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ASSESSMENT OF NATURAL PHYTOCHEMICALS, ANTIOXIDANT CAPACITIES, AND ANTIBACTERIAL PROPERTIES OF *CHENOPODIUM ALBUM* LEAVES

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ABSTRACT

Now a day antibiotic resistance of drug in the treatment of infectious diseases is a major concern. According to the many researches plants products exerts great potential to cure a variety of infectious diseases since time immemorial. The present work was attempted to study different characteristics of *Chenopodium album* (*C. album*) leaves in different solvents to understand its health benefits. Several tests were conducted to estimate different phytochemicals (such as phenols and tannins, flavonoids, saponins, glycosides, steroids, terpenoids, alkaloids, amino acids and anthroquinones). High phenol and flavonoid content were observed in methanol extract of the leaves. Maximum antioxidant activity was also reported in methanol extracted *C. album* leaves determined by DPPH scavenging activity. The *in vitro* antibacterial activity of *C. album* leaves was determined using agar well diffusion method. The leaf extract exhibited higher antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* (gram positive bacteria), and *Escherichia coli* and *Klebsiella pneumoniae* (gram negative bacteria). Results were also compared with standard antibiotic drug. All the extracts showed potential effective antibacterial activity.

Keywords: *Chenopodium album*, antibacterial activity, plant phenols, antioxidants

INTRODUCTION

Worldwide progression of microbial infectious diseases is the major health concern. The use of antibiotic drugs and the proper hygiene rule was the very effective to cure but long lasting and inappropriate use of antibiotic drugs increases the drug resistance as result of the spread resistance phenotype (Djeussi *et al*., 2016) **[1]**. The progression of antibiotic resistance is a major concern in the treatment of microbial infectious disease worldwide **[2]**. According to the WHO reports, currently drug resistance cause 700,000 deaths every year and it will be rise 10 million deaths each year by 2050 **[3]**. There is need to search alternative therapy to combat Multi drug resistant bacteria. Since ancient times, edible plant leaves and other plant parts are known for their medicinal properties **[4]**. Most of the world population (~80%) is relying on the use of plant products (as traditional medicines) for primary health care **[5]**. Natural and biologically active extracts are of keen interest to the scientists in fighting against deadly diseases **[6]**. The extraction and characterization of potential active compounds in such extracts resulted in the development of new drugs **[7, 8]**. The secondary metabolites produced by plants such as flavonoids, alkaloids, tannins, and terpenoids are responsible to give antimicrobial properties to the medicinal plants **[9]**.

C. album is known to be a rich source of flavonoid, glycosides, terpenoids, and phenolic acid **[10]**. Plants belonging to genus Chenopodium are resistant to adverse climate and edaphic conditions. Chenopodium species have been cultivated for centuries as leafy vegetables and fodder due to high protein content **[11, 12]**. It is also recognized as a key source of Vitamin-C and beta-carotene present in young shoots and mature plants **[13]**. According to researches *C. album* showed the pharmacological properties such as anti-inflammatory, antiallergic, immunomodulating, antiseptic, antifungal and antiviral activity **[14, 15]**. The study was conducted to evaluate the antioxidant, phytochemical potential of the crude extracts and the antibacterial activity of *C. album* leaves against the bacterial strains namely *Escherichia coli Staphylococcus aureus, Klebsiella pneumoniae* and *Bacillus subtilis*.

MATERIAL AND METHODS

Plant material and Preparation extracts

C. album leaves were procured from a local market in Ghaziabad district of Uttar Pradesh (India). The leaves were washed thoroughly under tap water and air dried under shade. The dried leaves were grounded in a mixer grinder. The plants material was taken in a soxhlet apparatus for extraction in different solvents (ethanol, methanol and distilled water). The obtained extracts were evaporated on a water bath at

100ºC until the semi-solid extracts were produced. Prepared the stock of the leaves extract (25, 50, 75 and 100 mg/ml) with respective solvent and dissolved with the help of dimethyl sulfoxide (DMSO). The leaves extracts were stored at 4ºC for further studies **[16, 17]**.

Phytochemical screening

Phytochemical screenings were done for the qualitative determination of secondary metabolites of plants **[4, 17-19]**.

Qualitative Phytochemical screening

Phenols and Tannins

Leaves extract was mixed with 2 ml of 2% ferric chloride ($FeCl_3$) solution. The presence of phenols and tannins confirmed with the blue-green or black coloration.

Alkaline reagent test for flavonoids

Leaves extract was mixed with 2ml of 2% sodium hydroxide (NaOH) solution. Addition of few drops of diluted acid formed the intense yellow colour which became colourless which confirm the existence of flavonoids.

Saponins

Leaves extract was mixed with 5ml of distilled water and shaken vigorously. The existence of stable foam confirms the presence of saponins.

Liebermann's test for Glycosides

Leaves extract was mixed with chloroform and acetic acid (1:1). Sulphuric acid (concentrated) was added in the mixture carefully. The sequential colour change from violet to blue to green showed the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Steroids

Leaves extract was mixed with 2 ml of chloroform and then concentrated H_2SO_4 was added in the mixture. The presence of steroid confirms with the generation of red colour in the lower layer of chloroform.

Terpenoids

Leaves extract was dissolved in chloroform (2ml) and evaporated to dryness. Concentrated H_2SO_4 (2 ml) was added and then heated for 2 min. A greyish colour confirmed the presence of terpenoids.

Alkaloids

2ml of 1% hydrochloric acid (HCl) was added into the leaf extract mixture and heated gently. Mayer's reagent was then added to the extract mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Amino Acids

Few drops of ninhydrin reagent was added in the 1 ml of leaf extract. The purple colour indicates the presence of amino acids.

Anthraquiones

Leaf extract (5 ml) was hydrolysed with diluted H_2SO_4, benzene and ammonia solution. Appearance of rose-pink colouration confirms the presence of anthraquinones.

Quantitative phytochemical screening

Determination of Total Phenolics

Total phenolic content in leaf extract was determined using the Folin-Ciocalteu colorimetric method **[17, 20, 21]**. The values are expressed as mg gallic acid equivalents (GAE) /100g extract).

Determination of Total Flavonoids

Total flavonoid content was determined using a colorimetric method **[17, 20]**. The values are expressed in mg of quercetin equivalents per g of extract.

In vitro Antioxidant assays*: *Evaluation of free radical scavenging activity by DPPH method

This method measures the free radical scavenging capacity of the extracts (4,22) at 517 nm wavelength. The percent radical scavenging activity is calculated as:

Scavenging effect (%) = $100 \times (A_b - A_s)/A_b$

Where A_b is the absorbance of the blank; A_s is the absorbance of the sample.

Antimicrobial activity

The test organisms *Escherichia coli* (MTCC1234), *Staphylococcus aureus* (MTCC1144), *Klebsiella pneumoniae* (MTCC4030) and *Bacillus subtilis* (MTCC1133) were collected from the Codon Biotech Laboratory Noida, U.P. The cultures were sub-cultured on sterilized nutrient agar and stored at 4°C for the further study. Nutrient agar medium was used for antibacterial activity. Nutrient agar medium plates were prepared and freshly prepared microbial broth culture (about 0.1 ml) was spread over the agar plates. 6 mm diameter wells were made in each plate with the help of borer. 0.1 ml of leaf extract was loaded individually in the wells and plates were incubated for 24 h at 37ºC in the incubator. After incubation, the diameter of clear zone of inhibition was measured (in mm) and compared with standard drug.

RESULTS AND DISCUSSION

In the present study, leaves of *C. album* are screened for their phytochemical constituents as well as antimicrobial and antioxidant potential. Quantitative analysis of total phenolic content (TPC) and total flavonoid content (TFC) in the plant leaves is also done.

Phytochemical screening

Table 1: Phytochemicals present in different extracts of *C. album* leaves (+ Presence, - Absence)

		Chenopodium album		
S. No.	**Test name**	**Methanol extract**	**Ethanol extract**	**Aqueous extract**
1	**Phenols**	+	+	+
2	**Tannins**	+	+	+
3	**Flavonoids**	+	+	+
4	**Saponins**	+	+	-
5	**Glycosides**	+	+	-
6	**Steroids**	+	+	-
7	**Terpenoids**	+	+	+
8	**Alkaloids**	+	-	+
9	**Amino acids**	+	+	+
10	**Anthroquinones**	-	-	-

Total Phenol Content in *Chenopodium album* extracts

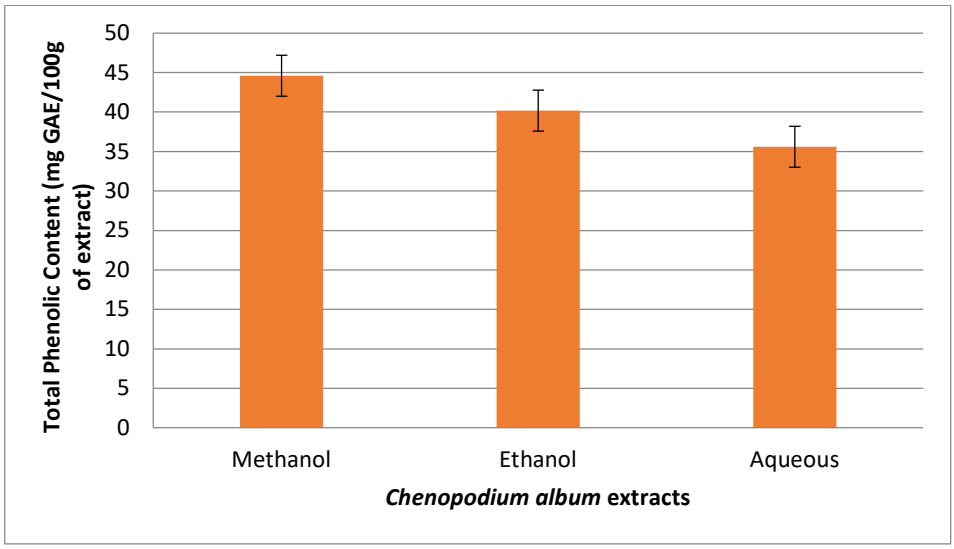

Figure 1: Total phenol content in *C.album* leaf extracts. Phenolic content is reported in terms of mg of Gallic Acid Equivalents per 100g of the extract

Total Flavonoid Content in *Chenopodium album* extracts

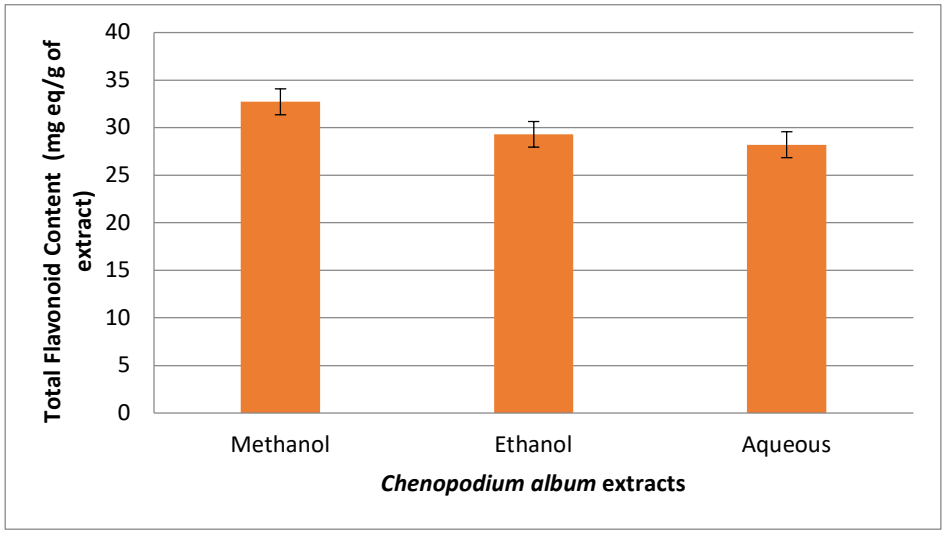

Figure 2: Total flavonoid content in *C.album* leaf extracts. Flavonoid content is reported in terms of mg of Quercetin Equivalents per gram of the extract

Antioxidant activity

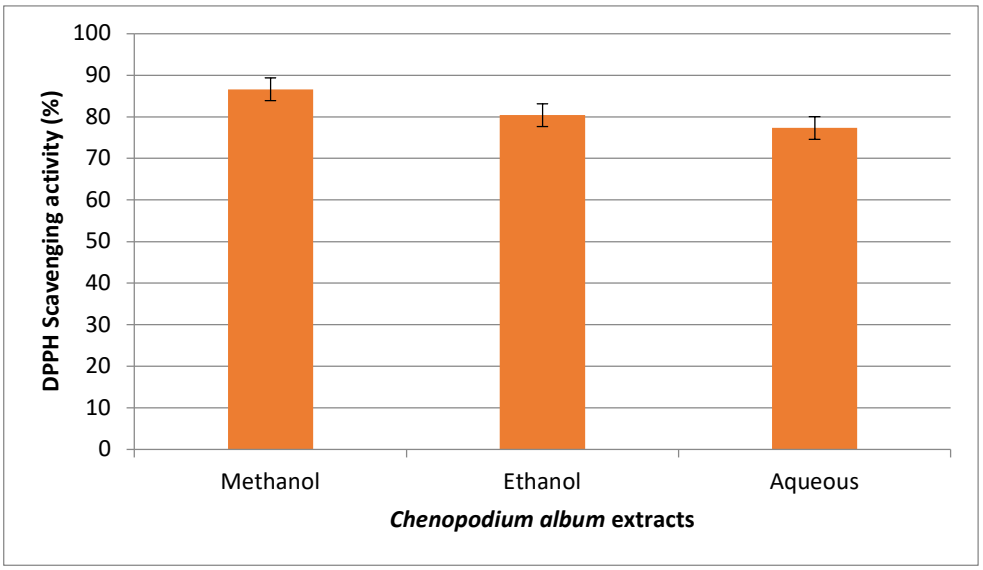

Figure 3: Percentage DPPH Scavenging activity of *Chenopodium album* leaf extracts

Antimicrobial activity

Table 2: Antibacterial assay of *C. album* leaf extracts by agar well-diffusion method and comparison with control

Diameter of zone of inhibition (mm)													
Ethanol extract (mg/ml) *C. album*					Methanol extract (mg/ml) *C. album*				Aqueous extract (mg/ml) *C. album*				Piperacillin/ Tazobactam (control)
	25	50	75	100	25	50	75	100	25	50	75	100	100/10 mcg/disc
Escherichia coli	8	10	13	13	12	13	14	15	-	-	12	14	22
Klebsiella pneumoniae	8	10	10	12	8	10	12	14	8	9	10	11	18
Staphylococcus aureus	10	10	12	13	8	9	12	15	11	12	14	16	20
Bacillus subtilis	10	10	11	12	10	11	11	13	7	9	9	11	16

Antioxidant activity of the leaves is measured in terms of percentage DPPH scavenging activity. Methanol extract of the leaves has high DPPH scavenging ability which means that methanolic leaves extract of *C. album* showed strong antioxidative property in comparison to ethanolic and aqueous leaves extract, which may be due to presence of phenols and flavonoids. Studies reviled the potential of Phenolic and flavonoids as an antioxidants which is shows the free radical scavenging activity **[23, 24]**. Phytochemical screening of the leaves extract revealed the considerable proportion of important phytochemicals that are easily detected by qualitative tests. In our analysis, it was found that methanol extract of *C. album* leaves are rich in phenols, steroids, terpenoids, alkaloids, and amino acids. The important thing is that all extracts contain two common and abundant secondary metabolites, phenols and

flavonoids. Flavonoids have wide range of anti-inflammatory, antibacterial, antiviral, antiallergic, cytotoxic and antitumor properties **[25]**. Alkaloids and phenolic compounds along with hypoglycemic, anti-diabetic properties also exhibit anti-inflammatory, antimicrobial and antioxidant effects **[16-26]**. Saponins exhibit various biological activities such as cell membrane permeability, lowering the serum cholesterol levels, and antidiabetic properties **[27]**. Tannins exhibit cardio-protective, anti-inflammatory, anticarcinogenic, and antimutagenic properties **[28]**. Quinones inhibit HIV1 reverse transcriptase and shows antitumor and immunomodulatory activities **[29]**. In our study, it is found that anthroquinones are absent in all the extracts.

Quantitative tests were carried out to estimate the TPC and TFC in the different extracts of *C. album*. The maximum phenolic **(Figure 1)** and flavonoid **(Figure 2)** content was found in the methanol extract of *C.album* **(Figure 1)**. The antimicrobial activity of ethanol, methanol and aqueous extracts were determined against two gram-positive and two gram-negative bacterial strains. The range of concentration used was 25, 50, 75 and 100 mg/ml. The antibacterial activity against each bacterial strain was found to be varied. The inhibition zones of the bacterial strains were in the range from 7mm to 16mm. The antibacterial activity varied depending on the species of microorganism and the type of extracts and fraction. Antimicrobial activities of the plant species have been highly investigated. Studies stated the role of secondary metabolites (Polyphenols, Alkaloids) in the inhibition of microorganisms **[30, 31]**. Results showed that increasing concentration of extracts raised the zones of inhibition.

Further study, however, is still warranted to explore the active compound of *C. album* which are responsible for the inhibition of growth of microorganism other than studied.

CONCLUSION

Traditional usage of the edible plants and their extracts possess biological active compounds that exhibit antimicrobial properties. With the increased resistance towards synthetic antibiotics in pathogenic microorganisms, plant products can potentially be a better alternative to synthetic antibiotics. This not only prevent from infections but also cure the disease. The present study thus revealed significant antimicrobial and antioxidative potential of the common edible plant leaf, *Chinopodium album*. It has potential as a novel and cost-effective antibacterial agent against multiple drug resistant microorganisms and also acts as an antioxidant agent. However, further investigations regarding the isolation of individual compound from most effective

extracts may help in offering the natural substitutes to treat diseases.

Conflict of Interest

None declared.

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