Antimicrobial And Phytochemical Potential Of Chenopodium Album Linn.

Rashi Saini, Dinesh Kumar, Amita Mittal

Abstract: Chenopodium album Linn. (Family: Chenopodiaceae) is a wildly growing weed in Africa, Asia, Europe and North America. In folk medicine, this plant has been used as antiparasitic, diuretic, hepato-protective, laxative and sedative. Its leaves possesses antirheumatic, anthelmentic, antiphlogistic, mildly laxative and odontalogic properties, applied as wash or poultice to bug bites, rheumatic joints, sunstroke, and swollen feets. This plant is used as vegetable in India and has high biological potential in addition to basic nutritional benefits. Previous scientific studies have reported the anthelmintic, antipruritic, contraceptive and sperm immobilizing properties of this plant. Hence, this plant possesses a great potential for detailed biological screening. So, in order to elucidate the potential traditional use of this plant, the present study was undertaken to evaluate its phytochemical and antimicrobial screening along with its free radical scavenging effect. Phytochemical investigations confirmed the presence of alkaloids, flavonoids, anthocynidine, saponins, glycosides, tannins and carbohydrates in the plant leaves extract. Antimicrobial effect tested by well diffusion method proved that methanolic leaf extract of the plant possesses considerable inhibitory activity against Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans, and Candida glabrata.The antioxidant activity was studied by DPPH free radical scavenging activity, total phenolics content and ascorbic acid estimation. The decolorization of purple colour in DPPH assay indicated the free radical scavenging activity. This activity was dose dependent and maximum activity (80.58%) was observed 50 µg/ ml. The total phenolic content in C. album is found to be 0.115g of phenols, and is calculated by plotting O.D. on standard graph of catechol. The ascorbic acid in leaf extract of C. album is calculated as 1.29 mg of ascorbate / g of leaf. Total phenolic content and ascorbic acid in the leaf extract justify the nutritional and biological significance of C. album.

Index Terms: Phytochemical, antioxidant, antimicrobial, P. aeruginosa, C.album.

1 INTRODUCTION

HERBS are still used by 80% of world population, because of their easy availability and very negligible side effects. From ancient period medicinal plants are used for pharmacological studies. Knowledge of therapeutic value of these plants acts as sources for lead compound development [1]. Chenopodium album Linn. (C. album) belonging to family Chenopodiaceae, is commonlycalled as Bathua(Hindi), Chandanbetu (Bengali), Parupukkirai (Tamil), Pappukura (Telgu) and Katuayamoddakam (Malyalam) in India. It is afast growing annual plantwhich wildlygrows as weed in Africa, Asia, Europe and North America. Tender branches and leaves are used as a vegetable in various parts of world. In India, it is locally used in curd known as “Raita”. Leaves are often dried and stored for later use. Infusions of leaves were also used as a laxative. Hindu physicians recommended C. album for treatment of splenic enlargement and liver diseases [2]. In folk medicine, this plant has been used as antiparasitic, diuretic, hepato-protective, laxative and sedative. Its leaves possesses antirheumatic, anthelmentic, antiphlogistic, mildly laxative and odontalgic properties, applied as wash or poultice to bug bites, rheumatic joints, sunstroke, and swollen feet [3].

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This plant is used as vegetable in India and has high biological potential in addition to basic nutritional benefits. Previous scientific studies have reported the anthelmintic, antipruritic, antinociceptive, contraceptive and sperm immobilizing properties of this plant [4]. Hence, this plant possesses a great potential for detailed biological screening. So, in order to elucidate the potential traditional use of this plant, the present study was undertaken to evaluate C. album leaf extracts for phytochemical and antimicrobial screening along with its free radical scavenging effect.

2 MATERIALS AND METHOD

2.1 Collection of plant material

C. album leaves were taken from village Surkhpur (Kurukshetra, Haryana) in December 2018**.** After drying in shade, the leaves were coarsely powdered.

2.2 Preparation of the plant extracts:

For extract preparation, the leaf powder was soaked in various organic solvents (methanol, chloroform, n-hexane, petroleum ether and acetone) in the ratio of 1:10 (20gm in 200ml solvent) for 72 hr. at 30ºC. After maceration of 72 hr, extracts were filtered. Complete evaporation of solvent was done in water bath according to the boiling temperature of respective solvent. In the residual powder 5% (w/v) DMSO was added and stored at 4ºC till further investigation.

2.3 Study of the Antibacterial Activity

Culture collection

The microbial strains were obtained from MTCC Centre, IMTECH, Chandigarh. The pure cultures were preserving on nutrient agar for growth. Cultures were stored at 4ºC in refrigerator for further use.

Preparation of extract dilutions

1 mg ml^{-1} extract concentration was prepared by adding 10 mg of extract in 10 ml of 5% dimethylsulfoxide, and further diluted to 0.5 mg/ml with DMSO.

Agar well diffusion method
In this method, lawn culture of bacteria (B. subtilis, E. coli, P. aeruginosa) and fungi (C. albicans and C. glabrata) were spread on the Muller Hinton agar using spreader. The wells were taken from agar plates using a cork borer. The extracts were poured into the well. The plates were placed in BOD incubator at 37ºC for 24 hrs. After incubation, the zone of inhibition was determined [5].

2.4 Phytochemical Screening
Prepared C. album extracts were screened for various biochemical parameters [6].

2.5 Antioxidant activity

DPPH radical scavenging method
In this method, solution of 0.1 mM DPPH was prepared in methanol and from this solution 1ml was mixed with 3 ml of extract solution prepared in methanol having 10, 20, 30, 40 and 50 µg/ml of leaf extract. Reaction mixture was swirled thoroughly. After 30 minute incubation, absorbance of the mixture was observed at 517 nm in Double beam UV-Visible spectrophotometer [7]. L-ascorbic acid was taken as standard. The scavenging ability of sample was estimated as per reported method [8].

% Inhibition = [(Ac-Aa)/Ac] X 100

Where Ac and Aa represent the absorption of the control and extract respectively.

Estimation of total phenols
500 mg leaves of C. album were mixed with 80% ethanol (10 times) followed by centrifugation at 10,000 rpm for half an hour. Supernatants were mixed together and evaporated to dryness. Again, remains were dissolved in a known quantity of distilled water and transferred into test tubes by pipette. Final volume was made 3.0 ml with distilled water. 0.5 ml of Folin-Phenol reagent was added to each test tube and mixed well. 3 minute later, 2.0 ml of 20% sodium carbonate solution was added. After mixing, tubes were placed in boiling water for 1 minute and then cooled. Absorbance was noted at 650 nm in UV-Vis spectrophotometer against blank [9].

Ascorbic acid Estimation
Ascorbic acid content existing in C. album leaves was determined as per the previous method [10] [11].

3 RESULTS AND DISCUSSION

3.1 Antimicrobial activity:
The antimicrobial potential of different leaf extracts of C. album is mentioned in Table 1. The results shows inhibitory effect of extract on growth of microbial species: E.coli, P. aeruginosa (Gram-ve), B. subtilis (Gram +ve) and C. albicans, C. glabrata (Fungi). The methanolic leaf extract showed maximum activity against all strains with highest inhibition zone of 31 mm against gram positive B. subtilis, while the chloroform and acetone extracts were also active against test samples with active range from 14-28 mm and 11-16 mm respectively. n-Hexane extract had no effect on any microbial species except E. coli and C. albican with 10 mm inhibition zone, while petroleum ether extract showed no activity. Highest antimicrobial activity was also observed by Külcü et al in C. album [12]. Ramproshad et al. also reported great antimicrobial activity against various pathogenic microbes [13].

Table 1: *Antimicrobial potential of C. album leaves extracts.*

Micro organisms	Diameter of inhibition zone (mm)									
	Methanol		Chloroform		Acetone		n-hexane		Pet. Ether	
	0.5 $mgml^{-1}$	1 $mgml^{-1}$	0.5 $mgml^{-1}$	1 $mgml^{-1}$	0.5 $mgml^{-1}$	1 $mgml^{-1}$	0.5 $mgml^{-1}$	1 $mgml^{-1}$	0.5 $mgml^{-1}$	1 $mgml^{-1}$
E. coli	14	**25**	14	21	11	15	10	--	--	--
P. aeruginosa	18	24	20	23	12	16	--	--	--	--
B. subtilis	15	**31**	25	**28**	12	--	--	--	--	--
C. albicans	17	21	16	20	12	15	10	--	--	--
C. glaburata	18	24	19	23	11	15	--	--	--	--

3.2 Phytochemical analysis:
Main phytoconstituents such as flavonoids, tannins, carbohydrates, proteins, phenolic compounds, alkaloids and sterols were found to be present in the aqueous and methanolic leaf extracts (table 2). Results are in accordance with the former studies [14]. Result shows the presence of alkaloids, phenolic, flavonoids, saponin, tannin, protein, carbohydrates, and glycoside, in the leaf extract of C. album. It has been already reported that alkaloids are potent anesthetic and spasmolytic agents whereas saponins improves the immunity [15] [16]. The finding of the study shows that this plant has a number of chemical constituents, which might be responsible for the different pharmacological activities [17]. Therefore, presence of phenolic compounds in the leaves could be responsible for their free radical scavenging activity.

Phytochemicals	Aqueous extract	Methanolic extract
Alkaloids	-	+
Amino acids	+	-
Proteins	+	-
Carbohydrates	+	+
Flavonoids	+	+
Anthraquinone	-	-
Cardiac glycosides	+	-
Starch	+	+
Saponin	+	+
Anthocynidine	+	+
Steroids	+	+
Tannins	+	+
Phenolic compounds	+	+

Table 2: Phytochemical screening of aqueous and methanolic leaf extracts of C. album. ("+ve" indicates presence of compound where as "-ve" indicates absence of compound)

3.3 Antioxidant assay

DPPH free radical scavenging activity:

Scavenging activity of methanolic extract of C. album is shown in table 3. A significant drop in the concentration of DPPH free radicals was observed due to scavenging ability of extract. The activity was dose dependent. Maximum scavenging activity 80.580 was observed at 50 µg/ml concentration. The total antioxidant capacity in the leaf extract was determined by the formation of phosphomolybdenum complex, which was able to reduce the stable DPPH radical to the yellow colored diphenylpicrylhydrazine. Free radical scavenging activity of this plant has also been reported previously [18]. Lone et al. (2017) reported 45%-73% DPPH activity of methanol extract of C.album [19]. 9.28% to 24.90% activity was observed by Külcü et al [12].

Total phenolics content:

Hydroxyl group of Phenolic compounds is responsible for scavenging ability of plant. It was observed by the formation of deep blue colour. The total phenolics content in C. album is 0.115g of phenols, calculated by plotting O.D. (Optical Density) on standard graph of catechol. Phytochemical studies demonstrated that C. album methanolic extract have flavanoids, alkaloids, tannins and to a very little extent of carbohydrates and glycosides etc. Flavonoids and phenolics or their combinations found to be liable for antioxidant potential of plant. The phyto constituents like polyphenol, flavonoid and isoflavonoid have gained considerable interest in improvement of human health, especially in tumor reduction. Phenolic compounds have been found significant due to their accepted role in the prevention of various human health problems [20]. Estimation of ascorbic acid (Vitamin C): A naturally present antioxidant in fruits, vegetables, medicinal plants and whole grains, plays a principal role in averting free radicals. The value was estimated by plotting the O.D. at 650 nm on standard graph of ascorbic acid and is calculated as 1.29 mg of ascorbate / g of leaf. Vitamin C being immunity booster has positive effect on our health and body.

Concentration (µg/ml)	Absorbance at 517 nm	% Inhibition of c. album
10	560	45.098
20	428	58.039
30	392	61.560
40	224	78.039
50	198	80.580
Standard (control)	1020	

Table 3: *DPPH scavenging activity of methanolic extract of C. album*

4 CONCLUSIONS:

Owing to the frequent use of this plant in Indian cuisine and because of its health benefits, study was performed with the aim to explore antimicrobial, phytochemical and antioxidant potential. Resultof the study indicates that Chenopodium has potent antimicrobial potential and very good antioxidant effect. The activity of this plant might be due to the occurrence of one or more of the chemical constituents reported in extract. The activity may be useful in the treatment of various health problems and diseases.

5 ACKNOWLEDGEMENTS:

Authors are thankful to Director, UIET for providing the necessary laboratory facility for carrying out the work.

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