# ANTIBACTERIAL ACTIVITY OF HEXANE EXTRACT OF *CORCHORUS DEPRESSES* AGAINST *STAPHYLOCOCCUS AUREUS, BACILLUS CEREUS, SALMONELLA TYPHI* AND *ESCHERICHIA COLI*

**AHMED SHAHID MIRZA[1], MIRZA TASAWER BAIG[1*], AMBREEN HUMA[1], SADAF IBRAHIM[1], UZMA SHAHID[2], AISHA JABEEN[1], MEHWISH MURAD ALI[1], RASHEEDA FATIMA[1], ADEEL ARSALAN[1] AND SABA SHAIKH[1]**

*[1]Faculty of Pharmacy, Ziauddin University, Karachi Pakistan*
*[2]Faculty of Medicine, Ziauddin University, Karachi Pakistan*
**Corresponding Author email: mirzatasawerbaig@gmail.com*

**خلاصہ**

کورچورس ڈپریسس ایک فعال طور پر فعال پودا ہے اور دوا میں کامیابی کے ساتھ استعمال ہوتا ہے۔اس مطالعے کا مقصد چار مائکرو جنزم دو گرام مثبت (اسٹیفیلو کوکس اور مینسس اور بیسیلس سیرس) اور دو گرام منفی (سالمونیلا ٹھیفی اور ایسچریچیا کولی) کے خلاف کورچورس ڈپریسس کے ہیکسنولک نچوڑ کی اینٹی بیکٹیریل سرگرمی کا تعین کرنا تھا۔ کورچورس ڈپریسس پتیوں کی اینٹی بیکٹیریل سرگرمی کا اندازہ ڈسک بازی کے طریقہ کار سے کیا گیا۔اینٹی بائیوٹکس ؛ Cefixime (5 µg) ، Cefriaxone (30 µg) ، Ciprofloxacin (5 µg) اور Co-amoxiclav (30 µg) کورچورس ڈپریسس کے ہیکسین نچوڑ کا موازنہ کرنے کے لئے استعمال کیا گیا تھا جو چاروں سو کشمجیووں کے خلاف اچھی اینٹی بیکٹیریل سرگرمی کی نمائش کرتا ہے۔ایس آوریس کے خلاف سیپروفلوکسین (23 ± 5.7) کے مقابلے میں، کورچورس ڈپریسس کے ہیکسین نچوڑ کے روکنے والے زون کے نتائج زیادہ (26 ± 1.6) تھے۔نتائج سے پتہ چلتا ہے کہ کورچورس ڈپریسس کے ہیکسین نچوڑ کے sCIM گرام پازیٹو ایس اور یئرس (0.321 ± 0.23) اور گرام منفی ایس ٹائفی (0.26 ± 0.07) کے خلاف اعلی ریکارڈ کیے گئے۔یہ نتیجہ اخذ کیا گیا تھا کہ کورچورس ڈپریسس جرثوموں کے خلاف گہری اینٹی بیکٹیریل سرگرمی کی نمائش کرتا ہے۔

**Abstract**

*Corchorus depressus* is therapeutically active plant and successfully used in medicine. The purpose of this study was to determine the antibacterial activity of hexanolic extract of *Corchorus depressus* against four microorganism two gram positive (*Staphylococcus aureus* and *Bacillus cereus*) and two gram negative (*Salmonella typhi* and *Escherichia coli*). Antibacterial activity of *Corchorus depressus* leaves were assessed by disc diffusion method. Antibiotics; Cefixime ($5\mu g$), Cefriaxone ($30\,\mu g$), Ciprofloxacin ($5\,\mu g$) and Co-amoxiclav ($30\,\mu g$) were used to compare the Hexane extract of *Corchorus depressus* that exhibit good antibacterial activity against all four microorganisms. The results of inhibitory zones of Hexane extract of *Corchorus depressus* were high (26±1.6) as compared with Ciprofloxacin (23±5.7) against *S. aureus.* The results shown that the MICs of hexane extract of *Corchorus depressus* was recorded high against gram positive *S. aureus* (0.321±0.23) and gram negative S. typhi (0.26 ± 0.07). It was concluded that *Corchorus depressus* exhibited profound antibacterial activity against microbes.

**Key words:** *Corchorus depressus*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, Antibacterial activity, Medicinal plants, Hexane extract

## Introduction

Common name of the *Corchorus depressus* L (CD) is Bauphali, belongs to family Malvaceae [1-3] and is known as a good sand binder. It is a perennial herb, spread typically in tropical and subtropical region of South-Asia and North America [4-5].This plant is also found in Pakistan, India, Africa and Cape Verde Islands [6]. The height of the plant is 6-9 inches. The plant is tiny and its color resembles with chocolate, having capsule shaped fruit of size 8-15 mm. The phytochemical constituents of CD include triterpenoids, sterols, phenolics, fatty acids, cardiac glycosides, carbohydrates, sitosterol,glucoside, apigenin, luteolin, oleanolic acid, cordepressic acid, cordepressin, cordepressinic acid, α amyrin, kaempferol, saponin, flavonoids and alkaloids [7-10].

**Therapeutic uses of the Plant:** *Corchorus depresses* has been used in medicine and is a therapeutically active plant. It is utilizedas tonic and cooling medicine in fevers. Roots of the plantare effective for relief in migraine [11]. The plant has also been used in traditional medicinal to increase the thickness of the seminal fluid and to set-up menstrual disorder [12].Extract paste of the plant has been used for the healing of wounds. The plant has been used as antibacterial, antifungal, anthelmintic drug in folklore medicine [13]. It has been used as cardio-

tonic and found effective in malaria, treatment of gonorrhea, as an anti-cancer, diuretic [14-18]. The plant is sweet hot sharp acrid helpful to removes tumors, relief pain also improved piles. In fever it provides cooling treatment. The leaves have emollient effect[19]. Biological studies on this plant revealed that the whole plant exhibited several therapeutic effects. It has antipyretic, analgesic, antioxidant, hepato-protective, antimicrobial, antifungal, aphrodisiacand anti- inflammatory properties [20-26].

**Antimicrobial properties of *Corchorusdepressus (Mundair)*:** *Corchorus depressus* showed resistance to *Klebsiella* species and *E.coli* species while showed sensitivity against *Shigella*species, *Salmonella* species and *Clostridium* species [27].

**Materials and Methods**

**Collection of *Corchorus Depressus*:** The plant was collected from the Nangarparker, Thar Desert, Sindh Pakistan from October 2017 to March 2018. The plant species was selected on the bases of literature data [28].

**Identification of *Corchorus Depressus*:** The plant specimens were authenticated and identified with the help of Department of Botany, Federal Urdu University of Arts, Sciences and Technology Karachi, Pakistan. Herbarium specimens were also deposited.

**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 *Escherichiacoli* (ATCC 25922) obtained from Oxoid (Basingstoke, UK).

**Preparation of Hexane Extracts of *Corchorus Depressus*:** 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),500gpowdered plant material was soaked for 24 hours in 2.5 L 100% hexane 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* using a 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 -21ºC in chiller [29].

**Preparation and Storage of Stock Solution of Hexane Extracts of *Corchorus depressus*:** To formulate the stock solution of the extract, dried residue was mixed in 200 mL of 1% dimethyl sulphoxide (DMSO) at a concentration of 51.2 mg/mL which was reserved at80 degree Celsius in Amber McCartney bottles until tested [29-30].

**Preparation of Nutrient Agar Plates:** Nutrient agar (NA) 28 was weighed using (CQT 202, Adam) electrical balance and then it was dissolved in 1000 mL of distilled water in 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 Nutrient agar Plate [28].

**Sub-culturing of Organism:** The cultures of the four micro organisms were streaked onto Nutrient agar (NA) plates inside the laminar flow chamber which was then incubated for 24 hours at 37ºC before use [28].

**Preparation of Mueller Hinton Agar (MHA):** 38 gram of Mueller-Hinton agar (MHA) was weighed and suspended in distilled water in a conical flask. The flask was placed on a Bunsen burner with medium flame and with continuous. The appearance of bubbles indicates that the solution has reached boiling point. The flask was enclosed with aluminum foil, and was autoclaved at 121ºC 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 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]. We selected three sterile MHA plates and were used to lawn the bacterial culture (0.5 McFarland standard) with the help of sterile swab. Plates were dried for 15 minutes in a sterile environment before performing the sensitivity test. The discs which had been filled concentration of about 50mg/mL of Hexane extract of *Corchorus depressus* was used to place on MHA plate surface. Each test plate contained six discs which were equidistance to each other and were marked at outer surface of the plate. Four positive controls (standard commercial antibiotic disc of 30µg Co-amoxiclav, 5µg Ciprofloxacin, 5µg

Cefixime and 30µg Cefriaxone), negative control (1% DMSO), and treated disc of Hexane extract of *Corchorus depressus*. The plates were allowed to incubate 24 h at 37ºC.After 24 hours the plates were observed for antibacterial activity and with the help of Vernier Caliper, zone of inhibition was measured for each disc. To confirm the reliability of the results tests were three times repeated.

**Measurement of Zone of Inhibition:** Zone of inhibition was measured as the clear area of a presence on the MHA plate surround an antibiotic disc or disc containing *Corchorus depressus* extract and signifies the antibacterial activity of the antibiotic as well as of the extract. The diameter of zone of inhibition was measured three times and the average value was calculated using MS Excel.

**MIC and Total Antibacterial Activity Assay**

The minimum inhibitory concentration of Hexane extract of *Corchorus depressus* assessed by standard sensitive serial dilution microplate method against the four bacterial strains in triplicate. This assay was selected because of its simplicity, reproducibility, and sensitivity, low cost and fast procedure. The selected bacterial cultures were grown during the night and were adjusted to McFarland standard, which was equivalent to 3.00 x $10^8$cfu/mL (*Staphylococcus aureus),* 1.30 x $10^8$cfu/mL (*Bacillus cereus*), 3.70 x $10^8$cfu/mL (*Escherichia coli*) and 3.50 x $10^8$cfu/mL (*Salmonella typhi*). The extract of *Corchorus depressus* was dissolved in 5 mL of 1% DMSO to obtain a concentration of 10.0 mg/mL. 100 µL of the above mentioned solution was added to the first well of a Ninety six well micro-titer plate and was then serially diluted 1:1 with 1% of DMSO. 100µL of the selected bacterial culture was added to each well. Starting with an extract concentration of 10 mg/ml, the bacterial culture was 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 bacteria has never been inhibited by 1% DMSO [32]. Microplates at 37°C in 100 %relative humidity were incubated over night. To indicate the growth, 40 µl of 0.2 mg/ml p-iodo-nitro-tetrazolium(INT)as a growth indicator dissolved in hot water, was then added to the microplate wells. The plates were incubated at 37 °C for 2 h. The MIC was determined visually as the lowest concentration that led to growth inhibition after 2h [33].

**Table: 1 Zone of Inhibitions *Corchorus depressus* /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±1.15 | 23.67±2 | 29±1.1 | 15±1.2 |
| Zone of Inhibition by Ceftriaxone (30mcg) | 31.67±1.45 | 24.33±4.3 | 30.67±0.8 | 22±2.6 |
| Zone of Inhibition by Ciprofloxacin (5mcg) | 20±1.53 | 20.67±2.4 | 23±1.3 | 35±2.1 |
| Zone of Inhibition by Co-amoxiclav (30mcg) | 19.67±3.84 | 24.67±1.5 | 18.67±2.8 | 13.33±0.9 |
| Zone of Inhibition by Hexane Extract of *Corchorus depressus* (mundair) | 16±0.58 | 21±3.5 | 26±1.6 | 23±5.7 |
| Zone of Inhibition by Control (1 % DMSO) | 1.67±0.67 | 2.33±1.3 | 1.6±2.7 | 1.33±0.7 |

**Table 2: Minimum Inhibitory Concentration and Total Antibacterial Activity (TAA) of Hexane extract of *Corchorus depressus* against test cultures**

| Cultures | Gram | MIC (mg/ml) | TAA (ml/g) |
|---|---|---|---|
| *Staphylococcus aureus* (ATCC 25923) | Gram Positive | 0.32±0.23 | 714.46 |
| *Bacillus cereus* (ATCC 14579). | Gram Positive | 0.22±1.32 | 319.82 |
| *Salmonella typhi* (ATCC 19430) | Gram Negative | 0.26±0.07 | 461.46 |
| *Escherichia coli* (ATCC 25922) | Gram Negative | 0.13±0.04 | 922.82 |

**Results and Discussion**

Antibacterial activity of leaves of *Corchorus depressus* was assessed by disc diffusion method. Cefixime (5µg), Cefriaxone (30µg), Ciprofloxacin (5µg) and Co-amoxiclav (30µg) were used to compare the Hexane extract of *Corchorus depressus* that exhibit good antibacterial activity. In our study, Hexane extract *Corchorus depressus* of leaves showed different antibacterial activity. Zone of inhibition of Hexane extract of *Corchorus depressus* (16±0.58) effective against *B. cererus* and this result is comparable with Co-amoxiclav (19.67± 3.84)

mentioned in Table 1. The extract exhibited good antibacterial activity against *E. coli* and resulted zone of inhibition of *Corchorus depressus* (21±3.5) (Table 1) also comparable with result of ciprofloxacin (20.7±2.4). Zone of inhibition of Hexane extract of *Corchorus depressus* (26±1.6) was found effective against *S. aureus* and this activity is comparable with Ciprofloxacin (23±5.7) mentioned in Table 1. Zone of inhibition of Hexane extract of *Corchorus depressus* (26±1.6) was found effective against *S. typhi* and it also this activity is comparable with Ceftriaxone (22±2.6) mentioned in Table 1.The results of minimum inhibitory concentrations (MICs) of hexane extract of *Corchorus depressus*are shown in Table 2. The results shown the MICs of hexane extract *Corchorus depressus* was recorded against gram positive *S. aureus* 0.321±0.23mg/ml and B.cereus 0.22±1.32mg/ml respectively, While MICs of hexane extract *Corchorus depressus* against gram negative 0.26±0.07mg/ml and 0.13±0.04 mg/ml *S. typhi* and *E. coli*, respectively. The total antibacterial activity (TAA), is a function of the extraction produce in mg per 1 g of plant material and the minimal inhibitory concentration (MIC), expressed ml/g. TAA indicates the volume of water or solvent, when added to 1 gram of the extract that still inhibit the growth of microbes. *Staphylococcus aureus, Bacillus cereus, Salmonella typhi* and *Escherichia coli* had higher activities in the antibacterial assay, with TAA values of714.46ml/g, 319.82ml/g,461.46ml/g and 922.82ml/g respectively (Table 2). The MIC and TAA values are useful parameter for measuring the activity of extracts in mg/ml (potency) for separating bioactive compounds and total activity on ml/g is beneficial for theright collection of plant [33].

## Conclusion

Hexane extract of *Corchorus depressus* exhibited antibacterial activity against gram positive bacteria *Staphylococcus aureus* and *Bacillus cereus* and gram negative bacteria *Salmonella typhi* and *Escherichia coli*.

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