Antibacterial Activities of *Peganumharmala* (Harmal) Seeds Against Pathogenic *E. Coli* strain

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ABSTRACT

In this study two experiments were carried out to investigate whether extracts and fractions of *Peganumharmala*(P.H) have antibacterial activity against pathogenic *E.coli*. Furthermore, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined. The results of this experiment showed that *E.coli* is resistant to gentamycin and the ethanolic extract of P.H. In **experiment II**, the ethanolic extract of P.H seeds was further fractionated with n-hexane, chloroform, ethyl acetate, n-butanol and water. The results of this experiment showed that *E.coli* is highly sensitive *(P<0.001)* to fractions of n-butanol(MIC=50mg/ml; MBC=50mg/ml) and chloroform (MIC= 100 mg/ml; MBC = 100mg/ ml). Furthermore, the sensitivity of *E.coli* increased with increment of concentrations of these fractions. The remaining fractions showed no antibacterial activity against *E.coli*. In conclusion, the n-butanol and chloroform fractions of P.H seeds showed good antibacterial activity against pathogenic *E. coli* even at low concentrations.

Keywords- Antibacterial activities, *Peganumharmala,* seeds, fractions.

1. INTRODUCTION

The problem of microbial resistance to antibiotic is growing and the future of using antimicrobial drugs against pathogenic microbes is ambiguous [1]. *E. coli* is a cause of many gastrointestinal disorders in Arab world and many herbal plants are used to cure these disorders [2]. However, the mode of action of these herbal plants is unknown. Whether these herbal plants have antimicrobial or functional activities remain to be investigated. *E. coli* resists to many of the common antibacterial agents [3]; a condition that necessitates development of new, save and cheap antibacterial agents.

Peganumharmala, commonly called Esf and, Wild rue, Syrian rue, African rue, Harmel, or Asp and is a plant of the family Zygophyllaceae. P.H is widely used as a traditional medicine to cure the diseases of the respiratory and digestive systems[4].The increased and in-judicial use of antibiotics for treatment of human and animals created many multidrug resistant microorganisms[1,5,6]. Herbal plants; found in various parts of the world; are endowments of nature to cure many diseases affecting mankind [7]. Although there are many formulated medicines; herbal plants remain the best reservoir for discovering new medicines. Recently, there is an increasing interest to use herbal plants as antimicrobial agents particularly in Arab world and developing countries[8]. Scientists from divergent fields are investigating plants for their antimicrobial activities [9,10,11,12, 13,14].Although P. His widely used as medicine in the Arab world there is sparse information about its antimicrobial activity [15,16,17].Therefore, the objectives of this study are to investigate the antibacterial activity of extracts and fractions of P.H. against pathogen *E.coli* strain.

2. MATERIALS AND METHODS

2.1. Collection and identification of plant materials

The plant *Peganumharmala* seeds" AlHarmal*"* was purchased from the local market of Sudan. The plant was authenticated and identified by specialist from department of medical and aromatic plants, National Institute of Research, Sudan.

2.2. Extraction and fractionation procedures

The P. H*seeds* sample was taken and grinded in a mortar with pestle under aseptic condition.100 grams of the grinded substances were extracted by soaking in 500 ml of 80% ethanol for about seventy two hours, then filtered and evaporated aseptically. The evaporation process was done under reduced pressure using rotor vapor (model IKA RV 10 basic). The yield percentage was calculated according to the following equation:

$$\text{The yield \%}=\frac{\text{Extracted weight}}{\text{Sample weight}}\times 100.$$

Each 100 grams of grinded P.H. seeds gave 18.5 grams extract. Thereafter 200 grams of the evaporated ethanolic extract of P.H were fractionated with n-hexane, chloroform, ethyl acetate, n-butanol and distilled water respectively according to the standard fractionation method described elsewhere[18,19]. Each 200 gm of evaporated ethanolic extract of P.H. seeds yielded 8.965 gm n-hexane fraction, 12.963gm chloroform fraction, 0.308gm ethyl acetate fraction, 13gm n-butanolfractionand160 gm water fraction.

2.3. Microorganism Preparation

Local clinical strain of pathogenic *E.coli* was obtained from the microbiology laboratory of King Khalid Hospital, Najran region, Saudi Arabia. The gram negative organisms were further identified by microbact™ to ensure their purity. The isolates were maintained on agar slant at 4C° and sub-cultured on afresh appropriate agar plates for 24hrs prior to any antimicrobial test.

2.4. Antibacterial assay

The antibacterial assay was carried out according to the standard method of[20,21]. Discs of 6 mm diameter were made from the sterile Whatman filter paper no. 1. The discs were placed in multi well sterile plates (96 well,). From the ethanolic extract and the different fractions (n-hexane, chloroform, ethyl acetate, n-butanol and water) 300µg/ml, 400µg/ml, 500µg/ml and 600µg/ml were dissolved in absolute ethanol and transferred to the multi well plates where in the discs are. Thereafter the discs were allowed to evaporate and stored. Muller Hinton agar plates were prepared according to the manufacturer (MHA, Difco). The bacterial inoculums were prepared in Muller's Hinton broth in test tubes and adjusted to 0.5 Mc according to Farland turbidometry[22]and were then placed in the incubator at 37°C for 4 hrs. Then sterile swabs were immersed in the broth and seeded on Muller Hinton agar plates. Thereafter, the discs were aseptically placed over the seeded plates. The plates were incubated in an upright position at 37ºC for 24 hrs and the inhibition zones were measured in mm with a ruler after 24h of growth. Two control plates were prepared with dried discs previously impregnated with gentamycin or ethanol. This experiment was repeated four times.

2.5. Minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) assay methods

The MIC and MBC were determined by the macro broth dilution assay method described elsewhere [23,24,25]. The fractions that gave different inhibition zones were used to determine their MIC and MBC. A sevenfold serial dilution of each fraction in MHB was made (50, 25, 12.5, 6.25, 3.125, 1.563 and 0.781 mg/ml). The test organism was incubated in MHB for 4hrs to obtain a concentration of 5 cfu/ml^{-1}. Thereafter 50 µl of the inoculated broth was transferred into each dilution tube and the dilution tubes were incubated at 37 °C for 24hrs. The MIC was determined visually as the least concentration of the fraction that completely inhibited the growth of the test organisms. The MBC was determined by culturing one standard loop of the tubes with no apparent growth on MHA and subsequently incubated at 37 °C for 24 hrs. Two control tubes were prepared with gentamycin and free sterile solution of Muller-Hinton Broth. This experiment was of 4 replicas. The least concentration that killed *E. coli* as express by no colonies formation on agar was considered as MBC for the fraction.

3. RESULTS

3.1. The inhibitory effects P.H.

The ethanolic extract of P. H has no effects on *E. coli.* Only chloroform and n-butanol fractions of P.H. seeds have inhibitory action on *E. coli*. (Table 1).The inhibitory effect of n-butanol fractions was significantly ($p< 0.001$) higher than that of chloroform. Furthermore, when the concentration of these fractions was increased the inhibitory effect significantly ($p< 0.05$) increased (Fig. 1).

Table1:The inhibitory effects of the different concentrations of P.H seeds fractions.

	Zone of inhibition in mm			
Fractions	300µg	400 µg	500 µg	600 µg
n. hexane	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Chloroform	8.5±0.3^{a}	9.8 ±0.3^{b}	10.0±0.0^{b}	12.5±0.3^{c,b}
Ethyl acetate	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
n. butanol	10.5±0.7^{e}	11.3 ±0.6^{f}	11.5±0.7$^{f, g}$	12.5±0.3^{g}
Water	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

$^{a-g}$ Values with different superscripts differ at $p< 0.05$.

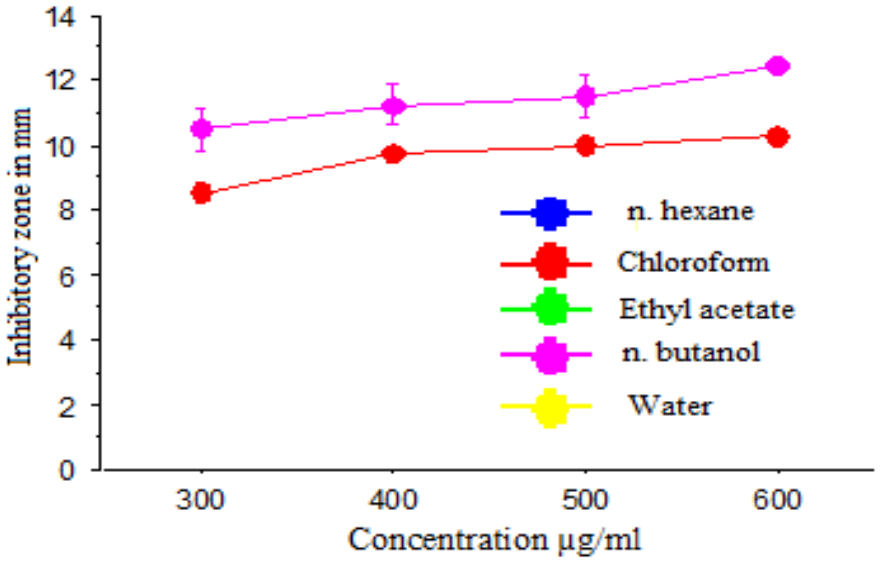

Figure 1:Inhibitory activity of *Peganumharmala* fraction (Sudan origin) against the tested local strain of pathogenic *E. coli.*

3.2. MIC & MBC of P. H. fractions

Both the MIC and MBC of chloroform fraction of P. H. on *E. coli* are≥100mg/ml. Also the MIC and MBC of n. butanol fraction of P. H. are equal (50mg/ml).

4. DISCUSSION

The results of the present study indicated that fractions of *Peganumharmala* seeds have antibacterial activity against pathogenic *E. coli* and the most active fractions were n-butanol and chloroform fractions. However, n. hexane, ethyl acetate and water fractions as well as ethanolic extract have no inhibitory effects on pathogenic *E. coli*. In this study when the ethanolic extract of P.H was tested against pathogenic *E. coli* no antimicrobial activity was observed. The P.H ethanolic extract has been reported to has potent effect on *E. coli*[26,27,28,29].This difference could be attributed to the difference in concentrations employed.

Contrary to this finding, when smoked P.H was fractionated with using gas chromatography and mass spectroscopy analysis to obtain harmine the extract was found potent against *E. coli* strain[30]. The difference between the two findings is probably due to the difference in the method of extraction and/or smoking of P.H before extraction. Also, the effects of the high concentration of the harmine fraction employed by Ahmad, *et al.* cannot be ignored. In this study the fractions were used at low conc.(0.3-0.6 mg/ml), while in the study of Ahmad, *et al. harmine* fraction was used in high conc. (0.2 - 5 mg/ml).Furthermore, in this study the inhibitory effect of n-butanol fraction was superior to that of chloroform fraction. This finding is in agreement with the result of Prashanth and John[31] who reported that the chloroform fraction inhibitory effect on pathogenic *E. coli* is inferior to that of methanolic fraction. Although Prashanth and John used different fractionation method their results supports the findings of this study. This study confirms that increasing the concentrations of n-butanol and chloroform fractions increased their inhibitory effects. This finding is in agreement with the findings of Mansour and Soudabe[28] who reported that higher concentrations induce greater inhibition. Additionally, MIC and MBC values of chloroform and n. butanol fractions of P. H. on *E. coli* were near or equal to each other. These results are in agreement with the result of Reuben, Esmaeil[14,32]who reported that the MIC and MBC values for the seeds of the P.H. against *E. coli* are the same or often near or equal to each other.

It is concluded that small amounts of n-butanol and chloroform fractions of *Peganumharmala* seeds "Al Harmal*"* have antibacterial activity *against* pathogenic *E. coli.* Furthermore, increasing the concentrations of these fractions increases their antimicrobial activity.

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