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Research Article

**PHYTOCHEMICAL INVESTIGATION AND BIOLOGICAL  
ACTIVITIES OF FIVE MEDICINAL PLANTS OF  
BALOCHISTAN****Saima Maher\*<sup>1</sup>, Zarbakht Dar<sup>1</sup>, Suad Naheed<sup>2</sup>, Shazia Iqbal<sup>1</sup>, Noureen Khan<sup>1</sup>, Farida Behlil,<sup>1</sup> Nadra Naheed,<sup>3</sup> Najma Sabzal<sup>1</sup>, Mah Gunj Bakhshi<sup>1</sup>.**<sup>1</sup>Chemistry Department, Sardar Bahadur Khan Women University, Quetta, Pakistan.,<sup>2</sup>Department of Biotechnology, Jinnah University for Women Karachi-Pakistan., <sup>3</sup>H.E.J. Research Institute of Chemical Science, University of Karachi-Pakistan.**Abstract :**

*The studies show the medicinal plants with emphasis on their antimicrobial, antioxidant properties of Foeniculum vulgare, Cuminum cyminum, Pegnum harmala, Rosmarinus officinalis and Chenopodium album. Agar-well diffusion method was used for antimicrobial and antioxidant activity which was performed by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The extracts of C. cyminum, C. album and P. harmala showed promising inhibitory effects against Pseudomonas aeruginosa with zones of inhibitions 27 mm, 22 mm and 17 mm respectively. C. cyminum and P. harmala exhibited highest antibacterial potential against Klebsella pneumonia. Whereas, among the crude methanolic extracts of the tested plants only F. vulgare, and P. harmala were also used for antifungal analyzation. Both plants showed the potential effects against the selected fungus (Aspergillus Niger) with inhibition zones of 18 mm and 13 mm respectively. On the other hand, all the selected plant showed significance antioxidant potential up to 98 % RSA. The study showed that a selected medicinal plant possesses potential against microbes and therapeutic significance.*

**Keywords:** *Foeniculum vulgare, Cuminum cyminum, Pegnum harmala, Antibacterial activity, Antioxidant activity.***Corresponding author:****Dr. Saima Maher,**

Department of Chemistry,  
Sardar Bahadur Khan Women University,  
Quetta Balochistan, Pakistan  
Email: [saimamaher@yahoo.com](mailto:saimamaher@yahoo.com)

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## INTRODUCTION:

The history of medicinal plants encourages its implementation in countless herbal formulations. Therefore, allow their use in different drugs discovery [1, 2]. Pakistan is included third world country and facing many health concern issues. The people of developing countries totally dependent on essential classification of suppository with the deep interest in medicinal plants therapies related to their health concerns [3, 4]. Herbal therapies have been showed a fruitful result with no side effect and potential effect against many diseases [1].

Traditional Remedy is used worldwide and is swiftly growing in economic significance. In third world developing countries, traditional medicine is usually the accessible and affordable treatment. According to the WHO, reports that traditional medicine is the main health care system for important percentage of the population in developing countries. About 71% of the population has used traditional medicine. Like, In various Asian countries Traditional Medicine is extensively used. While in Japan about 60 – 70% doctors recommend traditional medicines for their patients [5].

However, the effective challenge intended for manipulation and handling of expected possessions of ancient territories. Asian kingdom consumes the nutritious plants for the treatment of dreadful and hazardous diseases [6].

Infectious diseases signify vital source of morbidity and mortality among the overall population, mostly in developing countries. Consequently, pharmaceutical companies have been interested to discovery new antimicrobial drug. The microorganism species has a tendency genetically to acquire and transmit resistance against antibacterial subsequently [7, 8]. There are numerous reports on the isolation of microbes that are recognized to be sensitive, usually used in drugs and developed resistant to other medicines available in the market. So, the new strategies has adopted by pharmaceutical companies to produce new antimicrobial drugs. Which include changing the molecular structure of the existing medicines with more efficient and have ability to bacterial resistance mechanisms [9].

The new research has been conducted to examine the new antimicrobials isolated from plant. A large number of plants have been used from all over the world for hundreds of years by different populations. The medicinal plants examination and inquires desires to cure disease [11]. As defined in the earlier report, it has also resulted in the generation of

multiple-drug-resistant (MDR) microorganisms that are threatening to become untreatable. The success and low cost of broad-spectrum antibiotics have reduced the incentive to develop alternative antimicrobial strategies, such as enhancing host responses during infectious disease. Which reduce the complications and agree the direction of uncritical usage of powerful medicines that fight bacterial infections as a result. However, the situations are amazing in the countries to counter these harmful diseases [12].

In the management and treatment of transmittable diseases or complaints like, cancerous and Human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) patient. For the last 70 years, the effort to prevent or treat infectious diseases has depended on severely on targeting microorganisms themselves with antimicrobial drugs. That approach has been very successful, especially against bacterial infections [13].

The current research was conducted to find the presence of phytochemical constituents in selected medicinal plants of Balochistan. It encloses the effect and capacity of antibacterial, antifungal and antioxidant activities. However, the Spectroscopic techniques (UV/VIS and FT-IR) were used to identify the secondary metabolites.

## MATERIALS AND METHODS:

### Collection and Preparation of Plant Extracts

Fresh leaves of *R. officinalis*, *P. harmala*, & *C. album* and seeds of *F. vulgare* & *C. cyminum* were collected from Garrison Academy Quetta Cantt, SBKWU, Hazar Ganji Chiltan National Park and Ziarat valley respectively. All the five plant samples were identified by the proficient plant taxonomist Dr. Rasool Baksh Tareen University of Balochistan. The plants were washed with distilled water and then air dried in shade. The sample was crushed into powder through manual grinding to enhance the effective contact of solvent with the plant biomass and by methanol soaking for 17 days. The liquid part is collected to get extracts (CME) of the five plants.

### Preliminary phytochemical considerations

The main chemical in medicinal plants constituents is, the secondary metabolic compounds. The chemical structures and functional properties allow the appropriate organization of photochemical [14]. The chemical tests were performed on the crude

methanolic extracts to identify the bioactive constituents of plants by using standard procedures as described by Safowora (1993) [15] and Harborne (1973) [16].

### Microbial Examine

Ten Pathogenic (Gram positive & Gram negative) bacteria strains such as; *Pseudomonas aeruginosa*, *Klebsella pneumonia*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* along with a fungal strain *Aspergillus niger*. The identified and purified microbial isolates were obtained from the Agha Khan Labs Karachi. Then deriving a loop full of bacteria from stock culture was spread on sterile agar plate and incubated. The aqueous diphenic acid (DPA) was consumed to formulate fungal cultures. The inoculum was taken from 8 day's old and the fungal slant flowed by the totaling of 10 mL sterilized 0.05% (w/v) monoxal. From this fresh spore suspension, 1 mL was cultured in about 10 mL of cultivation medium. There occurs the transference to sterile nutrient broth medium which was incubated at 37°C for 24h. Spores were enumerated by hemocytometer. Crude extract loaded on the disk by using sterilized forceps and the loaded disc on the cultured media. Amoxicillin disc serves as positive control.

Observations of antibacterial and antifungal effects of CMEs against the stated stains were carried out after 18-24 hours on bacterial growth and 3 days for fungal growth.

### Antibacterial activity

CME of the concerned plants was be processed by agar well diffusion methodology for recognition of antibacterial activity [17]. All microbes are being through the progression of immunization, suggest that Mueller Hinton broth (PH 7.1) for approximately 12hours. The operation of a spectrophotometer allow concentration of the suspensions was adjusted to 0.5 (optical density). Isolates will be seeded on Mueller Hinton agar plates by benefiting with the sterilized cotton swabs.

Agar surface bored and sterilized gel borer intended to form wells (6 mm diameter). 100 µL of the test extract and 100 µL of sterilized distilled water serve as negative control. The contents poured into separate wells. The standard or positive regulator was attended as Amoxcilin (antibiotics). The standard antibiotic disc afterwards placed on the agar surface as positive management. The approximate incubation period of plates nearly at 37°C for 48 hours. Triplicate plates allow access to every single mortal.

The expansion inhibition zones related to CME of curative plants were measured or evaluated in millimeters (mm).

### Antifungal activity

The CME of desired plants were tested against fungal strains comprises of *Candida albicans* & *Aspergillus niger* were followed by measurement of the inhibition zone which follow the exact methodology as implemented in the antibacterial activity.

### Antioxidant activity using DPPH Method

Antioxidant activity of the CMEs were done by method explained by McCune & Johns [18] with few modifications. In this method various concentration of plant extracts (1mL) was treated with 2 mL solution of 0.004% 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol. The solution was incubated for 1 hour in dark at room temperature. After one-hour absorbance was measured at 517 nm using UV/VIS spectrophotometer. Control was made by taking equal volume of DPPH and methanol. Ascorbic acid was used positive control. Methanol was used as blank in this method. The formula implemented for the calculation of percentage scavenging activity:

$$\text{Percentage scavenging} = (A_c - A_e / A_c) \times 100$$

Where;  $A_c$  is the absorbance of control and  $A_e$  is the absorbance of plant extracts

## RESULTS AND DISCUSSION:

The phytochemical analysis of the selected medicinal plants are shown in the table 1. Terpenoid were present all the selected plant samples. Flavonoids were found to be present in selected plant samples except in *C. cyminum* and *R. officinalis*. Saponins were absent only in *P. harmala* plant. Tannins was absent in *C. ablum*. Steroid was present in *C. cyminum*, *P. harmala* and *C. ablum* but absent in *R. officinalis* and *F. vulgare*. Phenolic compounds were present in only in *C. cyminum* and *P. harmala*. Alkaloids were found to be present only in *C. ablum* and *R. officinalis*. Glycosides were present in all selected plant samples except in *F. vulgare*. Proteins were found to be present in all selected plants except *C. cyminum*. Carbohydrates were present in *F. vulgare*, *P. harmala* and *R. officinalis* but were absent in *C. cyminum* and *C. ablum*. Phytochemical analysis resulted that the all selected plant samples contain majority of phytochemical constituents and could play significant role in the field of medicinal and physiological [19].

Table 1. Phytochemical assessment of constituents of five Plant extracts (CME)\*

Phytochemical	<i>C. ayminum</i>	<i>F. vulgare</i>	<i>P. harmala</i>	<i>C. ablum</i>	<i>R. officinalis</i>
<b>Analysis</b>					
Terpenoid	++++	++++	++	+++	++++
Flavonoid	-	++++	++++	++++	-
Saponin	+++	+++	-	++++	++++
Tannins	++++	+	+++	-	+++
Steroid	+++	-	++++	+++	-
Phenolic	++++	-	++++	-	-
Alkaloid	-	-	-	++++	+++
Glycoside	++++	-	+++	+++	+++
Protein	-	++++	+	++++	+
Carbohydrates	-	++++	++++	-	+++

\* - =Not present, + = slightly present, ++ = moderate present, +++ = Strong present, ++++ = Very strong present

The application against gram positive and gram-negative bacteria being made on using crude methanolic extracts by means of agar well diffusion method. The extracts of the said plants exhibited different antimicrobial potential, owing to different composition of secondary metabolites. The result of the antibacterial activity against bacteria serves to be auspicious. The extracts of *C. cyminum*, *C. album* and *P. harmala* showed promising inhibitory effects against *P. aeruginosa* with zones of inhibitions 27mm, 22mm and 17mm respectively. *F. vulgare*, and *P. harmala* exert antifungal effects with

inhibition zones of 18mm and 13mm respectively. Whereas the CME of *C. Ablum* showed good antibacterial activity against *Proteus mirabilis* with zone of inhibition of 17mm. Against *E. coli* the *C. Cyminum* and *P. Harmala* showed significant inhibition with 15 and 14mm zones as shown in table-2. On the other hand, among the crude methanolic extracts of the tested plants only *Foeniculum vulgare*, and *P. harmala* exert antifungal effects with inhibition zones of 18mm and 13mm (Table-3) respectively.

**Table 2:** Anti-bacterial activities of crude methanolic plant extracts (CME)

Samples	Inhibition zone (mm)*			
	<i>E. coli</i>	<i>P. mirabilis</i>	<i>K. pneumonia</i>	<i>P. aeruginosa</i>
<i>C. cyminum</i>	15	00	26	25
<i>F. vulgare</i>	11.6	07	10.33	00
<i>C. cblum</i>	10	17	11	22
<i>P. carmala</i>	14	00	18	17
<i>R. officinalis</i>	00	00	14	NA

**Table 3:** Anti-fungal activities of crude methanolic plant extract (CME).

Samples	<i>Aspergillus Niger</i> (mm)
<i>C. cyminum</i>	00
<i>F. vulgare</i>	18
<i>P. harmala</i>	13
<i>C. ablum</i>	00
<i>R. officinalis</i>	00

Furthermore, all the plant showed excellent antioxidant activity. The absorbance value of DPPH decreased with the accepting of hydrogen atom resulting in the change of colour from purple to

yellow. This method is so sensitive that it can detect active compound at even low concentration and in a short period of time can detect many samples. The DPPH of plant extracts and standard ascorbic acid were calculated (Figure 1) [20].

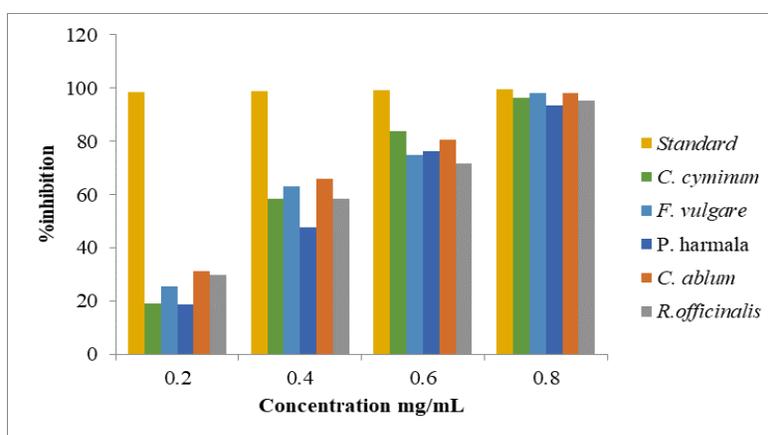


Figure: 1 The antioxidant activity of the five plant extracts

**CONCLUSION:**

This research and investigation demonstrated that the medicinal plants are the rich source of diverse classes of natural products exhibiting a range of

pharmacological activities. Due the variation of phytochemical composition, different plant showing different effected against the same strains of microbes. The results showed that the plants are

active against various strains of bacteria and the fungus and showed excellent antioxidant potential as well. This is an evidence of the medicinal value of the tested plant collected from Balochistan, Pakistan.

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