

The Biological Performance of *Crataegus songarica* Against Certain Infectious Fungal and Bacterial Diseases

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Abstract

The goal of the present study was to evaluate the antibacterial and antifungal of the crude extract/fractions of *Crataegus songarica* against six bacterial and fungal strains. The extract/fractions demonstrated significant susceptibility against tested bacteria namely *Escherchia coli*, *Bacillus subtilis* and *Shigella flexeneri* illustrated the most susceptibility were with MICs 150 µg/mL, 390 µg/mL and 220 µg/mL respectively. Meanwhile Antifungal activity was also recorded table and the crude extract and fractions showed marked activity against *Trichophyton longifusus*, *Aspergillus flavus*, *Microspoum canis* and *Fusarium solani* with MICs 220 µg/mL, 180 µg/mL, 110 µg/mL and 160 µg/mL respectively. Based on the obtained results, *C. songarica* could be considered a new natural healing agent for the treatment of various infectious diseases.

Keywords: *Crataegus songarica*; Antibacterial; Antifungal

Introduction

Numerous sources have been practiced to find out new anti-microbial compounds such as micro-organisms from animals and plants and their compounds [1]. During the course of time, biological control has gained incredible significance over synthetic antimicrobials [2]. Since long, medicinal plants continue to be extensively used as major sources of drugs for the treatment of many health disorders all over the world. Pakistan being rich in indigenous herbal resources offers a great scope for ethnobotanical studies [3-6]. Presently we require to convert this need able heritage of plant based therapies into practice, as Pakistani medicinal plants possesses tremendous therapeutic values [7-11] to overcome the increasing demand of indigenous populations in addition to earn foreign exchange from their export.

C. songarica is commonly known as Hawthorn member of genus *Crataegus* (*Rosaceae*) encompasses approximately 200 species. In Pakistan, it is common in Boni (Chitral), Swat, Astor, Gilgit and Muree [12]. Additionally, it also found in Afghanistan and Uttarr Pardesh 1500-2700 m [13]. The barriers of the plant possessed antihypertensive and cardio tonic potential. It improved cardiac activity in patients with congestive heart failure [14]. The antispasmodic activity of the plant has already been reported. It relaxed the uterus and intestine smooth vessel however, constricts the bronchi and coronary vessels [15]. The fruits are used as a popular remedy for diarrhea or sligh phlegmasia [16].

It may be used as tincture and has also got antioxidant properties [17]. In the Arab traditional medicine, leaves and unripe fruit has been formulated in the form of decoction for the treatment of cancer, diabetes and sexual weakness [18]. Phytochemically, different groups of compounds have been reported such as vitamin C, flavonoids, glycosides, anthocynaidins, saponins, tannins, antioxidants and phenolics [19,20]. Keeping in view the strong pharmacological and phytochemical backgrounds therefore the current study was aimed to investigate the antibacterial and antifungal activities.

Materials and Methods

Plant material

C. songarica as a whole plant was collected from Upper Boni

(Chitral), Khyber Pakhtonkhawa (Pakistan) during the month of October-November 2005. The plant material was authenticated by Prof Dr Abdul Rashid, plant taxonomist Botany Department University of Peshawar.

Extraction

After the preliminary necessary treatments like collection, drying under-shad for three weeks, the plant sample was cut into small pieces and pulverized in to a fine powder. The powdered of fruits of plant material (Berries 10 kg) was macerated in distilled ethanol (80% v/v) with infrequent stirring, at ambient temperature. After 2 weeks, the extract soluble in ethanol was filtered off through filter paper. The procedure was done in triplicate and the filtrate was concentrated under vacuum at low temperature (45°C) using a Buchi rotary evaporator to offered a dark brown extract. The crude ethanolic extract (40 g) was dissolved in distilled water and sequentially extracted with hexane (11% w/w), chloroform (31.9% w/w), n-butanol (38.8% w/w) and finally the water (18.2% w/w) fraction was obtained.

Microorganisms

The reference bacterial strains in the test were *E. coli* ATCC 25922, *B. subtilis* ATCC 6633, *S. flexeneri* (clinical isolate), *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 and *S. typhi* ATCC 19430. The tested fungal strains includes *T. longifusus* (clinical isolate), *C. albicans* ATCC 2091, *A. flavus* ATCC 32611, *M. canis* ATCC 11622, *F. solani* 11712 and *C. glaberata* ATCC 90030. The pathogens were maintained on agar slant at 4°C. They were activated at 37°C for 24 h on nutrient agar (NA)

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or Sabouraud glucose agar (SGA) respectively for bacteria and fungi, prior to any screening.

Antibacterial activity

The crude extract/fractions were screened for antibacterial potential against certain pathogens (*E. coli*, *B. subtilis*, *K. pneumoniae*, *S. flexneri*, *S. aureus* and *S. typhi*) by agar using well diffusion method [21]. In the procedure, nutrients broth (10 mL) was inoculated with the test organism and incubated at 37°C for 24 h. With the help of a sterile pipette, the broth culture of the test organism (0.6 mL) was introduced to a 60 mL of molten agar, which had been cooled to 45°C the reaction components were mixed and introduced into a sterile petri dish (for the 9 cm Petri dish, 0.2 mL of the culture was added to 20 mL of agar). Three plates were used for test each organism. The agar was permitted to set and become firm. In the medium, the requisite number of wells was dug by means of a sterile metallic cork borer guaranteed appropriate division of the wells in the side-lines and one in the center. Agar stoppers were detached. Stock solutions of the test samples (1 mg/mL) were prepared in the sterile dimethyl sulfoxide (DMSO) and 100 µL and 200 µL of each dilution was added in their respective wells. DMSO was used as control while Imipinem as a standard drug in the final concentration of 100 µL and 200 µL in each well was employed as standard drug. For diffusion of the samples, the plates were kept at room temperature for 120 min mid incubated face upwards at 37°C for 24 h. The activity was noted by measuring diameter of the inhibition zone (mm).

Antifungal activity

The antifungal activity of the crude extract/fractions of *C. songarica* berries were studied using agar tube dilution method [1,22]. The samples in the concentrations of 24 mg/ mL were dissolved in the sterile dimethyl sulfoxide (DMSO). 32.5 g sabouraud, 4% glucose agar and 4.0 g of agar-agar in 500 mL were mixed with distilled water for the preparation of sabouraud dextrose agar (SDA) as medium on a magnetic stirrer. The SDA (4 mL) was spread on screw cap tubes which were autoclaved (120°C for 15 min) followed by cooling to 15°C. The

uncongealed SDA media was treated with stock solution (66.6 µL) in order to get the final samples concentration of 400 µg per mL of SDA. Later on, the tubes were permitted at room temperature to congeal in the slanted position. The tubes were inoculated with a piece (4 mm diameter) of inoculums obtained from a week old culture of fungi for non-mycelial growth; using an agar surface streak. In the assay, DMSO was used as control while Amphotericin B and Miconazole were standard drugs. After one week incubation at 28 ± 1°C and humidity (40-50%), zone of inhibition was calculated.

Determination of MIC (macrodilution method)

In 96 well microplate, samples in the concentrations of 10 mg/mL were dissolved in DMSO followed by serial dilution with sterile water placed in a laminar flow cabinet. Each well was filled with an equal volume of an actively growing culture of the test pathogen. The cultures were grown for 12 h in 100% relative humidity at 37°C. Each well was added tetrazolium violet and growth was shown by a violet color of the culture. MIC was rated as the lowest concentration of the test solution that inhibited absolute growth [23]. Acetone was used as control that had no effect on the growth even at the highest concentration. Imipinem, standard drugs as shown 1 and 2 Amphotericin B and Miconazole were used as standard drugs.

Results

Effect of antibacterial assay

Antibacterial activity was carried out for the crude extract and subsequent fractions. Zone of inhibition is presented in millimeters and standard drug was Imipenem as shown in Table 1. The crude extract of plant showed potential antibacterial activity against *E. coli*, *B. subtilis* and *S. flexeneri* with MICs 310 µg/mL, 760 µg/mL and 220 µg/mL respectively. The *n*-hexane fraction was active only against *E. coli* with MIC 270 µg/mL while the chloroform fraction was active only against *B. subtilis* with MIC 490 µg/mL. The ethyl acetate fraction exhibited significant activity against *E. coli* and *B. subtilis* with MICs 150 µg/mL and 390 µg/mL, respectively (Table 2). The *n*-butanol fraction was

Name of Bacteria	Zones of inhibition of bacterial growth (in mm) by various samples						
	Std. drug	SI-1	SI-2	SI-3	SI-4	SI-5	SI-6
<i>E. coli</i>	24	14	14	-	17	-	-
<i>B. subtilis</i>	23	9	-	12	13	17	-
<i>S. flexeneri</i>	28	15	-	16	-	-	12
<i>S. aureus</i>	27	-	-	-	-	-	-
<i>P. aeruginosa</i>	20	-	-	-	-	11	9
<i>S. typhi</i>	26	-	-	-	-	-	-

Std. drug: Imipenem, SI-1=Crude extract; SI-2=*n*-hexane fraction; SI-3=Chloroform (basic) fraction; SI-4=Ethyl acetate fraction; SI-5=*n*-Butanol fraction and SI-6=Aqueous fraction.

Table 1: Antibacterial activity of crude extract and the fractions of *Crataegus songarica*. Activity is represented in zones of inhibition of bacterial growth (in mm).

Name of Bacteria	Minimum Inhibitory Concentration (MIC, µg/mL)						
	Std. drug	SI-1	SI-2	SI-3	SI-4	SI-5	SI-6
<i>E. coli</i>	0.19	310	270	-	150	-	-
<i>B. subtilis</i>	0.22	760	-	490	390	159	-
<i>S. flexeneri</i>	0.13	220	-	161	-	-	470
<i>S. aureus</i>	0.17	-	-	-	-	-	-
<i>P. aeruginosa</i>	0.31	-	-	-	-	540	-
<i>S. typhi</i>	0.17	-	-	-	-	-	-

Std. drug: Imipenem, SI-1=Crude extract; SI-2=*n*-hexane fraction; SI-3=Chloroform (basic) fraction; SI-4=Ethyl acetate fraction; SI-5=*n*-Butanol fraction and SI-6=Aqueous fraction.

Table 2: Antibacterial activity of crude extract and the fractions of *Crataegus songarica* represented in Minimum Inhibitory Concentration (MIC).

active against *P. aeruginosa* with MIC 540 and water fraction was active against *S. flexneri* with MIC 470 µg/mL.

Effect of antifungal assay

The effect of antifungal activity crude extract and subsequent fractions of the plant is illustrated in Table 3. The crude extract and subsequent fractions of *C. songarica* showed marked antifungal activity against *T. longifusus*, *A. flavus*, *M. canis* and *F. solani* with MICs ranges from 670 µg/mL to 110 µg/mL (Table 4). On the other hand, both the *Candida* species; *C. albicans* and *C. glaberata* were neither inhibited by the crude extract nor by the fractions of the plant.

Discussion

The current study revealed significant antibacterial and fungal activity of fruits (berries) of *Crataegus songarica* against various pathogenic test microorganisms.

B. subtilis was the only sensitive bacterium among the tested Gram positive bacteria. Clinical studies declared *B. subtilis* as a nonpathogenic or less pathogenic bacterium and only few cases of its infections are reported. Researchers therefore paid little importance to the study of its resistance. However, in an immunocompromised patient recurrent septicemia has been reported due to probiotic strains of *B. subtilis* [24]. The extract/fractions of the plant showed significant susceptibility against *B. subtilis* and thus it could be a significant natural healing agent infections caused by it.

The extract/fractions of the plant were more susceptible to Gram negative tested pathogens (*E. coli*, *S. flexneri* and *P. aeruginosa*). *Escherichia coli* (*E. coli*) is a very harmful human pathogen which involved in the pathogenicity of several infection namely urinary tract infections, gastroenteritis, neonatal meningitis, hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and Gram-negative pneumonia [25]. *Shigella flexneri* is a gram-negative bacterium which causes the most communicable of bacterial dysenteries, shigellosis. Shigellosis causes 1.1 million deaths and over 164 million cases each

year, with the majority of cases occurring in the children of developing nations. The pathogenesis of *S. flexneri* is based on the bacteria's ability to invade and replicate within the colonic epithelium, which results in severe inflammation and epithelial destruction [26]. *P. aeruginosa* typically the causative agent of pulmonary tract, urinary tract, burns, wounds, and of the outer ear (otitis externa), and is the most frequent colonizer of medical devices (e.g., catheters) [27]. The clinical utility of synthetic antibiotic has been reduced by resistant developed against these strains [28-30]. Therefore, the significant sensitive of extract/fractions of the fruits of the plant could offer a potential natural healing agent against infections caused by these Gram negative bacteria.

In antifungal bioassay, the crude extract and subsequent fractions of the plant offered potential activity against tested fungi including *T. longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microspoum canis*, *Fusarium solani* and *Candida glaberata* as presented in Table 3. The crude extract and subsequent fractions of *C. songarica* showed marked antifungal activity against *T. longifusus*, *A. flavus*, *M. canis* and *F. solani* with MICs ranges from 670 µg/mL to 110 µg/mL. On the other hand, both the *Candida* species; *C. albicans* and *C. glaberata* were neither inhibited by the crude extract nor by the fractions of the plant.

Antimicrobial resistance is indeed a global challenge for clinicians. In order to address the current emergence of resistance to various bacterial and fungal strains, appropriate and rapid measures has been suggested in terms of focusing new and effective antimicrobial agents [31-34]. For this purpose, multiple agents of different sources are testing worldwide including medicinal plants being time tested and therefore could be the ideal therapeutic option.

Conclusion

It can be concluded on the basis of our findings in the present antimicrobial study that this plant species has great potential. In the light of outstanding outcomes, the plant can be subjected to further detail studies for designing clinically effective antimicrobial of plant origin especially isolation of pure secondary metabolites.

Name of Fungi	% Inhibition of Fungal Growth By Various Samples						
	Std. drug	SI-1	SI-2	SI-3	SI-4	SI-5	SI-6
<i>T. longifusus</i>	100 ¹	50	-	-	50	-	30
<i>C. albicans</i>	100 ¹						
<i>A. flavus</i>	100 ²	50	60	40	60	20	50
<i>M. canis</i>	100 ²	50	80	40	10	70	50
<i>F. solani</i>	100 ¹	40	70	50	30	40	60
<i>C. glaberata</i>	100 ¹	-	-	-	-	-	-

¹Standard Drug = Miconazole, ²Standard Drug=Amphotericin B, SI-1=Crude extract; SI-2=*n*-hexane fraction; SI-3=Chloroform (basic) fraction; SI-4=Ethyl acetate fraction; SI-5=*n*-Butanol fraction and SI-6=Aqueous fraction.

Table 3: Antifungal activity of the crude extract and fractions of *Crataegus songarica* represented in % inhibition of fungal growth.

Name of Fungi	Minimum Inhibitory Concentration (MIC, µg/mL)						
	Std. drug	SI-1	SI-2	SI-3	SI-4	SI-5	SI-6
<i>T. longifusus</i>	1.4 ¹	230			220		670
<i>C. albicans</i>	1.8 ¹						
<i>A. flavus</i>	2.3 ²	220	190	270	180	430	230
<i>M. canis</i>	1.6 ²	230	110	260	490	150	250
<i>F. solani</i>	1.1 ¹	280	160	210	310	270	180
<i>C. glaberata</i>	0.5 ¹						

¹Standard Drug = Miconazole, ²Standard Drug=Amphotericin B, SI-1=Crude extract; SI-2=*n*-hexane fraction; SI-3=Chloroform (basic) fraction; SI-4=Ethyl acetate fraction; SI-5=*n*-Butanol fraction and SI-6=Aqueous fraction.

Table 4: Antifungal activity of crude extract and the fractions of *Crataegus songarica* represented in Minimum Inhibitory Concentration (MIC).

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