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# EVALUATION OF ANTIBACTERIAL ACTIVITY AGAINST MULTIDRUG-RESISTANCE (MDR) BACTERIA AND ANTIOXIDANT EFFECTS OF THE ETHANOLIC EXTRACT AND FRACTIONS OF *CHENOPODIUM ALBUM* (SUB SP *STRIATUM*)

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**Keywords:**

*Chenopodium album* (sub sp *striatum*), Antibacterial activity, Multidrug-resistance (MDR) Bacteria, Antioxidant effect, Phenolic content, Flavonoid content

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**ABSTRACT:** *Chenopodium album* (sub sp. *striatum*) is a folk and nutritious medicine belongs to Chenopodiaceae family and widely used as antimicrobial agent. The aim of this study is to evaluate the *in vitro* antibacterial potential against multidrug-resistance (MDR) bacteria isolated from clinical spicemens, antioxidant activity, total phenolic and total flavonoids content of crude ethanolic extract and nine different fractions of *C. album* (sub sp. *striatum*). The *in vitro* antibacterial activity was evaluate against eight multidrug-resistant bacteria based on minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), agar well and disk diffusion methods. Antioxidant property was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl radical scavenging activity. The total phenol contents were measured by Folin-Ciocalteu and $AlCl_3$ assays. Our results showed that MeOH: $H_2O$ with 80:20 ratio (most polar) fraction had the highest antimicrobial effect on eight multidrug-resistant bacteria (MIC: 0.15-2.5 mg/ml, MBC: 0.31-5 mg/ml and had highest inhibitation zones (well: 24.33±0.57 mm and disk: 26.33±1.53 mm). In antioxidant assay, MeOH: $H_2O$ fraction also exhibited the highest radical scavenging activity [Antioxidant activity = 95.1±2.13 percent] and flavonoid content of crude ethanolic extract and most polar fraction were the highest [(0.892±0.011, 0.865±0.010) mg/ml and (4.873±0.029, 4.535±0.025) mg/ml]. According to our result, *C. album* (sub sp. *striatum*) has the greatest potential to be consider as antibacterial (against MDR bacteria strain) and antioxidant agent, but further *in vivo* research, isolation of pure compounds should be carried out to discover the modes of its action and to shed light on the effects.

**INTRODUCTION:** Over the past decades, usage of traditional plants for medicinal purposes provide a basis for the use of specific plants for specific medicinal conditions.

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Medicines got from plant extracts continues to provide health coverage for over 80% of the world's population, especially in developing world [1]. The significance of plants and plant extract because of their therapeutic properties have grown tremendously during the recent years because they have several advantages such as efficacy, safety, cultural acceptability, better compatibility with human body and lesser side effects. Namdo stated that about a quarter of all prescribed pharmaceuticals in advanced countries contain

compounds that are directly or indirectly derived from plants [2]. Medicinal plants have been used for centuries as remedies for human diseases, Nowadays there is a wide spread interest in medicines derived from plants, and it is reported that green medicine is safe and reliable because they contain components and secondary metabolites such as polyphenols, alkaloids, volatile oils and etc which have therapeutic properties so formulation of plants for standardization and regulation of phytomedicinal products is the most alternative way [3, 4].

On the other hand, in recent years, antibiotic resistance has become a serious and widespread problem in developing countries and it is result of inappropriate usage, abusive and over prescription of antibiotics causing mortality each year [5, 6]. Global emergence of resistant bacteria is the result of ineffectiveness of current antibiotics and drugs causing treatment failure [7]. Hence there has been a growing interest by using alternative therapy and the therapeutic use of natural products specially medicinal plant [3].

Free radical formation is the result of the normal metabolism of aerobic cells. Consumption of oxygen in cell growth leads to production of oxygen free radical [8]. Unfortunately, free radicals are involving in various type of diseases such as cancer, hypotension, diabetic, neurological disorder like alziemr [9, 10]. Recently a great interest has been focused on antibacterial and antioxidant agent with natural base which play an important role for inhibiting the growth of microorganism and formation of free radical and oxidative chain-reactions [11].

The family of Chenopodiaceae is one of the largest families (goose foot family) of the flowering and annual plants which is widely spread worldwide in the moderate and subtropical zone to arid and saline regions. Iran with the surface area of 1,648,000 km $^2$ has large area of saline and arid rangelands. Due to harsh and halophyte ecosystem could provide a suitable condition for growing and cultivation of many plant species such as *C. album* (sub sp. *striatum*) [12]. This family consists of 104 genus and more than 1400 species [13, 14]. *C. album* are known to be a rich source of flavonoid, glucosides, terpenoids and phenolic acid. The leaves of the plant are rich in carotenoids and their seeds in proteins and fats [15, 16]. Many species of *Chenopodium* were reported to numerous medicinal properties such as antipruritic, antibacterial, antifungal, anticancer but there are no reports about the study of these properties on Striatum sub species and MDR bacteria strains [17, 18]. Previous studies exhibited the presence of flavonoids, alkaloids, phytosteroids and etc [19, 20].

The present study was designed to determine the role of ethanolic crude extract and polar and non-polar fractions of as *C. album* (sub sp. *striatum*) for potential antibacterial activity according to standard protocols by MIC, MBC tests and agar-based method against some selected MDR microorganisms as gram-positive and gram-negative bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Shigella flexneri*, *Shigella sonnei*, *Shigella dysenteriae*, *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella infanti*s and also determining of antioxidant capacity of the plant extract and fraction based on DPPH method and total phenol and flavonoid contents.

The aim of this study was to screen the *in vitro* antibacterial activity and antioxidant effect of *C. album* (sub sp. *striatum*) as potential sources of natural antimicrobial and antioxidant agents.

## MATERIALS AND METHODS:

**Plant Material:** Through random sampling, healthy aerial parts of *C. album* (sub sp. *striatum*) were collected from Qazvin plain near Almut (Sep 2016). The plant was identified by Plant Physiology Laboratory and the voucher specimens were confirmed and deposited in Herbarium (No. 2565) at the Department of Pharmacy, Faculty of Pharmacy, Shahid Beheshti University of Medicinal Sciences. The plant materials washed thoroughly 2-3 times with running tap water and then once with distillated water, shade dried for period of 6-7 days at room temperature, subsequently ground into fine powder using motor driven grinding mill.

**Plant Extraction Procedure:** 300 g of powdered aerial parts of plant material was macerated with 70% ethanol at room temperature for 72 hrs by maceration method. The extract was filtered by whatman filter paper (No.1) and to the marc was

added fresh solvent and kept for another 72 hrs (repeated 3 times). Finally the filtered extracts were concentrated using a rotary evaporator at 40 °C and then stored at 4 °C for further assays [21].

**Fractions Preparation:** The aerial parts dry residue were repeatedly extracted with solvents of increasing polarity beginning with Hexane ($A_1$), Hex 1: Dichlorometahne (20:80) ($A_2$), Hex: DCM 1 (40:60)($A_3$), Hex:DCM (60:40) ($A_4$), Hex: DCM (80:20) ($A_5$), DCM 100% ($A_6$), DCM: MeOH (50:50) ($A_7$), MeOH1 100% ($A_8$), MeOH: $H_2O$ (80:20) ($A_9$) extraction was done by shaker at room temperature. Further filtration through filter paper N° 0.45 µm [22].

The extract were evaporated to dryness under reduced pressure and then stored at 4 °C. For antimicrobial and antioxidant assays, pooled fractions residues were adequately diluted in the dimethyl sulfoxide (DMSO) to 5% [19, 20].

**Determination of Antibacterial Activity:**

**Bacterial Preparation:** The eight clinical bacterial strains were used in this study. The gram-positive and gram-negative species were *Staphylococcus aureus, Escherichia coli, Shigella flexneri, Shigella sonnei, Shigella dysenteriae, Salmonella typhimurium, Salmonella enteritidis* and *Salmonella infanti*s. Bacteria species were taken from isolated specimens who exhibited resistance to some antibiotics in hospitalized patients. They were taken based on ethical clearance approval from the ethical committee in hospital. The bacteria were cultured over night at 37 °C on LB broth for the preparation of cell suspensions. The bacteria cell suspensions were homogenized and adjusted to 0.5 McFarland standards.

Commercial antibiotic disks of Ampicillin (10 µg/disc), Amikacin (30 µg/disc), Amoxicillin-clavulanic acid (30 µg/disc), Azithromycin (30 µg/disc), Cefazoline (30 µg/disc), Cefixime (5 µg/disc), Cefotaxime (30 µg/disc), Cefoxitin (30 µg/disc), Cefpiramide (30 µg/disc), Ceftazidime (30 µg/disc), Ceftizoxime (30 µg/disc), Ceftriaxone (30 µg/disc), Cephalotine (30 µg/disc), Chloramphenicol (30 µg/disc), Ciprofolxacine (5 µg/disc), Clindamycine (2 µg/disc), Doxycyline (30 µg/disc), Erythromycine (15 µg/disc) Gentamycin (10 µg/disc), Imipenem (10 µg/disc), Kanamycin (30 µg/disc), Nalidixic acid (30 µg/disc), Norfloxacin (10 µg/disc), Piperacillin (100 µg/disc), Rifampine (5 µg/disc), Streptomycine (10 µg/disc), Tetracyclin (30 µg/disc), Ticarcilline (75 µg/disc), Tobramycine (10 µg/disc) and Trimethoprim-sulfamethoxazole (25 µg/disc) were used for assessment of their activity and sensitivity against the tested bacteria strains. *In vitro* antibacterial activity was tested by determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using micro-dilution method and also agar well and disk diffusion method. The plant extracts were dissolved in FBS1 and DMSO (up to 5% total volume) [23]. The stock concentrations of plant extract were 10 mg/ml. The protocol used in this study was based on CLSI guidelines.

**Determination of Minimum Inhibitory Concentration (MIC):** In order to determining MIC, serial two fold dilutions plant extract and fractions were made in a concentration from 10 mg/ml to 0.005 mg/ml in sterile 96-well plates was prepared. These dilution were added to tubes containing 100 µL LB broth and 5 µL of bacterial suspension. Micro-plates were incubated at 37 °C for 24 h. The lowest concentration of fractions in broth medium that had inhibited the growth of the test microorganism was considered as MIC. Dimethyl sulfoxide was used as a control and LB broth as negative control [24].

**Determination of Minimum Bactericidal Concentration (MBC):** To determine the MBC, about 10 µL of broth from those tubes which did not exhibit any visible growth in the MIC assay was cultured on freshly prepared sterile Muller-Hinton agar and then incubated at 37 °C for 18-24 h. after incubation the highest dilution (least concentration) that inhibited colony formation on solid medium was consider as MBC [25].

**Agar Well Diffusion Method:** The antimicrobial activity of the crude ethanolic extract and nine different fractions of *C. album* (sub sp. *striatum*) were screened by using the agar well diffusion method as describe by Perez *et al.,* [26]. Bacterial strains were grown on Mueller-Hinton agar at 37 °C for 18 h and then suspended in LB broth adjusted to a turbidity of 0.5 Mac Farland standards [~$10^8$Colony Forming Units (CFU)/mL].

50 µl inoculum suspensions were swabbed uniformly to solidified 25 mL Mueller-Hinton agar for bacteria. Afterwards inoculum was allowed to dry for 5 min. Wells with 5 mm diameter were punched in the agar and finally filled with 50 µl of 35, 40, 45, 50, 55 and 60 mg/ml of crude extract and fraction solution. The plate were allowed to stand on the bench for 1 h for proper diffusion and then incubated at 37 °C for 24 h. The antibacterial activity was evaluated by measuring the inhibition zone diameter observed. The experiments were performed in triplicate.

**Agar Disk Diffusion:** The antibacterial activity was also performed by disk diffusion method. The methods for growing of bacterial strains as same as described in well diffusion method but instead of creating wells we used blank disk with 6 mm in diameter. Each disk was soaked with about 10 µl of 35, 40, 45, 50, 55 and 60 mg/ml of essential oil solution. Six disks were placed on each petri plate. The plates were incubated at 37 °C for 24 h and finally the inhibition zones in mm were measured. The test was replicated three times.

**Determination of Antioxidant Activity by DPPH Scavenging Assay:** The effect of crude extract and fractions on DPPH free radical was measured using the method of Sanchez-Moreno *et al.*, with slightly modification [27]. 3 ml of different concentration of the crude extract and fractions (0.2, 0.4 and 0.6 mg/ml) were added to 0.5 ml of a 1mM methanol solution DPPH. After 30 min incubation at room temperature for complete reaction, the absorbance was measured against a blank at 517 nm.

DPPH radical scavenging activity = $(A_{blank} - A_{sample}/A_{blank}) \times 100$

Where $A_{blank}$ is the absorbance of the control reaction which is containing of all reagent except the test compound. $A_{sample}$ is the absorbance of the test compound. $IC_{50}$ is defined as the concentration necessary to obtain 50% of a maximum scavenging capacity which was calculated from the plot of inhibitation percentage against concentration. All determinations were done in triplicate.

**Determination of Total Phenolic Content:** The total phenolic content of the *C. album* (sub sp. *striatum*) crude extract and fractions were determined using the Folin-Ciocalteu reagent by the method of Karamian and Ghasemlou. The reaction mixture contained: 100 µl of diluted extract, 1.5 mL distillated water and 0.75mL of freshly prepared diluted Folin-Ciocalteu reagent and 0.75 mL of 7.5 % sodium carbonate. Mixtures were kept in dark at ambient conditions for 90 min to complete the reaction. The absorbance at 765 nm was measured. Gallic acid was used as standard and the results were expressed as mg Gallic acid (GAE)/g extract. Using a BioTek® power wave XS spectrophotometer. All determinations were performed in triplicate [28].

**Determination of Total Flavonoid Content:** The total flavonoid content was determined according to the aluminium chloride colorimetric method. For this, the method of Chang *et al.*, was employed. Plant extract (0.5 mL) in ethanol 70% was mixed with 100 µL of 10% aluminium chloride, 100µL of 1 M acetic acid and 2.8 mL of deionized water. After the 30 minutes incubation at the room temperature, the absorbance of the reaction mixture was determined spectrometrically at 415 nm on a BioTek® Power Wave XS spectrophotometer. All determinations were performed in triplicate. Total flavonoids content was expressed as mg rutin equivalent per gram weight of sample [29].

**Statistical Analysis:** All the values from experiments as we mentioned before were carried out in triplicate. Results were express as means ± SEM. Correlation coefficient of total phenol and flavonoid contents was determined using Excel programme and SPSS statistical software Ver.18.

## RESULTS AND DISCUSSION:

**Extracts Yield:** The yield of crude extract and nine different fractions of *C. album* (sub sp. *striatum*) are listed in **Table 1**. MeOH:$H_2O$ (80:20) had the highest extract yield.

**TABLE 1: THE EXTRACT YIELD**

| Fractions | Yield (%)* |
|---|---|
| Crude extract | 5.15±4.51 |
| Hex (100%) | 2.35±0.28 |
| DCM:Hex (20:80) | 1.65±0.08 |
| DCM:Hex (40:60) | 0.50± 0.05 |
| DCM:Hex (60:40) | 0.39±0.04 |
| DCM:Hex (80:20) | 0.83±0.26 |
| DCM (100%) | 0.29±0.03 |
| DCM:Methanol (50:50) | 4.44±0.11 |
| MeOH 100%, | 1.15±0.24 |
| MeOH:$H_2O$ (80:20) | 7.40±2.32 |

* Mean±SD

***In vitro* Antibacterial Activity:** The antibacterial activities of nine different fractions were assayed *in vitro* by agar micro-dilution method against eight multidrug-resistant bacteria. The MIC assay of the *C. album* (sub sp. *striatum*) showed strong antagonist against nearly all of examined microorganisms. The antibacterial activity against each bacterium was observed to be varied. The MIC value ranged from 0.15 mg/mL to 2.5 mg/mL (**Table 2**). The most effective fraction that inhibited the growth of bacteria was the most polar fraction ($A_9$) followed by crude extract. MBC value of fractions ranged from 0.31 mg/mL to 5 mg/ml (**Table 3**). $A_9$ fraction followed by crude extract showed greater effects in inhibiting bacterial growth in this study. Hexane fraction had no antimicrobial effect against *S. aureus*.

**TABLE 2: MINIMUM INHIBITORY CONCENTRATION OF *C. ALBUM* (SUB SP. *STRIATUM*) NINE DIFFERENT FRACTIONS AGAINST MDR BACTERIAL STRAINS**

| Fractions | Bacterial Species | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***E. coli*** | ***Sh. flexeneriae*** | ***Sh. sonnei*** | ***Sh. dysenteriae*** | ***S. infantis*** | ***S. enteritidis*** | ***S. typhimurium*** | ***S. aureus*** |
| Crude extract | 0.62 | 0.62 | 1.25 | 1.25 | 1.25 | 1.25 | 0.62 | 0.31 |
| $A_1$ | 2.5 | 1.25 | 0.62 | 2.5 | 1.25 | 2.5 | 2.5 | nd* |
| $A_2$ | 2.5 | 1.25 | 0.31 | 2.5 | 0.62 | 1.25 | 0.62 | 2.5 |
| $A_3$ | 1.25 | 2.5 | 0.62 | 1.25 | 0.62 | 1.25 | 0.62 | 1.25 |
| $A_4$ | 1.25 | 0.62 | 2.5 | 1.25 | 1.25 | 2.5 | 0.31 | 0.62 |
| $A_5$ | 1.25 | 2.5 | 1.25 | 0.62 | 2.5 | 0.62 | 1.25 | 2.5 |
| $A_6$ | 2.5 | 2.5 | 0.62 | 2.5 | 1.25 | 1.25 | 0.62 | 0.62 |
| $A_7$ | 0.62 | 1.25 | 1.25 | 0.31 | 0.31 | 0.62 | 0.31 | 2.5 |
| $A_8$ | 0.31 | 1.25 | 1.25 | 0.62 | 0.31 | 2.5 | 1.25 | 0.62 |
| $A_9$ | 0.62 | 1.25 | 0.15 | 0.62 | 1.25 | 0.62 | 1.25 | 2.5 |

*Nd: Not detected

**TABLE 3: MINIMUM BACTERICIDAL CONCENTRATION OF *C. ALBUM* (SUB SP. *STRIATUM*) NINE DIFFERENT ACTIONS AGAINST MDR BACTERIAL STRAINS**

| Fractions | Bacterial Sp. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***E. coli*** | ***Sh. flexeneriae*** | ***Sh. sonnei*** | ***Sh. dysenteriae*** | ***S. infantis*** | ***S. enteritidis*** | ***S. typhimurium*** | ***S. aureus*** |
| Crude extract | 1.25 | 1.25 | 2.5 | 2.5 | 2.5 | 2.5 | 1.25 | 0.62 |
| $A_1$ | 5 | 2.5 | 1.25 | 5 | 2.5 | 5 | 5 | nd |
| $A_2$ | 5 | 2.5 | 0.62 | 5 | 1.25 | 2.5 | 1.25 | 5 |
| $A_3$ | 2.5 | 5 | 1.25 | 0.62 | 1.25 | 2.5 | 1.25 | 2.5 |
| $A_4$ | 2.5 | 1.25 | 5 | 2.5 | 2.5 | 5 | 0.62 | 1.25 |
| $A_5$ | 2.5 | 5 | 2.5 | 1.25 | 5 | 1.25 | 2.5 | 5 |
| $A_6$ | 5 | 5 | 1.25 | 5 | 2.5 | 2.5 | 1.25 | 1.25 |
| $A_7$ | 1.25 | 2.5 | 2.5 | 0.62 | 0.62 | 1.25 | 0.62 | 5 |
| $A_8$ | 0.62 | 2.5 | 2.5 | 1.25 | 0.62 | 5 | 2.5 | 1.25 |
| $A_9$ | 1.25 | 2.5 | 0.31 | 1.25 | 2.5 | 1.25 | 2.5 | 5 |

[a]Nd: Not detected

The inhibition zones of the bacteria strains were in the range of (7.0±0.0) mm to (24.33 ± 057) mm in well diffusion method and (7.0 ± 0.0) mm to (26.33 ± 1.53) mm in disk diffusion method (**Table 4-7**). Crude ethanolic extract and MeOH: $H_2O$ (80:20) fraction were found to be the most effective in each concentration (35, 40, 45, 50, 55 and 60 mg/ml).

The panel of test organisms for *in vitro* antibacterial screening in this study is summarized in **Table 8**. It is important to mention that the solvent (up to 5% DMSO) did not inhibit the growth of bacteria strains. The antibacterial activity varied depending on the species of microorganism, plant species and the type of extract and fractions.

Our results showed that increasing concentration of crude extract and fractions raised the zone of inhibition. MDR *S. aureus* was the most sensitive bacteria strain and inhibited by different extract and fractions of the plant.

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**TABLE 4: ANTIBACTERIAL EFFECT OF C. ALBUM (SUB SP. *STRIATUM*) CRUDE EXTRACT AND FRACTIONS BY AGAR WELL DIFFUSION IN SIX DIFFERENT CONCENTRATION (35, 40, 45, 50, 55 AND 60 MG/ML RESPECTIVELY)**

| | Bacterial Sp. | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *E.coli* | | | | | | *Sh.flexeneriae* | | | | | | *Sh.sonnei* | | | | | | *Sh.dysenteriae* | | | | | |
| | 60 | 55 | 50 | 45 | 40 | 35 | 60 | 55 | 50 | 45 | 40 | 35 | 60 | 55 | 50 | 45 | 40 | 35 | 60 | 55 | 50 | 45 | 40 | 35 |
| Crude Ext. | 18.67± 2.08 | 16.33± 1.53 | 15± 1 | 13.67± 1 | 12± 1 | 10± 0 | 15.33± 1.53 | 15± 1 | 14± 1.15 | 13.67± 1.15 | 11.33± 0.57 | 10.33± 0.57 | 17.67± 1.53 | 16.67± 2.08 | 14± 1 | 14.33± 0.57 | 13.67± 1.15 | 12.33± 0.57 | 20± 1 | 18.33± 0.57 | 18.67± 1.15 | 17.33± 1.53 | 16.33± 0.57 | 16 ± 0.0 |
| $A_1$ | 12.33± 1.53 | 11.66± 1.15 | 10.66± 1.15 | 7± 0 | - | - | 10± 1 | 8.66± 1.15 | 8± 0 | - | - | - | 11.66± 1.15 | 10± 1 | 8± 0 | 7± 0 | - | - | 13.66± 1.53 | 11± 1 | 10.33± 0.57 | 8± 0 | - | - |
| $A_2$ | 13.33± 2.08 | 12.33± 0.57 | 9.33± 0.57 | - | - | - | 11.67± 1.53 | 9± 1 | 8± 0 | - | - | - | 12.67± 1.15 | 10.33± 0.57 | 10± 0 | 9± 1 | 7± 0 | - | 14.33± 2.52 | 11.67± 1.15 | 8± 1 | - | - | - |
| $A_3$ | 14.67±. 1.15 | 13.67± 1.15 | 13.33± 0.57 | 10± 1 | - | - | 11.67± 2.52 | 8.67± 1.15 | 7± 1 | 7± 0 | - | - | 12.67± 2.08 | 12± 1 | 12.33± 0.57 | 9± 1 | 7± 1 | - | 14.33± 1.53 | 12.67± 1.15 | 11.33± 1.15 | 8± 1 | 7±0.0 | - |
| $A_4$ | 14.33± 2.52 | 12.67± 1.53 | 12± 1 | 10± 1 | - | - | 12.33± 2.52 | 10.33± 2.08 | 9.67± 1.15 | - | - | - | 13.33± 2.08 | 10.67± 1.15 | 10± 1 | 8.33± 0.57 | - | - | 14.33± 2.08 | 13.67± 1.15 | 12± 1 | 9.33± 0.57 | 8± 0 | - |
| $A_5$ | 15.33± 2.52 | 13.67± 2.08 | 10.67± 1.15 | 9.33± 0.57 | 9± 0 | - | 13.33± 2.08 | 12.33± 1.15 | 11.33± 0.57 | 10± 1 | 10± 0 | - | 13.67± 1.53 | 8± 1 | 8.33 0.57 | 7± 1 | 7± 0 | - | 15.67± 2.52 | 11± 1 | 9.67± 1.15 | 9± 1 | 8± 1 | - |
| $A_6$ | 15.67± 1.53 | 12.67± 1.15 | 11.33± 0.57 | 11.33 0.57 | 9± 1 | - | 13.67± 2.52 | 13.67± 1.15 | 12± 1 | 11± 1 | 10.33± 0.57 | - | 13.67± 2.08 | 12± 1 | 9.33± 0.57 | 8.33± 0.57 | 8± 0 | 7± 0 | 15.67± 2.52 | 13.67± 1.15 | 13± 1 | 12.22± 0.57 | 11.33± 0.57 | 9± 1 |
| $A_7$ | 15.67± 2.08 | 13.67± 1.15 | 12± 1 | 12.33± 0.57 | 10± 1 | - | 15.33± 2.08 | 14.33± 1.15 | 13.33± 1.15 | 13.33± 0.57 | 10± 1 | - | 14.67± 2.08 | 13.67± 1.15 | 11± 1 | 9± 1 | 8.33± 0.57 | 7± 0 | 16.67± 1.15 | 12.33± 0.57 | 10± 1 | 9.33± 0.57 | 9.33± 0.57 | 8± 0 |
| $A_8$ | 16.67± 1.15 | 14± 1 | 13± 1 | 12.67± 1.15 | 11.33± 0.57 | - | 16.33± 0.57 | 14.33± 1.15 | 13± 1 | 13.33± 0.57 | 11± 1 | 10± 0 | 15.67± 1.15 | 14± 1 | 14.33± 0.57 | 11± 1 | 9.33± 0.57 | 9± 1 | 17.67± 2.08 | 15.33± 0.57 | 13± 1 | 12± 1 | 10.33± 0.57 | 10± 0 |
| $A_9$ | 17.67± 1.53 | 14.67± 2.08 | 14.67± 1.15 | 13.33± 0.57 | 11.33± 0.57 | 10± 0 | 17± 2 | 17.67± 1.53 | 15± 2 | 14.67± 1.15 | 13.33± 0.57 | 11.33± 0.57 | 16.33± 1.53 | 15.67± 1.15 | 13± 1 | 12.67± 1.15 | 11.67± 1.15 | 1033± 0.57 | 18.67± 1.15 | 16.67± 1.15 | 14± 1 | 13± 1 | 12.33± 0.57 | 10± 0 |

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**TABLE 5: ANTIBACTERIAL EFFECT OF *C. ALBUM* (SUBSP. *STRIATUM)* CRUDE EXTRACT AND FRACTIONS BY AGAR WELL DIFFUSION IN SIX DIFFERENT CONCENTRATION (35, 40, 45, 50, 55 AND 60 MG/ML RESPECTIVELY**

| | Bacterial Sp. | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S.infantis* | | | | | | *S.enteritidis* | | | | | | *S.typhimurium* | | | | | | *S.aureus* | | | | | |
| | 60 | 55 | 50 | 45 | 40 | 35 | 60 | 55 | 50 | 45 | 40 | 35 | 60 | 55 | 50 | 45 | 40 | 35 | 60 | 55 | 50 | 45 | 40 | 35 |
| Crude Ext. | 16± 2 | 14.33± 1.53 | 13.67± 1.15 | 12± 2 | 9± 1 | 8.33± 0.57 | 13.33± 2.52 | 10.67± 2.08 | 9.33± 1.15 | 9± 1 | 7± 0 | - | 23± 1 | 21.67± 1.15 | 19.33± 0.57 | 16.33± 0.57 | 14± 1 | 13.33± 0.57 | 17.33± 1.53 | 15.67± 2.08 | 1433± 1.15 | 13.67± 1.15 | 10.33± 0.57 | 9± 0 |
| $A_1$ | 9± 1 | 9.33± 0.57 | 8± 0 | 7± 0 | - | - | 10.67± 1.15 | 8± 1 | 7.33± 0.57 | - | - | - | 12.33± 1.53 | 11.67± 1.15 | 11± 1 | 10± 1 | 8.33± 0.57 | - | 11.33± 2.52 | 10.67± 1.15 | 8.33± 0.57 | 7± 0 | - | - |
| $A_2$ | 11.33± 0.57 | 11± 0 | 10± 0 | 8± 0 | 7± 0 | - | 12± 2 | 11± 1 | 10.33± 0.57 | 8.33± 0.57 | 7± 0 | - | 13.67± 1.15 | 13.67± 1.15 | 11± 1 | 9.33± 0.57 | - | - | 13± 2 | 12.33± 1.53 | 10± 1 | 9.33± 0.57 | - | - |
| $A_3$ | 12± 1 | 10.33± 1.53 | 10.67± 1.15 | 9± 1 | 8.33± 0.57 | - | 13.33± 2.08 | 10.67± 1.15 | 9± 1 | 7.33± 0.57 | - | - | 14± 1 | 14.33± 0.57 | 12± 1 | 9.33± 0.57 | 8± 0 | - | 15.33± 1.53 | 13.67± 1.15 | 11.33± 0.57 | 10.33± 0.57 | 8± 1 | - |
| $A_4$ | 12.33± 2.52 | 9.33± 1.53 | 8.67± 1.15 | 8± 1 | 7± 0 | - | 13.67± 2.52 | 11.33± 1.53 | 10.67± 1.15 | 8.33± 0.57 | 8.33± 0.57 | - | 14.33± 1.15 | 12.33± 1.53 | 11.67± 1.15 | 10.33± 0.57 | 8± 0 | 7.33± 0.57 | 15.67± 2.08 | 14± 1 | 11.67± 1.15 | 9.33± 0.57 | 7.33± 0.57 | - |
| $A_5$ | 13± 2 | 10.67± 1.15 | 10.33± 0.57 | 8.33± 0.57 | 7± 1 | 7.33± 0.57 | 14.33± 2.08 | 13.67± 1.15 | 11.33± 1.53 | 9± 1 | 8± 1 | 7.33± 0.57 | 15.33± 1.15 | 12± 1 | 11.33± 0.57 | 9± 1 | 8.33± 0.57 | 8± 0 | 17.67± 1.15 | 15± 2 | 14± 1 | 13± 1 | 12.33± 0.57 | 10.33± 0.57 |
| $A_6$ | 15.33± 1.15 | 13.33± 0.57 | 12.67± 1.15 | 9.33± 0.57 | 9.33± 0.57 | 8± 1 | 14.67± 2.52 | 11.67± 1.15 | 10.67± 1.15 | 8.33± 0.57 | 8± 1 | 7.33± 0.57 | 15.33± 1.53 | 12.67± 2.08 | 10.33± 0.57 | 10± 1 | 9.33± 0.57 | 8± 0 | 19± 1 | 17.33± 1.15 | 16.33± 0.57 | 15.33± 0.57 | 13± 1 | 11.33± 0.57 |
| $A_7$ | 15.67± 1.53 | 14.33± 1.15 | 11± 1 | 10± 1 | 9.33± 0.57 | 8.33± 0.57 | 15.33± 1.53 | 13± 2 | 11.33± 1.15 | 9± 1 | 8.33± 0.57 | 8± 0 | 16± 1 | 14.33± 1.53 | 13.67± 1.15 | 12.33± 1.15 | 11.33± 0.57 | 10.33± 0.57 | 21± 1 | 19.67± 1.15 | 16.33± 0.57 | 14± 1 | 14.33± 0.57 | 13± 1 |
| $A_8$ | 16.33± 0.57 | 14.67± 1.15 | 13.33± 0.57 | 11.33± 1.15 | 10± 1 | 10.33± 0.57 | 17.67± 1.15 | 16.33± 1.15 | 14.33± 0.57 | 11± 1 | 10.33± 0.57 | 9.33± 0.57 | 18± 1 | 16± 1 | 15± 1 | 14.33± 0.57 | 12.33± 0.57 | 11± 0 | 23± 0 | 21± 1 | 18.33± 1.15 | 17.33± 0.57 | 16± 1 | 14.33± 0.57 |
| $A_9$ | 19.67± 1.15 | 18.33± 0.57 | 16.67± 1.15 | 15.33± 0.57 | 13.67± 1.15 | 11.33± 0.57 | 19.33± 0.57 | 17.33± 0.57 | 15.67± 1.15 | 14± 1 | 13.33± 0.57 | 12± 0 | 18.33± 0.57 | 17.33± 1.53 | 16± 1 | 14± 1 | 13.33± 0.57 | 13.33± 0.57 | 24.33± 0.57 | 22.33± 1.53 | 20± 1 | 19± 1 | 18.33± 0.57 | 16± 1 |

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**TABLE 6: ANTIBACTERIAL EFFECT OF *C. ALBUM* (SUB SP. *STRIATUM)* CRUDE EXTRACT AND FRACTIONS BY AGAR DISK DIFFUSION IN SIX DIFFERENT CONCENTRATION (35, 40, 45, 50, 55 AND 60 MG/ML RESPECTIVELY)**

| | Bacterial Sp. | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *E.coli* | | | | | | *Sh.flexeneriae* | | | | | | *Sh.sonnei* | | | | | | *Sh.dysenteriae* | | | | | |
| | 60 | 55 | 50 | 45 | 40 | 35 | 60 | 55 | 50 | 45 | 40 | 35 | 60 | 55 | 50 | 45 | 40 | 35 | 60 | 55 | 50 | 45 | 40 | 35 |
| Crude Ext. | 19.33± | 16± | 15.33± | 13.33± | 12± | 11± | 17.33± | 15.33± | 15± | 14.33± | 13.33± | 12± | 19± | 17.33± | 15± | 14.33± | 13.33± | 12.33± | 21.33± | 19.33± | 18.33± | 18± | 17± | 16± |
| | 1.53 | 2 | 0.57 | 1.15 | 1 | 0 | 1.15 | 1.53 | 1 | 0.57 | 0.57 | 0 | 1 | 1.15 | 2 | 1.53 | 0.57 | 0.57 | 0.57 | 1.15 | 1.15 | 1 | 0 | 0 |
| $A_1$ | 13.33± | 11.33± | 8± | 8.33± | 7± | - | 12± | 10± | 9.33± | 8.33± | 8± | 7± | 12.33± | 12.33± | 11.33± | 10± | 8± | - | 13± | 12± | 12± | 9.33± | 8± | - |
| | 0.57 | 1.53 | 2 | 1.15 | 0 | | 2 | 1 | 0.57 | 1.15 | 0 | 0 | 1.15 | 0.57 | 0.57 | 0 | 0 | | 1 | 1 | 1 | 0.57 | 0 | |
| $A_2$ | 14.33± | 12.33± | 11± | 10.33± | 9.33± | - | 13± | 11.33± | 10.33± | 9.33± | 8.33± | - | 13± | 12.33± | 9.33± | 8.33± | 8.33± | 7± | 14.33± | 13.33± | 12.33± | 10.33± | 8.33± | 8± |
| | 2.08 | 1.15 | 0 | 0.57 | 1.15 | | 1 | 2.08 | 1.15 | 0.57 | 0.57 | | 1 | 1.53 | 1.15 | 1.15 | 0.57 | 0 | 1.15 | 1.53 | 1.15 | 0.57 | 0.57 | 0 |
| $A_3$ | 15± | 13.33± | 12± | 10.33± | 9.33± | 9± | 13.33± | 11± | 10.33± | 9.33± | 7± | - | 14± | 14.33± | 13.33± | 10.33± | 9.33± | 8± | 14.67± | 12± | 11.33± | 9.33± | 9± | 8± |
| | 0 | 1.15 | 1 | 1.53 | 1.15 | 0 | 0.57 | 1 | 1.15 | 0.57 | 0 | | 1 | 0.57 | 1.15 | 1.53 | 1.15 | 0 | 1.15 | 1 | 1.15 | 0.57 | 0 | 0 |
| $A_4$ | 15.33± | 13.33± | 11.33± | 10± | 9.33± | - | 14± | 11.33± | 10.33± | 10± | 9± | - | 14.33± | 12± | 10± | 9.33± | 8.33± | - | 15.33± | 14.33± | 12.33± | 10.33± | 9.33± | 9± |
| | 0.57 | 2.08 | 1.15 | 0 | 0.57 | | 1 | 1.53 | 1.15 | 1 | 0 | | 0.57 | 1 | 1 | 0.57 | 0.57 | | 0.57 | 1.53 | 1.15 | 1.15 | 0.57 | 1 |
| $A_5$ | 15.67± | 13.67± | 11.33± | 11.33± | 10.33± | 9.33± | 15.33± | 13.33± | 12± | 11.33± | 11.33± | 10± | 14.67± | 11.33± | 10± | 9.33± | 8± | 7± | 14.67± | 12.33± | 11± | 10.33± | 9.33± | 9± |
| | 1.15 | 2.08 | 0.57 | 0.57 | 1.15 | 0.57 | 0.57 | 1.53 | 1 | 1.15 | 1.15 | 1 | 1.15 | 2.08 | 1 | 1.15 | 0 | 0 | 1.15 | 2.08 | 1 | 1.15 | 1.15 | 0 |
| $A_6$ | 16.33± | 14.33± | 13± | 11.33± | 10.33± | 9± | 15.67± | 13.33± | 11± | 10.33± | 9.33± | 9± | 15± | 14.33± | 14.33± | 13± | 10± | 8± | 15.33± | 13.33± | 11.33± | 11± | 10± | 10.33± |
| | 0.57 | 2.08 | 1 | 0.57 | 1.15 | 1 | 1.15 | 1.15 | 1 | 1.15 | 0.57 | 1 | 1 | 0.57 | 1.15 | 1 | 1 | 0 | 0.57 | 1.15 | 0.57 | 1 | 1 | 0.57 |
| $A_7$ | 15.33± | 14.33± | 11± | 11.33± | 10.33± | 8± | 17.33± | 16.33± | 14.33± | 13.33± | 11± | 10± | 15.33± | 13.33± | 11± | 10± | 9.33± | 8± | 15.67± | 14.33± | 14± | 12± | 10± | 9± |
| | 0.57 | 1.53 | 1 | 1.15 | 0.57 | 0 | 1.15 | 0.57 | 1.15 | 1.15 | 1 | 1 | 0.57 | 1.53 | 1 | 1 | 0.57 | 0 | 1.15 | 0.57 | 1 | 1 | 1 | 0 |
| $A_8$ | 17.67± | 15± | 14.33± | 14.33± | 12± | 11.33± | 18.67± | 16.33± | 14.33± | 13.33± | 12.33± | 11.33± | 17± | 15.33± | 13.33± | 12± | 11.33± | 11.33± | 16.67± | 14.33± | 13± | 12± | 11.33± | 10± |
| | 1.15 | 2 | 0.57 | 0.57 | 1 | 2.08 | 1.15 | 1.53 | 2.08 | 0.57 | 1.15 | 0.57 | 2 | 1.15 | 2.08 | 1 | 0.57 | 0.57 | 1.15 | 2.08 | 1 | 1 | 0.57 | 0 |
| $A_9$ | 18.33± | 15.33± | 14.33± | 14± | 13± | 11.33± | 19.67± | 17± | 16.33± | 15.33± | 14.33± | 13± | 18.33± | 16± | 1.33± | 14.33± | 11.33± | 11± | 19± | 18.33± | 17.33± | 15± | 13.33± | 12.33± |
| | 2.52 | 1.53 | 1.15 | 1 | 1 | 0.57 | 1.15 | 2 | 1.15 | 0.57 | 0.57 | 0 | 2.52 | 1 | 1.15 | 0.57 | 0.57 | 0 | 1 | 0.57 | 1.15 | 1 | 0.57 | 0.57 |

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**TABLE 7: ANTIBACTERIAL EFFECT OF *C. ALBUM* (SUBSP. *STRIATUM)* CRUDE EXTRACT AND FRACTIONS BY AGAR DISK DIFFUSION IN SIX DIFFERENT CONCENTRATION (35, 40, 45, 50, 55 AND 60 MG/ML RESPECTIVELY)**

| | Bacterial Sp. | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S.infantis* | | | | | | *S.enteritidis* | | | | | | *S.typhimurium* | | | | | | *S.aureus* | | | | | |
| | 60 | 55 | 50 | 45 | 40 | 35 | 60 | 55 | 50 | 45 | 40 | 35 | 60 | 55 | 50 | 45 | 40 | 35 | 60 | 55 | 50 | 45 | 40 | 35 |
| Crude Ext. | 17.33± 2.52 | 16.33± 1.15 | 15.33± 1.53 | 13± 1 | 11.33± 0.57 | 10± 0 | 15.33± 1.15 | 14.33± 1.53 | 12.67± 1.15 | 12.33± 0.57 | 10± 1 | 9.33± 0.57 | 24.33± 1.53 | 21± 2 | 18± 2 | 16± 1 | 15.33± 0.57 | 13± 1 | 19.33± 1.53 | 17.33± 1.15 | 16± 1 | 14± 2 | 12± 1 | 10.33± 0.57 |
| $A_1$ | 11.33± 0.57 | 10.33± 0.57 | 10± 1 | 9.33± 0.57 | 8.33± 0.57 | 8± 0 | 12.33± 2.52 | 10.33± 1.15 | 8± 2 | 8± 1 | 7± 0 | - | 12.33± 1.15 | 12± 0 | 11.33± 0.57 | 10± 1 | 10± 0 | 9.33± 0.57 | 13.67± 1.15 | 11.33± 1.53 | 10± 1 | 9.33± 0.57 | 9.33± 0.57 | 7± 0 |
| $A_2$ | 12± 0 | 11± 0 | 10± 1 | 9.33± 0.57 | 9± 0 | 8± 0 | 13± 0 | 12± 1 | 11± 0 | 10± 1 | 10.33± 0.57 | 9.33± 0.57 | 13± 0 | 12± 1 | 11± 1 | 10.33± 0.57 | 9.33± 0.57 | - | 15.33± 2.52 | 14.33± 1.15 | 11± 2 | 10± 1 | 9.33± 0.57 | - |
| $A_3$ | 13± 1 | 11.33± 1.15 | 10± 1 | 10± 0 | 9± 1 | 9± 0 | 15± 0 | 13± 1 | 11.33± 1.15 | 9± 1 | 8± 0 | - | 14± 0 | 13.33± 0.57 | 12± 1 | 11.33± 0.57 | 10± 1 | 9± 0 | 17± 0 | 15± 2 | 13.33± 1.15 | 10± 1 | 10± 0 | 9.33± 0.57 |
| $A_4$ | 13.33± 0.57 | 11± 2 | 10± 1 | 9.33± 0.57 | 9± 0 | 8± 0 | 15.33± 0.57 | 13.33± 2.08 | 11.33± 1.53 | 10± 1 | 9.33± 0.57 | 9± 0 | 15± 1 | 14± 1 | 13.33± 1.53 | 12± 1 | 10.33± 1.53 | 9± 0 | 17.33± 0.57 | 14.33± 1.15 | 11.33± 1.53 | 10± 1 | 9.33± 0.57 | 9± 0 |
| $A_5$ | 14.33± 2.52 | 13.33± 1.15 | 11.33± 2.08 | 10.33± 0.57 | 9.33± 0.57 | 8± 0 | 16± 0 | 15.33± 0.57 | 13.33± 1.53 | 11.33± 1.15 | 10± 1 | 9.33± 0.57 | 15.33± 0.57 | 14.33± 0.57 | 11.33± 2.08 | 10± 0 | 9.33± 0.57 | 9± 1 | 19.33± 2.08 | 17± 2 | 14.33± 1.53 | 13± 0 | 11.33± 2.08 | 11.33± 1.15 |
| $A_6$ | 16± 1 | 15.33± 1.15 | 13.33± 2.08 | 11± 1 | 10± 0 | 9.33± 0.57 | 15.33± 2.52 | 12± 2 | 10± 1 | 9.33± 0.57 | 8± 0 | - | 16.33± 2.08 | 13.33± 0.57 | 12± 1 | 11.33± 0.57 | 10± 1 | 9.33± 0.57 | 22± 1 | 20.33± 2.08 | 17± 1 | 15± 2 | 14.33± 0.57 | 13± 1 |
| $A_7$ | 17± 0 | 14± 2 | 13± 1 | 12± 1 | 10.33± 0.57 | 10± 0 | 17.33± 2.08 | 15± 1 | 12± 2 | 11± 1 | 9.33± 0.57 | 9± 0 | 17.33± 2.52 | 14.33± 1.15 | 13± 1 | 12.33± 0.57 | 11± 0 | 11± 0 | 23.33± 2.08 | 21.33± 1.53 | 18.33± 2.52 | 17± 1 | 16.33± 1.15 | 15± 0 |
| $A_8$ | 17.33± 2.08 | 15.33± 2.52 | 14.33± 1.15 | 13± 0 | 12± 1 | 11.33± 0.57 | 19.33± 0.57 | 17± 2 | 14.33± 2.52 | 13± 1 | 12.33± 1.15 | 11± 1 | 19± 2 | 18.33± 2.08 | 16.33± 1.53 | 15± 1 | 14± 1 | 13.33± 0.57 | 25± 0 | 23.33± 1.53 | 20± 2 | 18.33± 2.08 | 16± 1 | 15.33± 0.57 |
| $A_9$ | 20.33± 0.57 | 17± 2 | 15± 1 | 14.33± 1.15 | 13.33± 0.57 | 12± 1 | 20.33± 1.15 | 18.33± 1.53 | 15± 2 | 15± 1 | 14.33± 0.57 | 13± 1 | 20.33± 1.53 | 19± 1 | 17± 2 | 16± 1 | 16± 0 | 15.33± 0.57 | 26.33± 1.53 | 24.33± 1.15 | 21± 2 | 20.67± 1.15 | 19± 1 | 18.33± 0.57 |

**TABLE 8: PANEL OF TEST ORGANISMS FOR *IN VITRO* ANTIBACTERIAL SCREENING**

| Species | Antibiotic Resistance Pattern |
|---|---|
| *Staphylococcus aureus* | AN, AZM, FOX, CP |
| *E. coli* | AZM, CPM, CRO, CAZ, CTX, AM |
| *Sh. flexneri* | AMC, NA, CAZ, AM, TIC, CTX, CRO, TE, S, SXT, CF |
| *Sh. sonnei* | AMC, AM, TOB, TIC, CTX, CRO, TE, S, SXT |
| *Sh. dysenteriae* | AMC, AM, TIC, CTX, CRO, K, GM |
| *S. infantis.* | AMC, NA, CAZ, AM, TIC, CTX, CRO,CT, PIP, D, TE, S, SXT, CF |
| *S. enteritidis* | AMC, NA, AM, PIP, D, TE, SXT |
| *S. typhimurium* | AMC, AM, PIP, TE, S, C |

AN: Amikacin, AZM: Azithromycin, FOX: Cefoxitin, CPM: Cefpiramide, CP: Ciprofloxacin, CRO: Ceftriaxone, CAZ: Ceftazidime, CTX: Cefotaxime AM: Ampicillin, AMC: Amoxycillin-clavulanic acid, NA: Nalidixic acid, TIC: Ticarcillin, TE: Tetracycline, S: Streptomycin, SXT: Trimethoprim-sulfamethoxazole, CF: Cephalothin, TOB: Tobramycin, K: Kanamycin, GM: Gentamycin, CT: Ceftizoxime, PIP: Piperacillin, D: Doxycyllin, C: Chloramphenicol

**DPPH Radical Scavenging Activity:** Antioxidant activity of crude extract and nine different fractions were evaluate by DPPH radical scavenging. Polyphenol compounds are most effective antioxidants [30]. In the present study antioxidant activity of crude ethanolic extract and nine different fractions in different concentrations (0.2, 0.4 and 0.6 mg/ml) were tested and results are shown in **Table 8**. All of crude extract and fractions had antioxidant activity. The antioxidant activity was increased by the order of polarity so that MeOH:$H_2O$ (80:20) showed significant radical scavenging activity in each tested concentration. In this study syntethic antioxidant BHT used as standard antioxidant activity of crude extract and fractions to be compared. As shown in **Table 9**, the Antioxidant activity (Inhibition %) concentration of MeOH: $H_2O$ (80:20) fraction was similar to that of BHT. These results suggested that MeOH: $H_2O$ (80:20) fraction contains of compounds which exhibit strongest free radical scavenging activity.

**TABLE 9: DPPH SCAVENGING CAPACITY**

| Extract | Antioxidant activity (Inhibition %) |
|---|---|
| Crude Ext | 50.33±5.79 |
| Frc.1 | 20.27±0.07 |
| Frc.2 | 30.92±4.11 |
| Frc.3 | 72.01±0.38 |
| Frc.4 | 79.55±1.45 |
| Frc.5 | 84.36±2.69 |
| Frc.6 | 89.55±2.84 |
| Frc.7 | 91.02±5.17 |
| Frc.8 | 92.94±2.09 |
| Frc.9 | 95.1±2.13 |
| BHT(Standard) | 95.3±0.43 |

**Total Phenolic and Flavonoid Content:** Phenolic compounds are a class of antioxidant which play an important role as free radical terminator and their bioactivities may be related to their ability to do different mechanism such as inhibit lipoxygenase, chelate metals and scavenge free radicals. DPPH assay do as the last one mechanism [8]. The total phenol and flavonoid contents in *C. album* (sub sp. *striatum*) crude extract and fractions were measured spectrometrically according to the Folin-Ciocalteu method and expressed in terms of gallic acid equivalents ($R^2$=0.986). The concentration of flavonoids was determined by $AlCl_3$ assay and calculated as rutin equivalent ($R^2$=0.997). The total phenol and flavonoid contents of crude extract and nine different fraction of *C. album* (sub sp. *striatum*) are presented in **Table 10**.

**TABLE 10: PHENOL AND FLAVONOID CONTENT OF *C. ALBUM* (SUB SP *STRIATUM*)**

| Extract | Total Phenol [1] | Total Flavonoid [2] |
|---|---|---|
| Crude Ext | 0.892±0.011 | 4.873±0.029 |
| Frc.1 | 0.116± 0.001 | 1.864±0.034 |
| Frc.2 | 0.182±0.009 | 2.431±0.060 |
| Frc.3 | 0.210±0.002 | 2.739±0.034 |
| Frc.4 | 0.243±-0.007 | 2.814±0.028 |
| Frc.5 | 0.327±0.023 | 3.419±0.092 |
| Frc.6 | 0.386±0.007 | 3.696±0.053 |
| Frc.7 | 0.409±0.007 | 4.484±0.028 |
| Frc.8 | 0.533±0.013 | 4.365±0.049 |
| Frc.9 | 0.865±0.010 | 4.535±0.025 |

mg of GAE/g of extract
mg of RU/g of extract

The results demonstrated that the phenolic content of *C. album* (Sub sp. *striatum*) increased in the order of increasing of polarity. Therefore crude ethanolic extract followed by MeOH: $H_2O$ (80:20) fraction and methanolic fraction had a higher total phenolic content.

On the other hand, crude ethanolic extract followed by MeOH: $H_2O$ (80:20) and MeOH: DCM (50:50) fraction contained higher flavonoid concentration.

MeOH: $H_2O$ (80:20) fraction had higher phenol and flavonoid content and also possessed the highest DPPH scavenging. Therefore this fraction can be consider as an effective antioxidant.

In recent times, searching for new and effective antibacterial and antioxidant agents has become a very important and serious global crisis, considering escalating levels of antibiotic resistant among pathogenic bacteria species and some chronic disease like cancer which continuously challenges the scientific community [31, 32]. So scientifics are directed to seek more organic and natural compounds for this solution [33].

One of the efforts in this research is focused on the use of medicinal plants, which have been remedies for human disease for a long time because they contain components of therapeutic value [34]. Medicinal plants contain diverse classes of bioactive compounds such as tannins, alkaloids, flavonoids and polyphenolic compounds, which in turn are responsible for various pharmacological properties [35, 36].

These secondary metabolites play an important role in the medicinal properties of plants. Many reports are available on the antifungal, antiviral, antibacterial, antioxidant and anti-inflammatory properties of plants, thus therapeutic properties of medicinal plants are well recognized [37, 38].

It is imperative that less expensive antibacterial and antioxidant agents should be developed to cure all of patients, regardless of financial status so medicinal plants can be the best option.

As we mentioned before, some medicinal have been known for their antibacterial properties, but their efficacies against MDR bacteria have not been well-documented in the medicinal literature. *C. album* (Sub sp *striatum*) has been used locally for its traditional and medicinal properties however, its efficacies against MDR bacteria have not been studied.

The microdilution method was used in the present study because it is a quantitative reference method routinely used in clinical laboratories. In this method, susceptibility panels in 96 well microtiter plates contained various concentrations of antimicrobial inoculated into the wells of the microtiter plates and incubated overnight at 37 °C. Compared with agar-based methods, broth microdilution can decrease much labor and time [39].

As previousely mentioned, it has been surmised that the antibacterial and antioxidant activities of herbal plant extracts and essential oils are focused on the structures and also cellular membranes and due to the presence of various bioactive compounds and extensive chemical profiles, it is likely that the antimicrobial potency is not just caused by one solitary mechanism but rather by several events at a cellular level [40, 33.]

On the other hand phenolic compounds (*e.g.* phenolic acid, flavonoids, coumarin, quinone, etc) possess a wide range of antioxidant and antimicrobial activity [41, 42]. Various antioxidant and antibacterial agents have different polarity so we can isolate using different solvents [43].

*C.album* (sub sp *striatum*) showed significant antagonist activities against MDR gram-positive and gram-negative bacteria and also potent antioxidant effect.

**CONCLUSION:** Our results describe broad-spectrum antimicrobial and antioxidant activity of *C. album* (sub sp *striatum*) crude ethanolic extract and nine different fractions. These properties could indicate the use of *C. album* (subsp *striatum*) in medical and pharmaceutical applications as potential antimicrobial and antioxidant agents and as effective preservatives. Additional and complementary studies are required concerning phytochemical screening, physiological analysis, isolation, purification and quatification of bioactive components for its *in vivo* assessment. This study showed *C. album* (Sub sp *striatum*) potential as a novel and cost-effective antibacterial agent against MDR bacteria and also as antioxidant agent.

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**CONFLICT OF INTERESTS:** The authors declare that they have no competing interests.

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