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DETECTION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF EPHEDRA PROCERA IN DISTRICT HARNAI, BALOCHISTAN, PAKISTAN

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Abstract

Background: *Traditional medicines such as Ephedra procera have been in use by the medical fraternity since centuries especially in the developing part of the world such as Baluchistan, Pakistan. As the antimicrobial resistance increased, it is important to investigate the natural antimicrobial agents. E. proceri has been reported to possess the following bioactive compounds phenolics, flavonoids and alkaloid which possess antibacterial, antifungal activities.*

Objectives: *The aim of the study was to investigate the antibacterial and antifungal properties of E. proceri ethanolic extracts to Staphylococcus aureus, Escherichia coli and Candida albicans, and to determine the bioactive compounds causing these activities by FTIR of the active test compounds.*

Methods: *Aerial portions of E proceri were sampled mechanically one a month in January to March 2025 in District Harnail, Baluchistan. Solvent extraction was used to extract ethanolic extracts, after which rotary evaporator was used to concentrate the extracts. Antimicrobial activity was determined by disc diffusion method in Brain Heart Infusion Agar in case of bacteria and Saburou dextrose agar in case of fungi. Functional groups in the extracts were determined by FTIR spectroscopy.*

Results: *The ethanolic extract showed strong antibacterial activity against S. aureus (19.2 mm inhibition zone), E. coli (18 mm) and moderate activity against C. albicans (16 mm), and weaker activity against none. FTIR analysis revealed phenolics (O-H stretching at 3245.71 cm^{-1}), flavonoids (C=C stretching at 1606.31 cm^{-1}), and aliphatic compounds (C-H stretching at 2973.43 cm^{-1}), likely contributing to antimicrobial effects.*

Conclusion: *The ephedra derivative (Ephedra procera) had significant antimicrobial activity particularly with Staphylococcus aureus and E. coli. FTIR reinforced active compounds, justifying its traditional use and its potential as a natural source of antimicrobial that is worth studying.*

INTRODUCTION

There have been thousand years of exclusive usage of plants as source of remedies in the management of the diseases of the human being as well as that of animals (1). At the moment, medicinal plants (MPs) continue to remain the primary health care in the developing world (2). The World Health Organization (WHO) estimates that 80 percent of the world population especially in the developing world tends to use traditional medicines and especially the medicinal plants to meet their healthcare demands (3). In the western drug, however, approximately half of the latter merely have the plant bioactive compound or analog in the active components (4). Several infections (including infections of the skin, lungs, and sinus) may develop on contact with high levels of fungal spores especially in the immunocompromised (5). The creation of new medicines and more recent approaches are powerfully necessary to fight the battle against antibiotics resistant (6). Discovery of new antimicrobial drugs against the resistant pathogens is focused on by the WHO (7).

The isolated phytochemicals have demonstrated various levels of activities against the microbial pathogen, and it is considered that there are no or reduced side effects in comparison with synthetic antimicrobials (8). Other phytochemicals are able to "downregulate" or overturn the antimicrobial resistance (9), or cause an effect of synergy with common antibiotics (10). In fact, phytochemicals can play an antimicrobial beneficial role via various ways (11).

Ephedra genus (Ephedraceae) belongs to one of the oldest known medicines to human beings (12) and comprises 69 species which are mostly found in semi-arid areas in both Palearctic and Nearctic regions, but some of the species are spread in a few Neotropical countries (13). To be specific, ephedra is one of the oldest drugs the humanity has ever known since Ancient China, at least 5 thousand years ago (14).

The Pakistan proportion of ephedra is tentatively given as nine species whereas five species of genus Ephedra are distributed in the Balochistan province namely, *Ephedra procera*, E. gerardiana, E. intermedia, E. sarcocarpa and E. ciliata. This is an ethno-botanically and commercially significant plant that is locally referred to as, Nari oman (15). The rural community uses *E. procera* plant in treatment of asthma. Since medicinal use of plants on various diseases is a norm in Pakistani rural society (16).

E. procera contains the valuable sources of antimicrobial substance because of possessing the alkaloids, flavonoids, and phenolics as antioxidant compounds (17). *E. procera* has shown moderate inhibitory effects against a wide variety of Gram positive and Gram-negative bacterial isolates (18). The antibacterial effect has been accounted to the activity on the bacterial cell walls and membrane together with the destruction of the essential bacterial enzymes because the plant ephedra has very strong concentration of the antibiotics (19). The effectiveness of chemical derived of *E. procera* to be used in treatment plan of infection caused by fungi in the skin. Topical formulation of the extracts had been found to reduce Candida species infections and thus can viably be used as dermatological product (20).

It was demonstrated that *E. procera* extracts have antifungal properties particularly against certain pathogenic fungi. Among the best known pathogenic species of fungi there are those *Candida Albicans*, Aspergillus Niger, and Cryptococcus Neoformans (21). In a comparison of the influence of the use of *E. procera* revealed that the extract has enormous antifungal properties especially against *Candida albicans* and Candida glabrata. They were associated to phenolic compounds, which are credited with suppressing the germination of the fungal spores as well as growth of mycelium. (22).

According to a research by Kaushik (2021), secondary metabolites that are inclusive of phytochemicals are widely employed drugs with the use of severe diseases such as respiratory tract infections, urinary, skin, and gastrointestinal infections (23). Gonzalez-Juarez et al. (2020) note that Ephedra species have been traditionally use in different cultures to treat respiratory disorders, fever, and as an energy booster suggesting a long history of usage as a medication (24). There is a recent study which has indicated that *Ephedra procera* has great bioactive compounds, which contributes to its antimicrobial and antioxidant activity (25). Chroho et al. (2024) have indicated that *Ephedra procera*, are applied to breathing difficulties, to alleviate the signs and symptoms of bronchial asthma, cold, and influenza (26). Tang et al. (2023) state that

certain species of Ephedra are consumed to relieve pain and to treat low blood pressure (27).

2. Research Methodology

2.1. Study Design/Study Area

This paper developed as an experimental research project aimed to determine the capacity of *Ephedra procera* plant in fighting the bacteria and fungi. The District Harnai of Balochistan was used as study area because weather and climate of this area was good and adequate to the growth of different medicinal plants including *Ephedra procera*. The plant sample was gathered between January 2025 and March 2025 at random places.

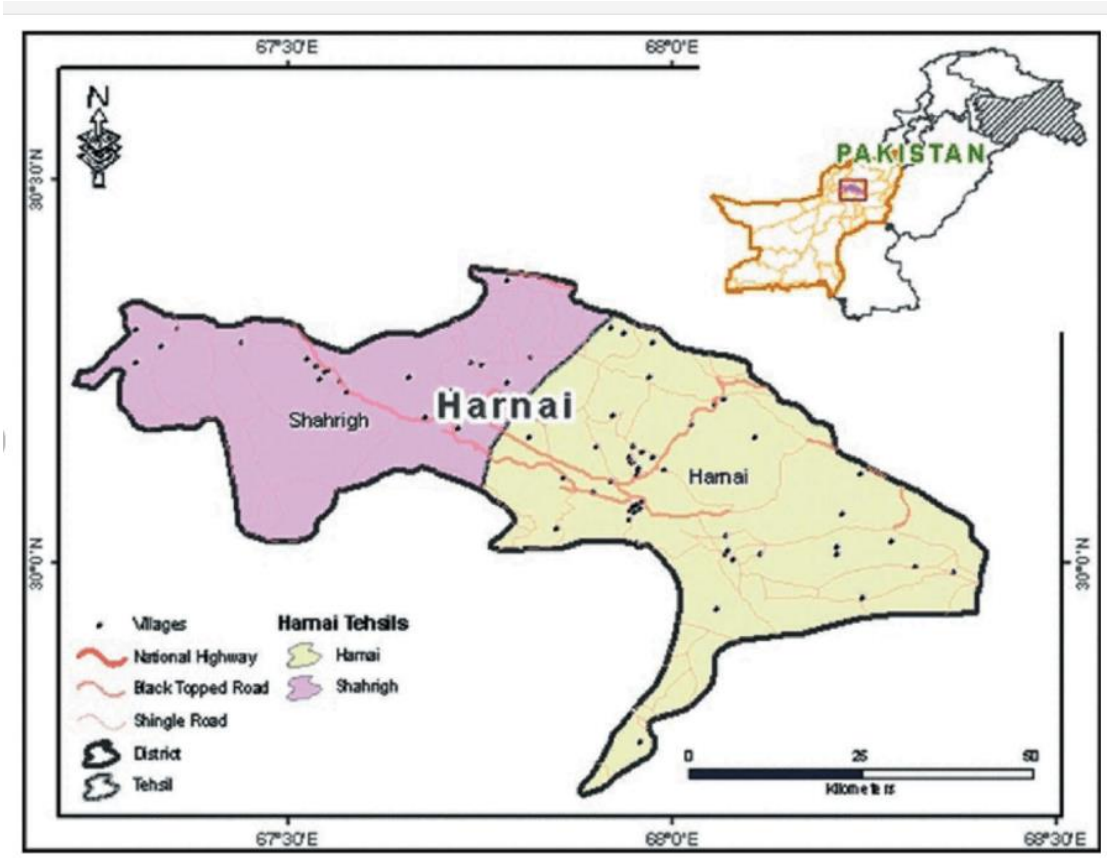

Figure 1: Map of study area District Harnai, Balochistan.

2.2. Sample Collection

Aerial parts of *E procera* have been gathered randomly in January to March 2025 at various regions in District Harnai. A taxonomist was a person hired to determine the species of the plant. Dust, debris and contaminants were removed by washing plant material by tap water and distilled water. The storage of the material took place at the Central Biochemistry Laboratory of University of Balochistan.

2.3. Drying of Samples

Samples of plants were dried at room temperature under even directed airflow until they were air-dried (in a room) up to three weeks so that degrading thermolabile compounds would be minimized. The samples were rotated every now and then so as to ensure they dry uniformly. The samples (dried) were then stored in a place that was free of moisture and they were used later.

2.4. Grinding of Plant Samples

The plant materials were then dried thoroughly afterward ground in powder using mechanical mills, best known as grinders. This was done to obtain a uniform grain sized powder which maximized the

amount of useful compounds extracted by using the solvent extraction process.

2.5. Mixing with Ethanol

In order to isolate the bioactive compounds, present in the plant materials, ground plant material was immersed in ethanol because ethanol is one of the popular solvents used in the extraction of both polar and non-polar compounds. It was extracted at a ratio of 1: 5 (500 grams of ground *E. procera* to 2500 ml of ethanol) and it was allowed to stand unfixed 72 hours with periodic shaking to increase the extraction.

2.6. Filtration of Ground Samples

Once the extraction period was over the plant residue and ethanol containing plant extract mixture was filtered using Whatman filters to isolate the plant extract that was now in a liquid form. This procedure ensured that compounds that were not soluble in ethanol were not allowed to go through the filter. Further concentration of the filtrate was carried out.

2.7. Separation of Crude Extract

Concentration of the ethanol extract with the rotary evaporator was done under 40 o C temperature. This was done to extract the ethanol and what remained was a crude form of plant extract that had a lot of bioactive phytochemicals. The concentrated ex-tract was quantified, placed in air-tight containers and refrigeration temperatures were used to preserve it until a later use.

2.8. Analysis of Bioactive Compounds by FTIR

The Fourier-transform infrared spectroscopy (FTIR) was used to identify the bioactive compounds of the crude extract. The methodology assisted in identification of functional groups in the compounds and their potential antimicrobial activity. Spectra received were compared with known standards in order to identify the type of com-pounds found in the extract.

2.9. Preparation of bacterial and fungal strains (Agar Plates)

Brain Heart Infusion Agar (BHI) and Sabouraud Dextrose Agar (SDA) were made by suspending the required quantities in distilled water after which they were auto-claved at 121oc in a 20 minutes setting. The media were then poured into sterile petri dishes and cooled at 45 50 C till they solidified. Individual bacterial and fungal strains were aseptic inoculated on BHI and SDA (_Lactose fermenting and SDA Strains_) using a clean loop. The plates were labeled, treated at proper temperatures of incubation and kept upside down.

2.10. Application of *Ephedra procera* Plant Extract on Bacterial and Fungal Agar Plates

In case of antimicrobial assay, *Ephedra procera* plant extract was extracted using ethanol, and subsequently, it was filter-sterilized. Bacteria species were grown on Brain Heart Infusion Agar (BHI) and fungi on Sabouraud Dextrose Agar (SDA). Filter discs of sterile filter paper impregnated using 50 muL per discs of the extract were added on the in-occulated plates. The plates were incubated and zones of inhibition recorded. The approach makes it easy to have uniform application and reproducibility.

Results

3.1 Antimicrobial Activity

In the disc diffusion technique, the Ethanolic extract of *Ephedra procera* showed antimicrobial activity against three microorganisms tested alongside. A high degree of antibacterial effect was observed against *Staphylococcus aureus* and *Escherichia coli*, with an average inhibition zone of 19.2 mm and 18 mm with their ranges such as, (range 17.7–20.7 mm), (16.5–19.5 mm) over two and four replicates respectively.

The extract exhibited moderate inhibitory effect against *Candida albicans*, with an average inhibition zone of 16 mm (14.5–17.5 mm) across eight replicates. These results indicate differential susceptibility of the tested pathogens to the *E. procera* ethanolic extract.

Table 1: Antimicrobial Activity of *Ephedra procera* Ethanolic Extract

Microorganism	Inhibition Zone (mm)	Range (mm)	Number of Replicates
Staphylococcus aureus	19.2 mm	17.7–20.7 mm	2
Escherichia coli	18 mm	16.5–19.5 mm	4
Candida albicans	16 mm	14.5–17.5 mm	8

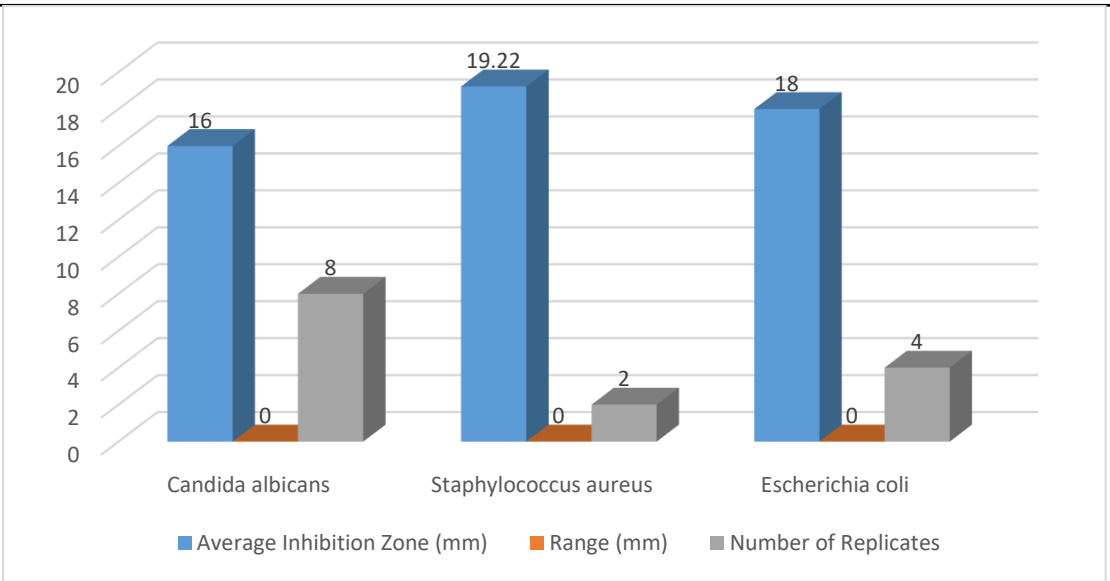

Figure 2: Antimicrobial Activity of *Ephedra procera* Ethanolic Extract

The Table 1 and figure 2 show the *Ephedra procera* ethanolic extract had an anti-microbial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, with varying results. With the inhibition areas *Staphylococcus aureus* showed the strongest antimicrobial response (19.2 mm), followed by *Escherichia coli* (18 mm), and *Candida albicans* (16 mm). Therefore, the inhibition against *S. aureus* was the highest and to that difference, *E. coli* moderately and the last pathogen tested *C. albicans* the least.

The inhibition zone range was widest for *Staphylococcus aureus* and *E. coli* such as, (17.7–20.7 mm), (16.5–19.5 mm) respectively. The extract had moderate potency against *C. albicans*. Thus, *S. aureus* and *E. coli* had the strongest inhibition, and *C. albicans* had moderate inhibition.

- **FTIR Analysis of Bioactive Compounds**

A fourier- transform infrared spectroscopy (FTIR) of ethanolic extract of *Ephedra procera* was used to determine the functional groups of bioactive compounds. There were various absorbance peaks in the spectrum representing an antimicrobial effect. A high absorption at 3245.71 cm -1 was ascertained to be O-H, which is typical of phenolic compounds or alcohols, which are antimicrobial agents. C-H stretching was also shown by a peak at 2973.43 cm 1. This showed that there were aliphatic chains present which were probably due to the presence of alkanes. The maximum at 1606.31 cm -1 was attributed to the C=C stretching of aromatic rings which was characteristic of flavonoid aromatic rings which reinforced the presence of phenolic structures. Further aliphaticity and oxygenated components were confirmed using additional peaks at 1444.96 cm -1 (C-H bending) and 1036.40 cm -1 (C-O stretching, probably in flavonoids ethers/alcohols). The latter is in line with the occurrence of phenolics, flavonoids, and even alkaloids expected of the *E. procera* which were likely to contribute to its antimicrobial actions against *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*.

Table 2: Summary of Antimicrobial Potency Based on Inhibition Zone Size

Inhibition Zone Size (mm)	Interpretation	Example Organism
≥ 18 mm	Strong activity	*Staphylococcus aureus*
14–17.9 mm	Moderate activity	*Escherichia coli*
< 14 mm	Weak or low activity	*Candida albicans*

The Table 2 shows the antimicrobial strength of *Ephedra procera* ethanolic extract by using the zone of inhibition. An inhibition zone of 18 or greater inhibition zone means that they are strongly active antimicrobially as in the case with *Staphylococcus aureus* and by *Escherichia coli*. Interzones between 1214 and 17.9 mm reflect moderate activity, exemplified by *Candida albicans*. Inhibition zones smaller than 14 mm suggest weak or low activity, as observed with none. This classification helps assess the extract's effectiveness against different microorganisms by comparing zone diameters formed during testing.

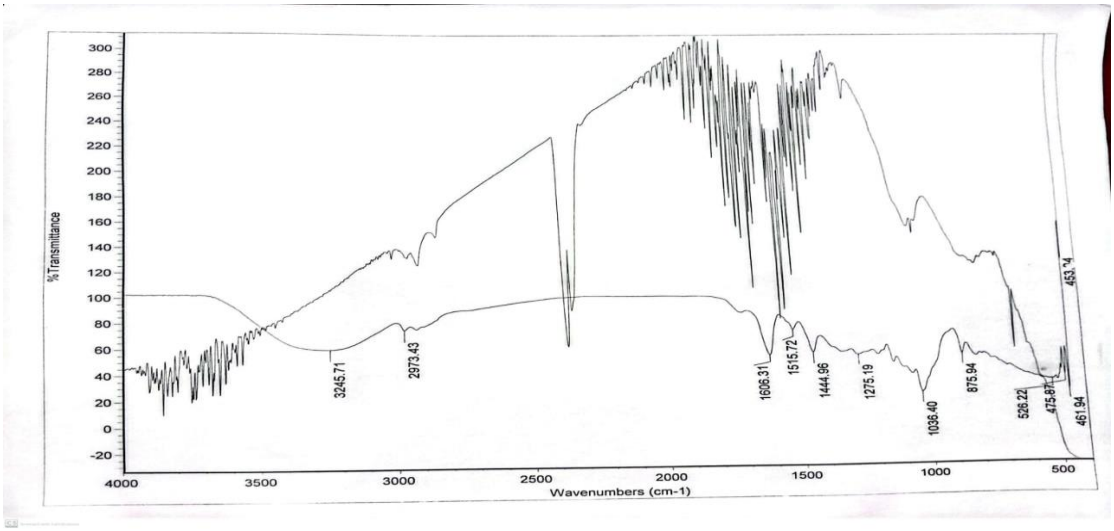

Figure 3: FTIR Spectrum of *Ephedra procera* Extract

Figure 3 shows the maxima to be 3245.71, 2973.43, 1606.31 cm -1 corresponding to O-H, C-H, C=O stretching, which shows phenolic, flavonoid, and alkaloid compounds.

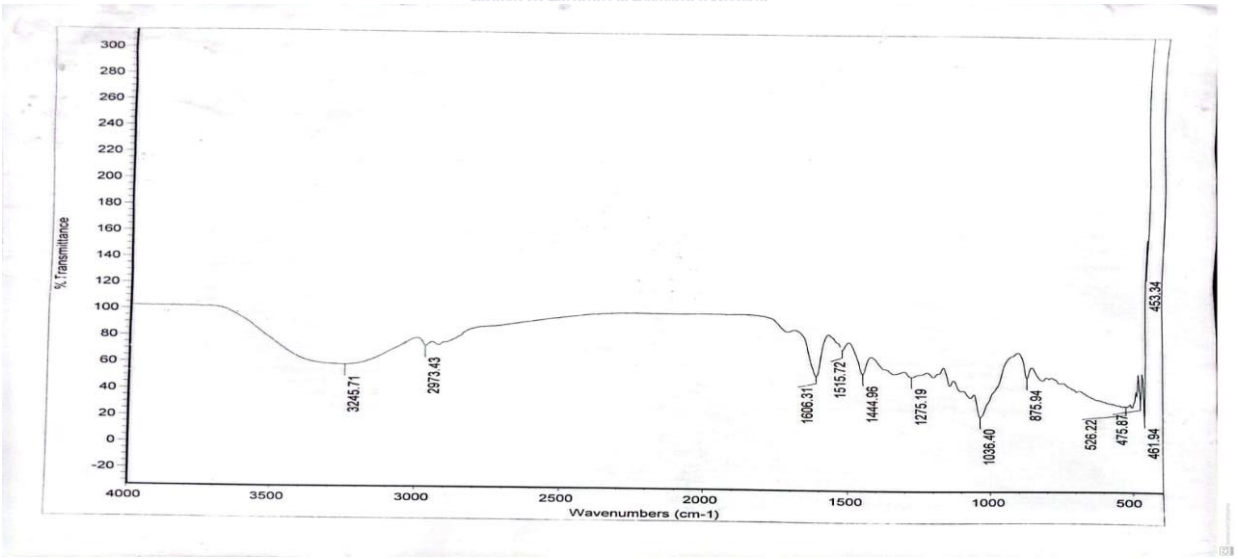

Figure 4: FTIR Spectrum of *Ephedra procera* Extract

Figure 4 indicates that 3241, 2927, and 1606 cm -1 show the presence of peaks which indicate the presence of bioactive compounds associated with antimicrobial activity.

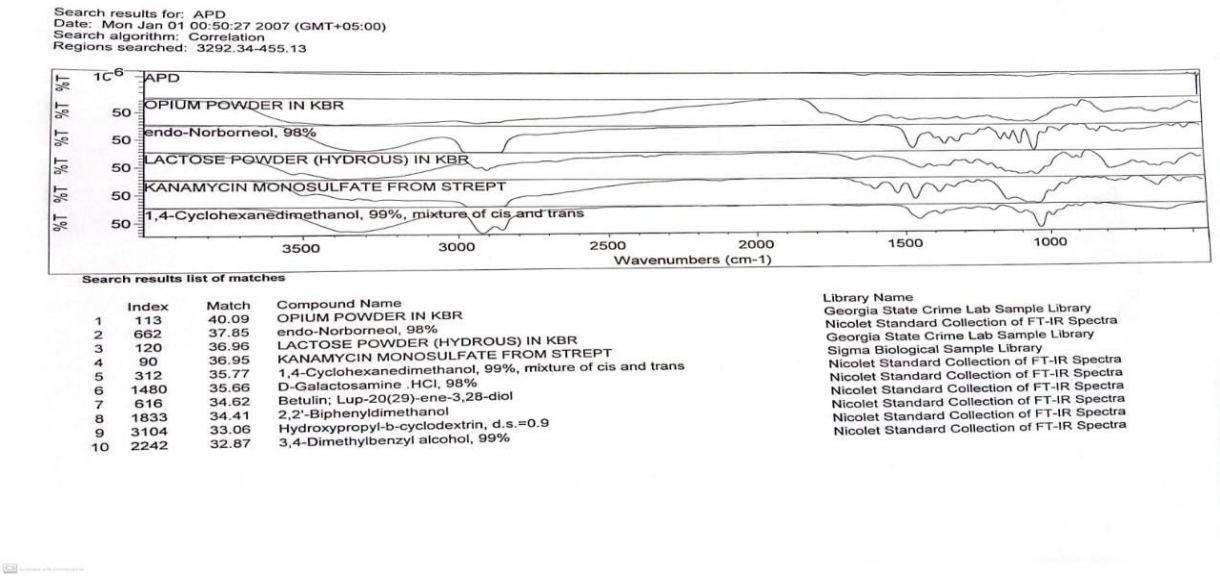

Figure 5: FTIR Spectrum Comparison of *Ephedra procera* Extract with Reference Compounds

A significant phytochemical profile is highlighted in the figure 5, which demonstrates the moderate matches of the opium powder and lactose.

Discussion

The results of this research showed that ethanolic extract of *Ephedra procera* indicated a different variable antimicrobial activity against the microorganisms that were used in the conducted experiment. Good action was noted on *Staphylococcus aureus* with an average inhibition zone of 19.2 mm and *Escherichia coli*, which showed an inhibition zone of 18 mm. In contrast Moderate activity was recorded against *Candida albicans*, with an inhibition zone of 16 mm. Such variations in the antimicrobial strength can be possibly attributed to the differences in the structures of the organisms. Gram-negative bacteria such as *E. coli* have an external layer of lipopolysaccharides and this membrane is less permeable to phytochemicals, however fungus cells such as the *C. albicans* can have different sensitivities because of the differing cell wall composition. The theory that phytochemicals contained in the Ephedra species can fluoridate microbial membranes, notably fungal ones, and bolster its antimicrobial potential, is confirmed by the previous studies (Anand & Sharma, 2022) (28).

E. procera has antimicrobial activities suggesting further use by ethnobotanical studies in Balochistan where it is called "Nari-oman" and used to treat asthma and respiratory diseases. The high level of activity against *C. albicans* demonstrates that *E. procera* has potential against the opportunistic infections especially among the immunocompromised individuals. The (Rahman & Ali 2021) report that traditional applications of medicinal plants in cases of respiratory disease have already been recorded in the literature of multiple cultures showing their anti-microbial and anti-inflammatory properties (29).

According to Ghorbanpour et al. (2018), the defense mechanism of phenolic metabolites which are present in Ephedra species consists in the activation of phytoalexins (which are antimicrobial compounds produced under the attack of a pathogen), instead of the reactive oxygen species (ROS). Such phytoalexins stop the growth and propagation of various pathogens hence increasing resistance of the plant to infections. The scientific basis explaining the antimicrobial activities in Ephedra extracts is the biochemical activity of these phenolic compounds. This process will underline the significance of phenolics not only as antioxidants but also as the major contributors to the defense responses against microbial invasion, which justifies their potential in the development of natural antimicrobial agents (30).

Singh et al. (2022) indicated that Ephedra extracts containing alkaloids such as ephedrine, alternatively contribute significantly to the plant's antimicrobial

activities. Investigations using related species like Ephedra foliata have established that these alkaloids also play a role in the suppression of growth of bacteria and fungi. This explains thickly alkaloid extracts are used to prepare efficient natural antimicrobial agents and provide arguments against application of Ephedra chemicals to fight infectious diseases (31).

Hemaiswarya et al. (2021) presented combining plant extracts with traditional antibiotics enhanced antimicrobial activity, representing a potential means of overcoming drug resistance. This type of synergy would particularly hold merit when utilized for Ephedra phytochemicals, which have myriad bioactives. Investigating these combinations potentially leads to new therapies that enhance treatment and diminish resistant microbial development (32).

Conclusion

The study articulates that the *Ephedra procera* ethanolic extract is the best antimicrobial agent with very strong inhibition against *Staphylococcus aureus* and *Escherichia coli*, moderate activity for *Candida albicans*. FTIR analysis represents the existence of phenolics, flavonoids, and alkaloids which are probably the main cause of its antimicrobial characteristics. These results are the confirmation of *E. procera* traditional medicine in Balochistan and at the same time they indicate the plant's potential as a source from which to develop natural antimicrobial agents. The scientists suggest further detail research for the isolation of particular compounds and their testing in a clinical setting against resistant microbial strains for their application.

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