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ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF *CAPPARIS DECIDUA* Edgew (Forssk.) AGAINST *STAPHYLOCOCCUS AUREUS*, *BACILLUS CEREUS*, *SALMONELLA TYPHI*, AND *ESCHERICHIA COLI*

Ahmed Shahid Mirza[1], Mirza Tasawer Baig[2*], Ambreen Huma[1], Sadaf Ibrahim[3], Uzma Shahid[4], Aisha Jabeen[3], Mehwish Murad Ali[5], Samina Sheikh[5], Nayel Syed[1], Rasheeda Fatima[6], Syeda Faryal Hassan[6], Shahzada Azam Khan[7], Saira Shahnaz[1] and Saba Shaikh[1]

1. ***Department of Pharmacognosy, Faculty of Pharmacy, Ziauddin University, Karachi Pakistan.***
2. ***Department of Pharmacy Practice, Faculty of Pharmacy, Ziauddin University, Karachi Pakistan.***
3. ***Department of Pharmacology, Faculty of Pharmacy, Ziauddin University, Karachi Pakistan.***
4. ***Department of Physiology, Faculty of Medicine, Ziauddin University, Karachi Pakistan.***
5. ***Department of Pharmaceutics, Faculty of Pharmacy, Ziauddin University, Karachi Pakistan.***
6. ***Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ziauddin University, Karachi Pakistan.***
7. ***NMC Royal Hospital Khalifa City Abu Dhabi, UAE***

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ABSTRACT

Capparis decidua Edgew (Forssk.) belongs to family Capparidaceae, the common name of this plant is Kirar. *Capparis decidua* Edgew (*Cd*E) therapeutically active plant and successfully used in medicine. This study aimed to assess the antibacterial activity of methanol extract of *Cd*E against four microorganisms two gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and two-gram negative (*Salmonella typhi* and *Escherichia coli*). The antibacterial activity of Capparis decidua Edgew leaves was assessed by the disc diffusion method. Antibiotics Cefixime (5mcg), Ceftriaxone (30mcg), Ciprofloxacin (5mcg), and Co-amoxiclav (30mcg) were used to compare the results of methanol extract of *Cd*E that exhibit good antibacterial activity against all three microorganisms except one i.e S aureus. The results of Zone of inhibition of Methanol extract of *Capparis decidua Edgew* high against B cereus (21.21±1.2) and this activity comparable with Ceftriaxone (21.21± 0.45). The results have shown the MICs of methanol extract *Capparis decidua Edgew* were recorded high against gram-positive S. aureus 1.21±3.1mg/ml and gram-negative E coli 1.08 ± 5.81mg/ml. In conclusion, *Cd*E *exhibit* profound antibacterial activity against microbes.

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Introduction

Capparis decidua Edgew (Kirar):

The kapparis is a Greek name from the Persian kabar, "caper". The plant is commonly known in English as Caperberry, Karira in Sanskrit, Kerada in Gujarati, Karer or Kurrel in Hindi, Nispatige in Kannad, and Enugadanta in Telegu, Titali in Urdu and its belong to the family Capparidaceae. The spineless buds of caper are wild-collected and cultivated in dry, sandy soil in the sun [1]. Capparis decidua is broadly dispersed in deserted dry lands showing to intense radiations with an altitude range, mean annual rainfall and temperature of 300–1200 m, 100–750 mm, and 18–48 C, [2-4]. It is very resistant to drought [5] salinity [6] soil erosion [7] and tolerates some frost [8] resulting in a plant that can grow with adaptation in dry

Corresponding Author: Mirza Tasawer Baig Ph.D., Department of Pharmacy Practice, Faculty of Pharmacy, Ziauddin University, Karachi Pakistan. Email: mirzatasawerbaig @ gmail.com

conditions [4]. Caper about 30–100 cm tall shrub, roots of the plant are 6 to10 m (long). Leaves are oval to elliptic, with a round base, mucronate, obtuse, and 2 to 5 cm long or emarginated apex. The fruit (caperberry) is ellipsoid, with a thin pericarp. Seed exposed when the ripe fruit is opened and the size of seeds are 3 to 4mm [9]. Phytochemical study of *Capparis decidua* showed high contents of Isothiocyanate glucoside, glucocapparin, stachydrine, n triacontane, β-carotene, and β-sitosterol and also shows the presence of compound n-triacontanol, n-pentacosane and phthalic acid [10]. The flowers of caper yield a volatile sulphur compound (0.4%), which is active against numerous microorganisms [1]. Many studies reported *CD*E to have profound therapeutic and pharmaceutical use [11].

Therapeutic uses of plant:

Plants are an important part of the earth. Peoples have used plants as medicine from the ancient years. After various observations, medicinal plants are identified as a source of imperative medicine [12, 13]. The numerous parts of Capparis decidua Edgew provide pharmacological activity such as antiparasitic, antihyperlipidemic, antioxidant, hepatoprotective, antidiabetic, antifungal, and antimicrobial activity. Roots act as antibacterial in several diseases. Multifarious interactions concerning several biological, sociological, and psychological factors trigger antimicrobial drug use [14]. Anti-microbial agents play a major role in maintaining good health [15-17]. The root bark is used in arthritis, gout, cough, asthma, dropsy, intestinal worms, and fever. The root powder is applied externally on malignant ulcers [18]. Traditionally this plant was used for the treatment of many diseases like rheumatism, asthma, cough, lumbago, dysentery, liver dysfunction, diarrhea, cardiovascular disorder, constipation, ulcer, renal dysfunction, and dermal diseases [19]. The plant also acts as carminative and aphrodisiac [20]. Effective as antifungal [21], effective as antidiabetic [22], used as anthelmintic [23, 24], antirheumatic [25], as anti-aging [26], anti-inflammatory [23] and analgesic [20].

Antimicrobial property of *Capparis Decidua* Edgew:

The antibacterial activity was determined against Gram-positive: Staphylococcus aureus and *Bacillus subtilis* and Gram-negative: *E. coli* and *Klebsiella pneumonia* using the dichloromethane root extract of *Capparis decidua* Edgew [1]. The ethanol extract was inactive against *Candida albicans* but showed activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* [27]. *CD*E has been reported for its antibacterial [20, 28].

Material and Methodology

Collection of *Capparis decidua Edgew*: The plant was collected from the Nangarparker, the Thar Desert, Sindh Pakistan from October 2017 to March 2018. The plant species were selected on the bases of literature data [29].

Identification of *Capparis decidua Edgew*: The plant specimens were authenticated and identified with the help of Chairperson Department of Botany, Federal Urdu University of Arts, Sciences and Technology Karachi, Pakistan. Herbarium specimens were also deposited via voucher specimen number FUU/Bot/213/2019

Microorganisms and media: Four bacterial strains were tested in this study. Gram-Positive included *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 14579). Gram-Negative included *Salmonella typhi* (ATCC 19430) and *Escherichia coli* (ATCC 25922) obtained from Oxoid (Basingstoke, UK).

Preparation of Methanol extracts of *Capparis decidua Edgew*:

The plant material was washed with distilled water at room temperature and shaded-dry for 3 days. Then the whole plant material was grounded uniformly using an electric grinder. With the help of weight balance (CQT 202, Adam), 500g powdered plant material was soaked for 24 hours in 2.5 L 100% methanol with continuous stirring through a flask shaker (SF, Biby Scientific Ltd., UK) at room temperature. Extracts were subsequently filtered through filtration assembly (GFL3005, Burgwedel, Germany) and concentrated *in vacuo* with the help of rotary vacuum evaporator R-200 (Buchi, Flawil, Switzerland) at 25 degree Celsius. Then it was scooped out into a vial that was previously autoclaved. This was then labeled and kept in -21°c in the refrigerator [30]

Preparation and Storage of stock solution of Methanol extracts of *Capparis decidua Edgew*: To formulate the stock solution of the extract, the dried residue was mix in 200 ml of 1% dimethyl sulphoxide (DMSO) at a concentration of 51.2 mg/mL which was reserved at 80 degree Celsius in amber McCartney bottles until tested [29, 30]

Preparation of Nutrient agar plates: Nutrient agar (NA) 28 gm media powder was weighed using (CQT 202, Adam) Balance, and then it was dissolved in 1000 ml of distilled water in a conical flask and was boiled until solution become clear. After cooling at room temperature, 15 ml of prepared NA was poured in each Petri dish and labeled as a Nutrient Agar Plate (NAP)

Sub-culturing of an organism: The four different microorganisms cultures were streaked on to Nutrient Agar (NA) plates inside the laminar flow chamber which was then incubated for 24 hours at 37ºc before use.

Preparation of Mueller Hinton Agar (MHA): As per the instruction stated on the bottle, 38 grams of Mueller-Hinton agar (MHA) powder was needed to prepare 1 liter of media. Keeping this a constant, the required amount of MHA was prepared each time. The required amount of MHA powder was measured on an electric balance machine, poured, and suspended in DW in a conical flask. The flask was placed on a Bunsen burner with medium flame and was continuously stirring with a glass rod to break down any clump of powder in the flask. The appearance of bubbles indicates that the solution has reached a boiling point. The solution must be heated until it is clear. The flask enclosed with aluminum foil and autoclaved at 121 degrees Celsius for 90-120 minutes. After that, the agar medium was poured on sterile plates inside a laminar flow chamber. The MHA medium was left to set for solidifying and then labeled properly with name as MHA Plate and preparation date. The media plates were then kept in the refrigerator until further use [30].

Procedure for performing the disc Diffusion test

Antimicrobial susceptibility by disc diffusion testing was performed according to the standard method [31]. To determine the presence of antibacterial activity of Methanol extract of *Capparis decidua Edgew*. In this method, we select three sterile MHA plates that were used to lawn the bacterial culture (0.5 McFarland standards) with the help of a sterilized swab. Plates were dried out for 15 minutes in a sterile environment before performing the sensitivity test. The discs which had been fill concentration of about 50mg/ml of Methanol extract of *Capparis decidua Edgew* were used to place on the MHA plate surface. Each test plate contains six discs that were equidistance to each other and were marked at the outer surface of the plate. Four positive controls which were standard commercial antibiotic disc of 30µg Co-amoxiclav, 5µg Ciprofloxacin, 5µg Cefixime, and 30µg Ceftriaxone), negative control (1% DMSO), and treated disc of Methanol extract of Capparis decidua Edgew. The plates were allowed to incubate 24 h at 37 degrees Celsius. After 24 hours the plates were observed for antibacterial activity and with the help of Vernier Caliper, we measured the zone of inhibition. To confirm the reliability of the results tests were three times repeated.

Measurement of the zone of inhibition:

The zone of inhibition was measured as the presence of a clear area on the MHA plate surround antibiotic disc or disc containing *Capparis decidua Edgew* extract and signifies the antibacterial activity of the antibiotic as well as of the extract. We take three times the diameter of the clear zone of inhibition and calculate the average value of the zone of inhibition.

Quantitative antibacterial activity assay by minimum inhibitory (MIC) and the entire activity

The minimum inhibitory concentrations of Methanol extract of *Capparis decidua* Edgew was assessed by standard sensitive serial dilution microplate method against the four bacterial strains in triplicates. This assay was selected for the reason that of its simplicity, sensitivity, reproducibility, and low cost along with the fast procedure. The selected bacterial cultures were grown during the night and were used to McFarland standard 1, which was equal to 3.00 x 10^8cfu/ml (*Staphylococcus aureus*), 1.30 x 10^8cfu/ml (*B. cereus*), 3.70 x 10^8cfu/ ml (*Escherichia coli*) and 3.50 x 10^8cfu/ml (*Salmonella typhi*). The extract of *Capparis decidua Edgew* was dissolved in 5 ml of 1% DMSO to obtain concentrations of 10.0 mg/ ml. Then 100 µl of the above-mentioned solution was used to the first well of Ninty six-well microtiter plates and then it was serially diluted 1:1 with 1% of DMSO. 100µl of the selected bacterial culture was introduced to each well. Starting Preliminary with extract concentrations of 10 mg/ ml, the bacterial growths were therefore subjected to final concentrations of 0.1 to 0.01 mg/ ml. DMSO 1% was utilized as a solvent control to which the maximum concentration the bacterial culture was subjected to in the first well and decreased two-fold in each subsequent well. It has been found that the growth of bacterial culture has never been inhabited by 1% DMSO [32]. Microplates at 37°C in 100% relative humidity were incubated all night. To specify the growth, 40 µl of 0.2 mg/ ml p-iodo-nitro-tetrazolium (INT) as a growth indicator was dissolved in hot water and then added to the microplate wells. The plates were incubated at 37°C for 2 hours. The MIC was determined by visually inspected the lowest concentrations that led to the growth inhibition after 2 hours [33].

Table 1: Zone of Inhibitions Capparis decidua Edgew /B cereus \CDPH

	Mean± Std. Error of Zone of Inhibition			
	B cereus	**E coli**	**S aureus**	**S typhi**
Zone of Inhibition by Cefixime (5mcg)	21.01±4.15	24.17±1.2	19±1.5	20.27±3.2
Zone of Inhibition by Ceftriaxone (30mcg)	21.21±0.45	23.32±4.3	29.07±0.8	30±2.6
Zone of Inhibition by Ciprofloxacin (5mcg)	25±3.7	19.27±2.5	31±3.3	24.34±2.1
Zone of Inhibition by Co-amoxiclav (30mcg)	16.37±1.7	21.51±3.5	29.14±4.8	19.32±1.9
Zone of Inhibition by Methanol Extract of *Capparis decidua* (Kirar)	21.21±1.2	19.44±1.08	10.52±1.08	19.23±6.7
Zone of Inhibition by Control (1% DMSO)	0.87±1.2	1.02±0.73	0.69±2.25	1.31±1.7

Table 2: Minimum Inhibitory Concentration and Entire Antibacterial Activity (EAA) of Methanol extract of *Capparis decidua Edgew* against test cultures

Cultures	Gram	MIC (mg/ml)	EAA (ml/g)
Staphylococcus aureus (ATCC 25923)	Gram-Positive	1.21±3.1	500.34
Bacillus cereus (ATCC 14579).	Gram-Positive	0.83±0.32	331.18
Salmonella typhi (ATCC 19430)	Gram-Negative	0.31±1.62	708.12
Escherichia coli (ATCC 25922)	Gram-Negative	1.08±5.81	675.09

Results and Discussion:

The disc diffusion method was used to assess the antibacterial activity of *Capparis decidua Edgew* leaves. Cefixime (5mcg), Ceftriaxone (30mcg), Ciprofloxacin (5mcg), and Co-amoxiclav (30mcg) standard antibiotics were selected to compare the Methanol extract of *Capparis decidua Edgew* that exhibit good antimicrobial activity. In our study, Methanol extract of *Capparis decidua Edgew* leaves showed various antibacterial activities. Zone of inhibition of Methanol extract of *Capparis decidua Edgew* (21.21±1.2) effective against *B. cereus* and this result similar to Ceftriaxone (21.21±1.84) mentioned in Table 1. The methanol extract of Capparis decidua Edgew exhibits good antibacterial activity against E coli and result of the zone of inhibition of Capparis decidua Edgew (19.44±1.08) (Table 1) also similar with result of ciprofloxacin (19±2.5). Zone of inhibition of Methanol extract of Capparis decidua Edgew (10.52±1.08) not so effective against S aureus and results are also not similar or comparable with any antibiotic mentioned in Table. Zone of inhibition of Methanol extract of *Capparis decidua Edgew* (19.32±3.7) effective against *S. typhi* and this activity result also similar to Ceftriaxone (19.23±6.7) mentioned in Table#1. The outcome of minimum inhibitory concentrations of methanol extract of *Capparis decidua* is shown in Table 2. The results have shown the MICs of methanol extract *Capparis decidua* were recorded against gram-positive S. aureus 1.21±3.1mg/ml and B. cereus 0.83±0.32mg/ml respectively; While MICs of methanol extract Capparis decidua Edgew against gram-negative 0.31±1.62mg/ml and 1.08±5.81 mg/ml *S. typhi* and E. coli, respectively. The entire antibacterial activity (EAA), is a function of the extraction produce in mg per 1 gram of plant material and the minimal inhibitory concentration (MIC), expressed (ml/g). EAA indicates the amount of water or solvents when added to one gram of the extracts that restrain the growth of microbes. *Staphylococcus aureus, Salmonella typhi, Bacillus cereus*, and *Escherichia coli* was found with higher activities in the antibacterial assay, with EAA values of 500.34ml/g, 331.18ml/g, 708.12ml/g and 675.09 ml/g respectively (Table 2). The MIC and EAA values are the helpful parameters for measuring the activity of extracts in mg/ml (potency) for separating biologically active compounds and total activity on ml/g (efficacy) is beneficial for the collection of right plants [33].

Conclusion:

The methanol extract of *Capparis decidua* exhibited antibacterial activities against gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus* and gram-negative bacteria *Salmonella typhi* and *Escherichia coli*.

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Department of Microbiology, Federal Urdu University

Author's contribution:

Conflict of interest:

Authors have no conflict of interest.

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