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# ANTIBACTERIAL, ANTIPARASITIC AND PHYTOCHEMICAL ACTIVITIES OF *CHENOPODIUM ALBUM* (BATHUA) PLANT EXTRACT

**MUHAMMAD SULEMAN[1], ABU UL HASSAN FAIZ* AND FAKHR-I-ABBAS[2]**

*Department of Zoology, Women University of AJ&K, BAGH, Pakistan*

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**Abstract**

Evaluation of the antimicrobial, antiparasitic and phytochemical potential of medicinal plant *Chenopodium album* (Bathua) leaves extract was carried out. The plant extracts were prepared by using distilled water and methanol as an extraction solvents. Pure methanolic extract of leaves showed 15 mm against *Staphylococcus auerus* after 24 hrs. Aqueous extract showed no activity against *S. auerus*. Similarly in case of *S. typhi* pure methanolic leaves extract showed 13 mm. While standard discs gentamicin showed (DIZ) 18 mm after 24 hrs, different dilution inhibition zone was maximum in case of 100% pure extract, and decreased with increasing dilution. Minimum DIZ was observed at 25% and no zone at 5% dilution. Anthelmintic activity of crude methanolic extract of *C. album* leaves were tested, all extracts showed maximum corrected mortality of 100%, aqueous extract showed better antiparasitic activity than methanolic extract. It can be a potential source of therapeutic agent. Further analysis is needed for clinical trial. Phytochemicals screening was carried out which reveals the presence of alkaloids, tannins, phenols and flavonoids in studied plant extract. The nutritional analysis indicated that *C. album* may be a rich source of carbohydrate.

*Chenopodium album* (Bathua) is one of the most important medicinal plant in Pakistan and its different parts are utilized in the traditional system of medicine (Jabbar *et al*. 2007). The leaves of studied plants are rich in proteins and have essential amino acids (lysine, leucine, and isoleucine), with calcium, vitamins A and C (Poonia *et al.* 2015). The plant is traditionally used as anthelmintics, cardiotonic, carminative, and digestive, diuretic, laxative and is also used for treatment of peptic ulcer, dyspepsia, flatulence. It is also used for the treatment of hepatic disorders, spleen enlargement, intestinal ulcers and in burns (Usman *et al*. 2010). Many plant tissues contain a variety of compounds called "secondary" grouped as glycosides, saponins, tannins, alkaloids, essential oils, organic acids and others (Fraenkel *et al*. 1959). The present study was designed to find to analyze the anti-microbial activities of plant extract against *Staphylococcus auerus*, *S. typhi* and against antihelminthes. The phytochemical screening of plant was also carried out.

The fresh leaves and stems of *Chenopodium album* were collected from various places of Bagh AJK, washed under running tap water and ethanol. The leaves were dried under shade for three weeks.

The dried leaves were then homogenized by using a grinder to make fine powder and stored in air tight bottles. The aqueous and solvent extract were prepared by dissolving 100 g of leaf dried powder in 1000 ml distilled water and methanol was added in separate conical flasks respectively, and then kept in a shaking incubator for 6 hrs. The extract was filtered by using Whatman No. 1 filter paper. The solvents were then evaporated to make the final volume (250 ml) one-fourth of the original volume and stored at 4ºC until for further use.

*Author for correspondence: <sabulhussan@gmail.com>. [1]Department of Chemistry, Riphah International University, Faisalabad Campus, Pakistan. [2]Bioresource Research Centre, Islamabad, Pakistan.

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The dilutions of stem and leaves extract was done in different organic solvents like ethyl acetate, ethanol, and DMSO (25, 50, 75 and 100%) and their activities were checked against bacteria and parasites. The sensitivity test was made by spread plate method for Gram-positive and Gram-negative bacteria (*Staphylococcus aureus, Salmonella typhi* and *E. coli* ).

Bacterial strains were grown in nutrient agar medium and Macconkey agar was used for Gram-negative bacteria. A 50 µl of bacterial suspension was introduced in to the sterile medium. The soaked discs were transferred to the inoculated plates by a sterile forceps. Antibiotic (Gentamicin) was used as a positive control and Petri plates were set in incubator for 24 hrs at 37°C.The growth was observed after 24 hrs and the diameter of inhibitory zone (DIZ) was measured.

For evaluation of anthelmintic efficacy, adult *Haemonchus contortus* worms were collected from the abomasii of freshly slaughtered goat. The motile worms were cleaned with lukewarm normal saline solution. The cleaned worms were transferred in beaker containing Lock's solution at 37°C. Different concentrations *viz*., 5, 25, 50, 75 and 100% of various extracts were prepared in Lock's solution for evaluation of their anthelmintic activity. Ten adult *H. contortus* were taken in each small Petri dish having different dilutions of test extract in Lock's solution *viz.,* 5, 25, 50, 75 and 100%. Total volume of each Petri dish was kept 15 ml restricted 15 ml Lock's solution, was used as control. It was then incubated at 37°C for hrs and number of live and dead adult worms was counted at 2, 4, 6, 8, 12 hrs interval. The minimum fatal time for all the ten worms in each extract was recorded.

For alkaloid screening, plant extract was treated with a few drops of Hager's reagent while Tannins screening plant extract was treated with 5% ferric chloride. The saponins screening was done by boiling plant extracts with distilled water. The terpenoids screening was carried out by treating plant extracts with 3 ml of chloroform and 2 ml of $H_2SO_4$ and noted color while flavonoids screening plant extract was treated with 10% acetic acid.

The nutritional analysis of *Chenopodium* leaves was done by following method of, Association of Official Analytical Chemists (AOSC) (1975).

Phytochemicals screening showed the presence of medicinally important bioactive: alkaloids, saponins, glycosides, steroids, terpenoids, flavonoids and tannins.

Table 1 shows the antibacterial activity of *Chenopodium album* leaves extract against Gram positive bacteria *Staphylococcus aureus. In vitro* antibacterial activity was carried out with different dilutions of extract with some solvents such as ethanol, ethyl acetate and dimethyl sulphoxid (DMSO). DMSO extract showed comparatively maximum inhibition zone 12 mm followed by ethyl acetate with inhibition zone 11 mm and then ethanol with inhibition zone 9 mm. All extracts exhibited that on increasing dilution antibacterial activity decreased. Sample having least concentration of extracts showed less activity while on high concentration activity also increased (Parkash *et al.* 2015). It was also worked with the similar dilution against *S. aureus* with inhibition zone 9.25, 11, 10.87 and 11 mm. The findings of anti-microbial activity are similar to those reported by Külcü *et al.* (2019). The zone of inhibition recorded in previous studies (*Staphylococcus aureus*) were 20.5 mm (Pandey *et al.* 2014).

The antimicrobial activity was also observed by Ramproshad *et al.* (2018) and similar results were reported. Similar anti-microbial activity was also reported by Kaur *et al*. (2018) on different bacteria (*E. coli* and *Lacto bacillus*) and found inhibition zone in the range of 19 mm.

Statistical analysis ANOVA single factor summary showed that similar results in case of ethanol (6 mm) and DMSO (6.6 mm) while ethyl acetate (4 mm) showed comparatively less activity. A single factor ANOVA result indicate p value 0.00326 which reject null hypothesis that methanol extract does not retard growth of both types of bacteria.

The results exhibit the antibacterial activity of *C. album* leaves extract for *S. typhi*a bacterium (Table 1). *In vitro* antibacterial potential of the leaf extract was performed with three solvents, namely ethanol, ethyl acetate and DMSO. Low concentration of extract showed no activity while on increasing concentration of extract in different solvents shows slight difference in activity against organism as in case of 100% ethanol maximum activity recorded as 10 mm, while in case of ethyl acetate 8 mm and DMSO showed 12 mm at 100%.

**Table1. Antibacterial activity of *C. album* leaf extract against a Gram-positive and a Gram-negative bacterium.**

| Conc. (%) | *Staphylococcus aureus* DIZ | | | *Salmonella typhi* DIZ | | |
|---|---|---|---|---|---|---|
| | Ethanol (mm) | Ethyl acetate (mm) | DMSO (mm) | Ethanol (mm) | Ethyl acetate (mm) | DMSO (mm) |
| 5 | No zone | No zone | No zone | No zone | No zone | No zone |
| 25 | 5 ± 0.02 | No zone | 6 ± 0.09 | 4 ± 0.03 | 4.3 ±0.08 | 7 ± 0.04 |
| 50 | 8 ± 1.09 | 5 ± 1.02 | 8 ± 0.7 | 6 ± 0.12 | 5 ± 0.02 | 10 ± 0.08 |
| 75 | 8 ± 0.03 | 7 ± 0.07 | 9 ± 0.2 | 8 ± 0.98 | 6 ± 0.4 | 11 ± 1.04 |
| 100 | 9 ± 0.04 | 8 ± 1.03 | 10 ± 1.02 | 10 ± 0.34 | 8 ± 0.9 | 12 ± 0.8 |
| Water | 0 ± 1.04 | 0 ± 0.03 | 0 ± 0.09 | 0 ± 1.02 | 0 ± 1.04 | 0 ± 0.3 |
| Antibiotic (amoxicillin) | 11 ± 0.09 | 10 ± 0.9 | 12 ± 0.4 | 15 ± 0.9 | 11.5 ± 0.45 | 17 ± 0.2 |

DIZ - Diameter of inhibitory zone.

Table 2 shows anthelmintic activity of *Chenopodium album* leaf extract in water and in methanol against *Haemonchus contortus.* Aqueous leaf extract and methanol extract showed 100% mortality at 100 and 75% concentrations. It might be due to effect of different phyto-chemicals present in plant extract. *Chenopodium album* may be used as an alternative treatment of gastrointestinal nematodes. These findings are in agreement with the findings of Jabbar *et al.* (2007) where they showed 100% mortality in aqueous and methanolic extract. These findings are also partially in agreement with the findings of Silveira, Chagas *et al*. (2012) where they showed 100% mortality in aqueous and methnolic extracts.

The anti-helmintetic and Schistosomiasis by *C. album* leaves was reported by Akhtar *et al.* (1999) and leaf extract was reported to be highly significant against parasites (Pal *et al.* 2011). The use of *C. album* leaves as anthelmintics medicine was also reported in eastern medicine and/or folk medicine (Kokanova *et al.* 2009). The antihelmintic activity was also studied on sheep parasite (*Haemonchus contortus*) and proved to be effective against control of mature parasites and its eggs (Jabbar *et al.* 2007)

Yellow precipitates confirmed the alkaloids while green precipitates confirmed the presence of tannins. Development of foam was sign of saponins. Development of reddish brown color of solution confirmed the terpenoids, Yellow solution turned to colorless was the sign of flavonoids. Formation of violet to blue color was indication of glycosides (Table 3). These findings are partially in agreement with the findings of Vijay *et al.* (2011) where they reported flavonoids, saponins, triterpenoids were the major constituents of the genus *C. album*. The presence of constituent (Flavonoids, saponins, triterpenoids) was also reported by Rashi *et al.* 2019. The results of phytochemical constituents are similar as reported by previous studies (Kaur *et al.* 2018). The concentration of phytochemical constituents was determined by calculating the plotting optical density by standard graph of catechol.

This study has a successful approach in the direction of new antimicrobial drugs discovery from plant origin. It has revalidated that *C. album* acts as a remedy for different microbial diseases not only useful for human being but also for animal and crops related diseases. These findings indicate that further studies on chemical and biological properties of this plant should be performed.

**Table 2. Ant-helminthic activity of *C. album* leaf extract against *Hameonchus contortus.***

| Treatment | No. of parasite exposed | Time of exposure No. of parasites found dead (hrs) | | | | | % corrected mortality |
|---|---|---|---|---|---|---|---|
| | | 2 | 6 | 10 | 14 | 18 | |
| Aq. leaves extract | 08 | 00 | 00 | 04 | 08 | - | 100 |
| Me OH 5% | 08 | 00 | 00 | 00 | 04 | 05 | 25 |
| 25% | 08 | 00 | 00 | 02 | 04 | 05 | 25 |
| 50% | 08 | 00 | 01 | 02 | 05 | 08 | 50 |
| 75% | 08 | 00 | 02 | 03 | 08 | - | 100 |
| 100% | 08 | 00 | 02 | 04 | 08 | - | 100 |
| Control | 08 | 00 | 00 | 00 | 00 | 04 | |

Mortality; ratio of deaths.

**Table 3. Phytochemical analysis of *Chenopodium album*.**

| Phytochemicals screening | Aqueous extract | Me OH extract | Concentration Ug/ml | Absorbance at 517 nm | % inhibition of *C. album* |
|---|---|---|---|---|---|
| Alkaloids | + | _ | 10 | 558 | 46.01 |
| Tannins | + | _ | 20 | 429 | 59.1 |
| Saponins | + | _ | 30 | 393 | 62.0 |
| Terpenoids | + | _ | 40 | 225 | 79.0 |
| Flavonoids | + | _ | 50 | 200 | 81.0 |
| Glycosides | + | _ | Standard | 1020 | |

+ Indicates the presence of constituent, - Indicates the absence of constituent.

**Table 4. Nutritional analysis.**

| Constituents | Chenopodium leaves (%) |
|---|---|
| Moisture contents | 7.01 |
| Ash | 23 |
| Protein | 30.1 |
| Fats | 4.9 |
| Carbohydrates | 42.1 |
| Dietary fibers | 0.5 |

The results of nutritional analysis is presented in Table 4 which shows low percentage of dietary fibers and high percentage of carbohydrates. The present finding of nutritional analysis is similar to the reports by Pandey *et al.* (2014).

Here, it is reported that *C. album* medicinal plant is cheap and rapid growing source for harvesting of phytochemicals (Flavonoids, saponins, triterpenoids) and substitute of costly antibiotics. The plant can be used to control parasite burden in domestic animals instead of expensive veterinary medicine. Furthermore the use of studied plant has low side effects and also can be used for nutritional value. Phytochemicals showed antimicrobial activities due to its complex structure and inhibit the growth of bacterial species by rupturing their cell walls.

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