, 60%, and 48%, respectively.

PHYTOCHEMICAL TESTS

The analysis of *Ephedra intermedia*, *Berberis baluchistanica* and *Achillea santolina* methanolic extracts showed the presence of different plant compounds like glycosides, tannins and saponins. The screening of methanolic extract revealed alkaloids, carbohydrates, glycosides, flavonoids, resins, saponins, tannins, unsaturated sterols, and triterpenes. These compounds have various properties and could contribute to the plant's potential health benefits.

TEST FOR ALKALOIDS

Nitrogen-containing compounds are often recognized for their pharmacological effects. To test for the presence of alkaloids in the samples, a 1 ml solution was used. The presence of alkaloids was confirmed by adding a few drops of Mayer's reagent, which produced an off-white or pale precipitate. (17).

TEST FOR SAPONINS

To test for the presence of saponins, 10 milliliters of pure water were mixed with one gram of the extract, and the mixture was shaken continuously for two minutes. The presence of saponins was indicated if foam developed and remained at a height of 1 cm for 30 minutes(18).

TEST FOR CARDIAC GLYCOSIDE

Glycosides are compounds formed by the combination of a sugar molecule with a non-sugar moiety (aglycone). To test for glycosides, 3 drops of lead acetate were added to 2 milliliters of plant extract, and the mixture was filtered. When 5 milliliters of chloroform were added to the filtrate and stirred, the chloroform coating disappeared. The remaining material was dissolved in glacial acetic acid, and a few drops of ferric chloride were added. Then, 1 milliliter of concentrated H_2SO_4 was introduced. The presence of deoxy-sugar cardenolides was indicated by the formation of a brown ring at the interface. A violet ring may form below the brown one, and a greenish ring may gradually appear through the thin layer (19).

TEST FOR TERPENOIDS

To test for terpenoids, 2 milliliters of chloroform were mixed with a 5 milliliter portion of a methanolic extract, then 3 milliliters of concentrated sulfuric acid were added. A reddish-brown color at the interface between the methanolic extract and chloroform layers indicated the presence of terpenoids (20).

TEST FOR FLAVONOIDS

Flavonoids are a class of polyphenolic compounds known for their various health benefits. To test for the presence of flavonoids, 1 milliliter of a methanolic extract was mixed with a few drops of magnesium (Mg) and concentrated hydrochloric acid (HCl). The appearance of a pink or magenta-red color upon mixing indicates the presence of flavonoids (18, 19).

TEST FOR TANNINS

To detect the presence of tannins, a 5 milliliter extract solution was tested by adding a few drops of ferric chloride ($FeCl_2$). The development of an intense purple, black, green, or blue color confirmed the presence of tannins. Additionally, a lead acetate test was performed by adding a few drops of 10% lead acetate to 5 milliliters of the extract. The formation of a precipitate indicated the presence of tannins (18).

DPPH RADICAL SCAVENGING ACTIVITY

To determine hydrogen providing capability of the root extract in scavenging DPPH stable radical, the following procedure was followed:

Various concentrations of the extract were prepared, ranging from 1 to 0.0625 mg/ml, using a stock solution. To prepare a 0.1 millimolar DPPH solution, 0.0039432 grams of DPPH were dissolved in 100 milliliters of ethanol. Next, 0.5 milliliters of the 0.1 millimolar DPPH solution were mixed with 50 microliters of the plant extract. This mixture was then shaken and incubated in the dark at room

temperature for 30 minutes. Ascorbic acid was used as the standard compound for comparison. The reduction in DPPH color was assessed by measuring the decrease in absorbance at 517 nm. Blank samples containing ethanol, ascorbic acid as the standard, and DPPH solution without the plant extract were used as controls in the experiment. The percentage inhibition of DPPH was calculated using the following formula:

$$\% \text{ inhibition of DPPH} = AC - AS / AC \times 100$$

Where:

*AC= the absorbance of the control (DPPH solution)

*AS = the absorbance of the extract

The IC50 value indicates the concentration of the sample extract necessary to scavenge 50% of the DPPH free radicals, thus reflecting the antioxidant capability of the extracts. A relationship curve was constructed by plotting the scavenging activities against different concentrations of the extract, expressed in mg/ml. This curve helps to visualize the antioxidant activity of the extract at different concentrations and determine its IC50 value.

BACTERIAL STRAINS AND REFERENCE DRUGS

Various bacterial strains include, Bacillus subtilis (ATCC 5230), Salmonella typhi (ATCC 14028), Escherichia coli (ATCC 8739), Staphylococcus aureus (NCTC 6571) and Pseudomonas aeruginosa (ATCC 9027) were identified and obtained from CASVAB, UOB, Quetta.

Stock solutions of *B. baluchistanica, E. intermedia, A. santolina* extracts as well as reference drug Gemifloxacin, were prepared at concentration of 300 µg/ml using methanol as solvent. The stock solutions were further diluted to concentrations of 30, 60 and 90 µg/ml in methanol, respectively.

The antibacterial activity of *B. baluchistanica, E .intermedia and A. santolina* was assessed using the Disc diffusion method. The procedure involved the following steps:

Fresh Muller Hinton Agar media was prepared and then sterilized. Subsequently, sterile agar was poured into plates and allowed to solidify and also incubated for 24 hours for any contamination.

Using a sterile swab or inoculating loop, streak the surface of the agar plates with the bacterial culture(s) to form a uniform lawn of growth. This is usually done by making several passes across the agar surface in different directions.

Put antibiotic disks onto the inoculated agar plates with care using sterile forceps.

Ensure that the disks are evenly spaced and pressed gently onto the agar surface to ensure proper contact.

The disk diffusion method was employed to evaluate the antibacterial activity of extracts obtained from *Berberis baluchistanica, Ephedra intermedia* and *Achillea santolina.* In this study, we used sterile 4.0 mm diameter disks available commercially. These disks were loaded with different dilutions of reference drugs, including gemifloxacin, to check their antibacterial effects. Additionally, the crude methanolic extracts from *Berberis baluchistanica, Ephedra intermedia* and *Achillea santolina* were also loaded onto separate discs.

Prior to use, the loaded discs were stored in a refrigerator to maintain their stability. Each disk, containing either the reference drug or the plant Extract (20 µl), was then placed onto nutrient agar plates for the assessment of antibacterial activity. These plates were subsequently incubated at 37°C for a period of 24 hours to facilitate bacterial growth and allow for the observation of inhibition zones.

DMSO served as a negative control group to ensure validity of the results. Entire procedure was conducted in triplicate to ensure consistency. After incubation, plates were examined for presence of clear zones (inhibition zones) around both controlled and extracted drug loaded discs. Using a calibrated ruler, the diameter of the zones was measured in millimeters (mm). The findings were interpreted in relation to the level of bacterial growth inhibition.

RESULTS

This study presents a comprehensive investigation into the phytochemical composition and bioactive properties of the methanolic extract derived from *Ephedra intermedia, Berberis baluchistanica* and *Achillea santolina*. The study has shown detecting the presence of Alkaloid, Saponins, Terpenoids,

Flavonoids, Tannins and Glycosides, shown in (Table I.), highlighting the diverse nature of their chemical constituents.

Table I. shows the detection of phytochemical groups in the methanolic extract of *Ephedra intermedia*, *Berberis baluchistanica* and *Achillea santolina*

Phytochemical Group	*E. intermedia*	*B. baluchistanica*	*A. santolina*
Alkaloid	Detected	Detected	Not detected
Saponins	Not Detected	Detected	Detected
Glycosides	Detected	Detected	Not detected
Terpenoids	Not Detected	Detected	Detected
Flavonoids	Detected	Detected	Detected
Tannins	Not Detected	Detected	Detected

ANTIBACTERIAL ACTIVITY

Antibacterial activities *of E. intermedi, A. santolina* and *B. baluchistanica* extracts were demonstrated against different bacterial strains. The diameter of inhibition zones in millimeters was measured at various concentrations (30, 60, and 90 µg/ml) of plant extracts and standard drugs (Table II), dissolved in DMSO. No inhibitory zones were observed in the negative control (DMSO). The highest inhibition zones were recorded for *B. baluchistanica*. Zones of inhibition of *B. baluchistanica* (10.88 ,12.67 and 13.02 mm), *A. santolina* (10.15,13.57 and 14.02 mm) and *E. intermedia* (9.8,10.67 and 12.2 mm) were observed against *Pseudomonas aeruginosa* a (Gram negative bacterium) as compared to the standard drug gemifloxacin (12.41 ,20.45 and 29.5mm) at 3 different concentrations respectively. Against a gram positive strain *Berberis baluchistanica* extract was found with better bacterial inhibitory profile (zone of inhibition of *B. subtilis* 16.45, 18.13 and 19.5 mm) than *E. intermedia* and *A. santolina.* Nevertheless, inhibition zones of all three extracts were lesser than reference drug (24.5, 27.59 and 33.93 mm for *B. subtilis*) at 3 different tested concentrations. Overall, the results suggest that Gemifloxacin had the highest inhibitory effect, followed by *B. baluchistanica* and *A. santolina* extracts, while *E. intermedia* extract showed the least inhibition against the tested pathogens.

Table II. Comparative Antibacterial activity of *Achillea santolina*, *Berberis baluchistanica* and *Ephedra intermedia*

Sample information		Diameter of zone of inhibition against target Pathogens in millimeters (mm)				
Sample name	**Conc.(µg/ml)**	***E. coli***	***P. aeruginosa***	***S. aureus***	***B. salitus***	***S. typhi***
Gemifloxacin	30	32.45	12.41	30.78	24.5	25.15
	60	34.67	20.45	34.12	27.59	28.38
	90	38.2	29.5	36.78	33.93	30.7
B. baluchistanica	30	11.35	10.88	10.12	16.45	10.32
	60	15.45	12.67	12.45	18.13	12.9
	90	17.67	13.02	15.45	19.5	14.15
A. santolina	30	9.87	10.15	13.19	11.45	10.98
	60	11.54	13.57	15.45	13.91	13.56
	90	12.67	14.02	16.71	14.95	15.09
E. intermedia	30	7.56	9.8	5.18	9.49	8.36
	60	8.51	10.67	6.4	10.78	9.9
	90	9.1	12.2	7.19	11.5	10.25

All values in table are mean ± SEM n=5 results (P<0.01) where * shows significant results (P<0.05) and ** show highly significant

Anti bacterial activities of three plant extracts are illustrated in Fig. 1. *Berberis baluchistanica* demonstrates moderate to strong antibacterial activity. Most effective against *B. salitus* with inhibition zone of (19.50 mm), while *Achillea santolina* exhibits moderate antibacterial activity. Particularly effective against S. aureus with zone of inhibition (16.71 mm) and *Ephedra intermedia* shows relatively low antibacterial activity compared to other samples, most effective against *P. aeruginosa* with inhibition zone of (12.20 mm). The standard gemifloxacin shows strong antibacterial activity against all tested pathogens.

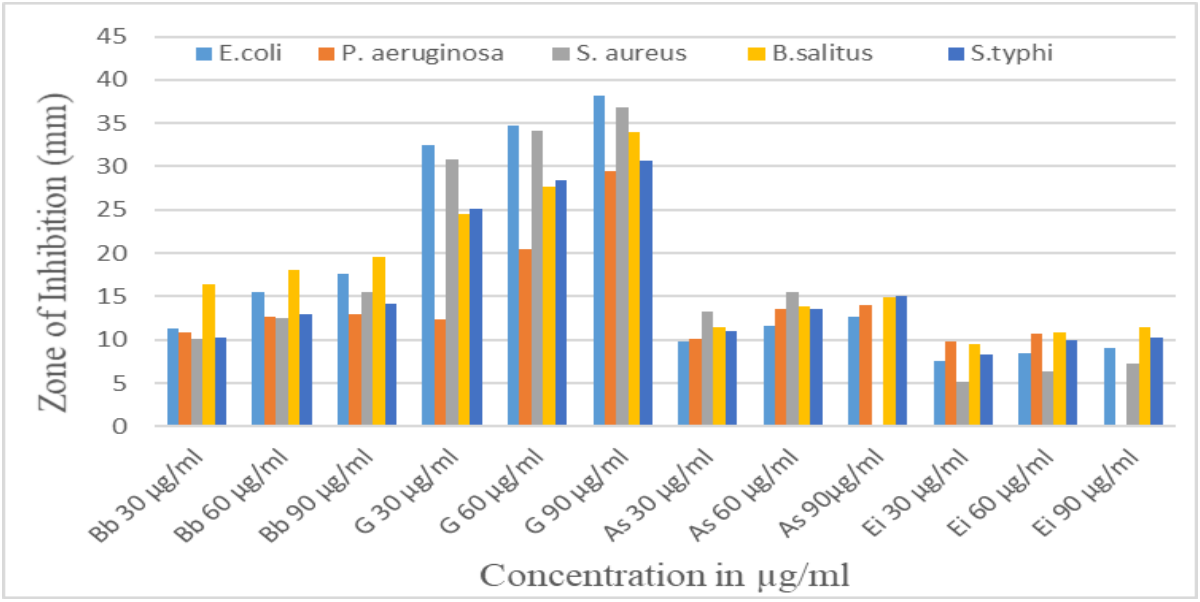

Fig. 1. Comparative Antibacterial activities of *Achillea santolina, Berberis baluchistanica* and *Ephedra intermedia* (Bb = *Berberis baluchistanica*, G = Gemifloxacin, As = *Achillea santolina*, Ei = *Ephedra intermedia*)

ANTIOXIDANT ACTIVITY

The study evaluated the extract of *Berberis baluchistanica, Ephedra intermedia* and *Achillea santolina* and for its ability to donate hydrogen in the presence of the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) and evaluated its reducing potential based on the concentration required to achieve 50% inhibition of DPPH free radicals. To evaluate the ability to donate hydrogen, we determined the concentration of the extract needed for scavenging 50% of DPPH free radicals. This was achieved by establishing a linear regression equation correlating the conc. of the extract with its percentage scavenging ability. The antioxidant activity of *E. intermedia, A. santolina and B. baluchistanica* extract was assessed using DPPH assay, with ascorbic acid serving as reference drugs. Various concentrations ranging from 0.0625 mg/ml to 1 mg/ml were tested.

The results revealed that *A. santolina* demonstrated significant antioxidant activity, with IC50 values of 0.378 ± 0.67 mg /ml than *B. baluchistanica* 0.482 ± 0.10 mg/ml and *E. intermedia* 0.915 ± 0.12. Notably, these values were lower than that of ascorbic acid (IC50: 0.271 ± 0.08 mg/ml). Overall, these findings highlight the promising antioxidant potential of these plant extracts, suggesting their potential utility as natural antioxidants in various applications.

Table III. Comparative DPPH % inhibition *of Achillea santolina*, *Berberis baluchistanica* and *Ephedra intermedia* at different concentration

Conc.(mg/ml	% inhibition Ascorbic acid ± SD	% inhibition *A. santolina* ± SD	% inhibition *B. baluchistanica* ± SD	% *inhibition E. intermedia* ± SD
1	96.56 ± 0.67	79.50 ± 0.29	56.19 ± 1.10	45.10 ± 1.10
0.5	88.41 ± 1.15	64.09 ± 1.54	47.13 ± 1.34	40.23 ± 2.34
0.25	65.17 ± 1.09	49.26± 0.87	34.69 ± 1.10	28.89 ± 1.19
0.125	48.20 ± 2.10	37.17 ± 1.05	23.26 ± 2.05	17.16 ± 1.65
0.0625	35.16 ± 1.18	28.15 ± 1.10	17.10 ± 1.09	12.10 ± 1.01

All values in table are mean ± SEM n=5 results (P<0.01) where * shows, significant results (P<0.05) and ** show highly significant

Fig. 2 presents the results of the DPPH % inhibition activity of three plant extracts (i.e.) *Achillea santolina, Berberis baluchistanica,* and *Ephedra intermedia* at various concentrations. The % inhibition of DPPH radical by each plant extract is compared to that of ascorbic acid. *Achillea santolina* showed significant DPPH % inhibition compared to other tested plants extracts while Ascorbic acid shows the highest % inhibition in all tested concentrations.

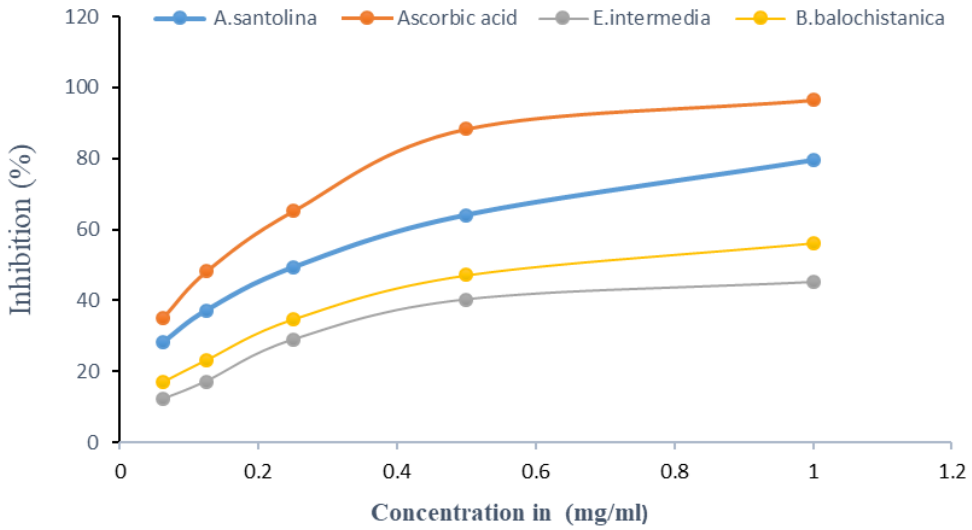

Fig. 2. Comparative DPPH % inhibition plot of *Achillea santolina, Berberis baluchistanica* and *Ephedra intermedia* with Ascorbic acid

Table IV provides a comparative analysis of the IC50 values of methanolic extracts obtained from three different plant species; *A. santolina, B. baluchistanica, and E. inermedia* along with the IC50 value of ascorbic acid, which serves as a reference antioxidant. The IC50 values were determined through the DPPH assay. The table showed IC50 values (mg/ml) ± SEM for methanolic extracts of *A. santolina* (0.378 ± 0.67), *B. baluchistanica* (0.482 ± 0.10), *E. inermedia* (0.915 ± 0.12), and ascorbic acid (0.271 ± 0.08) obtained from DPPH assay**.** Lower IC50 values signify stronger antioxidant activity, as they indicate that less concentration of the sample is required to scavenge 50% of the DPPH radicals. *A. santolina* extract showed significant antioxidant activity compared to other plant extracts tested but less than Ascorbic acid.

Table IV. Estimated IC50 of methanolic extracts of plants

Sample	DPPH assay IC50 (mg/ml) ± SEM
A. santolina	0.378 ± 0.67 mg /ml
B. baluchistanica	0.482 ± 0.10 mg/ml
E. inermedia	0.915 ± 0.12 mg/ml
Ascorbic acid	0.271 ± 0.08 mg/ml

*Ascorbic Acid = Standard

DISCUSSION

The current comparative study was conducted to study the Anti-bacterial and Anti-oxidant activity of the methanolic extracts of *Berberis baluchistanica, Ephedra intermedia* and *Achillea santolina* plants. Plants that are rich in phytochemicals offer numerous health benefits due to their capability to decrease the risk of various diseases, including diabetes, cardiac diseases and different types of cancers. In current study different phytochemicals were found in all three plants extracts including alkaloids, flavonoids, essential oils, tannins, saponins, and phenolic compounds are naturally occurring compounds found in plants which are not considered essential nutrients but have demonstrated beneficial effects on human health (21). Various bioactive compounds have been detected, including, cardiac glycosides, tannins, saponins, flavonoids, alkaloids and terpenoids. Several compounds, including phenolics, alkaloids, pakistanamine, flavanols, gallic acid, phlobatannins, anthraquinones, phenolics, carbohydrates, coumarin, steroids & berberine have been previously documented (22). The existence of phytochemicals provides justification for their utilization by local communities. Furthermore, the exploration of new types of drugs will enhance our understanding of the therapeutic properties of valuable compounds found in therapeutic plants (23). Each compound identified possesses recognized biological activity, including notable antioxidant, antiviral, antifungal, antiparasitic, and antibacterial properties (13). Alkaloids, known for their anti-inflammatory and pain-relieving properties, are vital in supporting the immune system and providing pain relief. They're

commonly utilized in the treatment of asthma, skin conditions, and snakebites. Alkaloids given basic structure, significantly contributed to the development of various antibiotics, each exhibiting diverse effects (24).

Flavonoids exert notable physiological effects, including their capacity to modify the body's response to viral infections, carcinogens, and allergies (25). Flavonoids also inhibit a variety of enzymes such as arylsulfatases, cAMP phosphodiesterase hyaluronidases, hydrolases, alkaline phosphatase, kinases, α-glucosidase, and lipase (26). The presence of flavonoid and phenolic compounds within the plant extract is responsible for its biological effects. Flavonoids, in particular, are highly efficient at scavenging oxidizing molecules, which include a variety of free radicals implicated in different diseases (27). The increased presence of phenolic compounds in plants enhances their antioxidant abilities, therefore the detection of Flavonoids and phenol along with other phytochemicals were investigated (Table I).

According to Raudone *et al.,* (2022) the phenolic and flavonoid constituents found in *A. santolina* are considered key metabolites due to their substantial impact on biological activity, with potential implications for antioxidant properties (28). Tannins exhibit antitumor and immune-defensive properties, thus demonstrating the capacity to reduce blood pressure, enhance blood clotting, and decrease serum lipid levels (29). Saponins possess diverse medicinal benefits, including antimicrobial, antidiabetic, antioxidant, antispasmodic, anti-inflammatory, analgesic and anticancer properties. Consequently, their presence in plant extracts underscores their therapeutic potential (30).

The antioxidant mechanisms of various molecules are multifaceted, primarily reliant on two key processes: single electron transfer (SET) and hydrogen atom transfer (HAT) or alongside their ability to chelate metals. Single electron transfer (SET) involves the transfer of a single electron from the antioxidant molecule to the free radical, forming a stable radical species on the other hand Hydrogen atom transfer (HAT) involves the scavenging of free radicals by donating a hydrogen atom, thereby interrupting the chain reaction of radical formation and propagation. This process effectively terminates the radical chain reaction and reduces oxidative stress (31).

In the context of the study, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was utilized to evaluate the radical scavenging potential of extracts from *B. baluchistanica, E. intermedia and A. santolina*. This assay relies on the reduction of the purple-colored DPPH radical to its yellow-colored reduced form by antioxidant compounds. The degree of discoloration is indicative of the scavenging activity of the test sample against free radicals. The methodology involved measuring the reduction in absorbance of the DPPH solution over time in the presence of the plant extracts. The concentration of the extracts required to reduction the initial absorbance of DPPH by 50% was determined and expressed as the IC50 value. Lower IC50 values indicate greater radical scavenging activity, thereby suggesting stronger antioxidant potential for the tested extracts.

Acillea santolina methanolic extract presented more radical scavenging effect comparing to *Berberis baluchistanica* and *ephedra intermedia* extract. The Literature shows that *A. santolina* possess different types of essential oil (32). Numerous research studies have highlighted the significant antioxidant properties found in essential oils extracted from plants (33). The antioxidant capability of essential oils is of significant interest in disease prevention, including conditions such as heart diseases, cancer, and immune system disorders, as well as in the preservation of food. Unlike synthetic antioxidants, essential oils are natural products. Common synthetic antioxidants like butyl hydroxytoluene butylated hydroxyanisole and are believed to have potential adverse effects on human health (34). In this study antioxidant activity of *Berberis baluchistanica, Achillea santolina and Ephedra intermedia* was checked by DPPH radical scavenging methods, the results are shown in Table II. At a concentration of 1 mg/ml, the % inhibition of *A. santolina* was 79.50% while *E. intermedia* and *B. baluchistanica* show 45.10% and 56.19% respectively. At a concentration 0.5mg/ml the *A. santolina* were able to inhibit 64.09 % while *E. intermedia* and *B. baluchistanica* showed 40.30 & 47.13 % inhibition respectively. As the concentration of compounds decreased, the inhibition percentages gradually decreased as shown in Table II. Additionally, the study included IC50 values. The results for the IC50 antioxidant activity of *A. santolina* while *E. intermedia* and *B. baluchistanica* are summarized in Table III.

Despite the fact that the calculated IC50 values for *Achillea santolina* were lower than ascorbic acid and higher than *E. intermedia* and *B. baluchistanica* in the DPPH radical scavenging assay indicating a good potency in inhibiting oxidative processes.The present results are coherent with the earlier studies of (32). The antioxidant of *E. intermedia* and B. *baluchistanica* is attributed due to occurrence of phenols and flavonoids (5, 13). Polyphenols and flavonoids are essential components contributing to the antioxidant potential of plant extracts. Their significance lies in their ability to scavenge reactive oxygen species, engage in redox reactions, donate hydrogen atoms, and chelate metal ions, all of which are vital mechanisms in combating oxidative stress (35). Phenolic compounds, which are present in both the terpenoid and phenylpropanoid families, are commonly recognized for their antioxidant capabilities. Additionally, the collective antioxidant effect observed in essential oils is the result of the synergistic interplay among their various constituents (36). However, antioxidant potential of all Methanolic plant extracts was less significant than ascorbic acid.

The growing prevalence of multi-drug resistant pathogens poses a significant challenge to conventional synthetic drugs, highlighting an urgent need for novel antibacterial agents. In response to this pressing concern, plant-based antimicrobials emerge as promising alternatives, renowned for their efficacy in combating various diseases (37). Furthermore, plant-based antimicrobials offer several additional benefits. They are often readily available, cost-effective, and culturally accepted in many traditional medicine systems worldwide. Moreover, compared to synthetic drugs, they typically have fewer adverse effects and lower toxicity, making them safer options for long-term use (13).

Antibacterial activity of methanolic extracts of three plants of Quetta which is traditionally used as medicine were tested against *Staphylococcus aureus* (NCTC 6571), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella typhi* (ATCC 14028) *Bacillus subtilis* (ATCC 5230) and *Escherichia coli* (ATCC 8739) to find out which plant extract has better coverage against these pathogens. Disc diffusion method was used for this activity, and the results revealed that all the plant extracts exhibited activity against all tested bacteria to varying degrees. The antibiotic Gemifloxacin was active against all tested bacteria.

Berberis baluchistanica showed the best antibacterial activity amongst all the studied plants. Its highest activity was against *Bacillus subtilis* (19.50 mm) and lowest was against *P. aeruginosa* (13.02mm), followed by *A. satolina* whose highest activity was against *P. aeruginosa* (16.71 mm) and lowest against *E. coli* (12.67 mm), whereas *E. intermedia* showed highest zone inhibition against *P. aeruginosa* (12.20mm) shown in Table IV. Inhibition zones less than 10 mm are classified as weak, those ranging from 10 to 16 mm are considered as moderate, and those exceeding 16 mm are categorized as active (38). The literature indicates that phenolic compounds, flavonoids and tannins have shown considerable action against Gram-negative bacteria (39). The findings of the present study are consistent with those reported in previous research (13). Nevertheless, *Pervez et al.* (2019) assessed the antibacterial efficacy of berberisinol, which demonstrated notable activity against *Escherichia coli, Staphylococcus aureus* and *Streptococcus pyogenes*, moderate activity against *K. pneumonia* and *B. subtilis* and weak activity against *P. aeruginosa*. Interestingly, these findings align with the recently reported antibacterial activity of the methanolic root extracts of *B. baluchistanica*, which exhibited zones of inhibition measuring 30 mm against *Staphylococcus aureus*, 22 mm against *Bacillus subtilis*, 19 mm against *Pseudomonas aeruginosa*, and 29 mm against Escherichia (40). The results show that all the extracts possess a wide range of anti-microbial capacity. The notable anti-bacterial effectiveness of the obtained extracts is likely attributed to presence of secondary metabolites like saponins, steroids, coumarin, terpenoids and alkaloids. Moreover, the extracts contain significant levels of phenolics and flavonoids, known for their involvement in inhibiting nucleic acid biosynthesis and other metabolic processes (26). The difference in antibacterial activity may be due to the bacterial morphology or presence of varying plant constituents.

CONCLUSION

The present comparative study was conducted to evaluate the anti-bacterial and antioxidant activities of *Berberis baluchistanica, Ephedra intermedia and Achillea santolina* extracts. This study revealed that

the methanolic extract obtained from *Berberis baluchistanica* showed substantial antibacterial activity against all tested pathogens. And achillea santolina exhibited significant antioxidant potential among all tested plant extracts. *B. baluchistanica, E. intermedia and A. santolina* be worthy for further scientific investigation on these preliminary studies to explore the bioactive compounds and their mechanisms of action, which underlie their effectiveness.

Conflict of Interest:

Authors have no conflict of interest.

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