STUDY ON NUTRITIONAL, PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES FROM THE LEAVES OF *CHENOPODIUM ALBUM* L. (MYU)

Khin Myo Win[1], Yin Yin Kyaw[2], Nway Nway[3]

Abstract

Chenopodium album L. is known as Myu belong to Chenopodiaceae. The plant was collected from Htan taw village, Amarapura Township, Mandalay Region during March 2023. The *Chenopodium album* L. was annual erect herbs. The leaves of *Chenopodium album* L. contain nutritional values such as protein, fat, fiber, carbohydrates and organic matter. The leaves of *Chenopodium album* L. involve saponins, flavonoids, tannins, alkaloids, carbohydrates, phenolic compounds etc. In antioxidant activity, the methanol extract was higher than ethanol and watery extract. The leaves of *Chenopodium album* L. has significantly antioxidant effects. Then, antimicrobial activities of the water extract of leaves of *Chenopodium album* L. showed that the highest inhibition zone (20mm) on *Escherichia coli, Xanthomonas oryzae* and *Pseudomonas fluorescens*. The extracts of *Chenopodium album* L. showed good antimicrobial activities against on test organisms. The aim of the study of *Chenopodium album* (L.) leaves is to know the nutritional contents, chemical constituents, antioxidant and antimicrobial activities. It was suggested that the leaves of *Chenopodium album* L. should be eaten because it contains medicinal properties for human health.

Keywords: *Chenopodium album* L. (Myu), nutritional value, phytochemical, antioxidant and antimicrobial activities.

Introduction

Chenopodium album L. belongs to the family Chenopodiaceae (Goose foot family). *Chenopodium album* L. was originating from Western Asia. This plant was widely distributed all over the world and has about 250 species. The plant of *Chenopodium album* L. naturally grows as a weed in the field of Htantaw village, Amarapura Township, Mandalay Division during summer and winter season crops. Htantaw village lies between 21˚53'45"N 96°04'11"E. This plant was found around Mandalay and Taunggyi in Myanmar. This plant is small annual herb having fleshy leaves.

The plant of *Chenopodium album* L. is a significant food crop and valuable plant in Ayurveda in tropical and subtropical countries and is abundantly used as food and herbal medicine around the world. The medicinal property of *C. album* is mainly found in its leaves and seeds. (Saini & Kant Saini, 2020).

The tender shoots of *Chenopodium album* L. are eaten raw in salad or cooked as a vegetable. The plant possesses various activities such as antibacterial, anti-inflammatory, anticancer, antiulcer, hepatoprotective, antioxidant, anthelmintic, spasmolytic and analgesic, sperm immobilizing agent, antipruritic and antinociceptive. Hence, this plant provides a significant role in the prevention and treatment of a disease. (Aman *et al*., 2016).

Chenopodium album L. is an important medicinal plant in Ayurveda that cures leukoderma, epilepsy, fever, cough and abdominal pain. The species are cultivated as a grain or vegetable crop as well as animal feed in Asia (Ratre *et al*., 2020). Herbal medicine is called as

[1] Department of Botany, Yadanabon University
[2] Department of Botany, Yadanabon University
[3] Department of Botany, Yadanabon University

botanical medicine or phyto medicine. Phytochemical studies demonstrated that *C. album* L. methanolic extract have flavonoids, alkaloids, tannins and to a very little extent of carbohydrates and glycosides etc. (Saini *et al*., 2019).

The leave extract of *Chenopodium album* L. was very good antioxidant effect. (Saina *et al*., 2019). Many vegetables contain free radical inhibitors with preventive potential regarding a large number of human diseases that have been defined as nutraceuticals (Chludil *et al.,* 2008). The leaves of *Chenopodium album* L. had showed auticuicrobial effects against *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans and Candida glabrate* (Saina *et al*., 2019).

Therefore, the leaves of *Chenopodium album* (L.) (Myu) were studied for the nutritional, phytochemical constituents, antioxidant and antimicrobial activities to obtain a thorough knowledge about their properties and constituents.

Materials and Methods

(a) **Plant collection and identification of plant specimens**

The leaves of *Chenopodium album* L. were collected from Htan taw village, Amarapura Township during March 2023. The specimen was studied for morphological characters and identified with the help of literature Backer, 1962.

(b) **Preparation of Powdered sample**

The leaves were thoroughly washed under tap water. The clean sample was dried in shade. Then, the leaves were coarsely powdered using a mixer grinder, sieved and stored in an air tight container for further use.

(c) **Nutritional values of the Leaves of *Chenopodium album* L.**

The leaves of *Chenopodium album* L. were evaluated for its nutritional values such as moisture, organic matter, Ash, crude protein, fats, crude fiber and nonstructural carbohydrate by using Association of Official Analytical Chemists (AOAC) method (Horwitz, 1980) in University of Veterinary Science, Physiology and Biochemistry Department, Yaezin.

(d) **Preliminary study on Phytochemical constituents in Leaves of *Chenopodium album* L.**

The leaves of *Chenopodium album* L. were studied for phytochemical constituents by various chemical tests. The presence or absence of alkaloids, saponins, tannins, reducing sugars, glycosides, starch, carbohydrates, phenolic compounds and flavonoids were studied according to method Harbone (1998).

(e) **Antioxidant activities of the leaves of *Chenopodium album* L.**

Screening of antioxidant activity by using DPPH free radical scavenging assay

DPPH (1,1–diphenyl -2- picryl- hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of sample extracts. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenol in sample. DPPH (0.002 g) was thoroughly dissolved in ethanol (100 mL). This solution was freshly prepared in the brown coloured bottle and must be stored in refrigerator for no longer than 24 hours. The extracts (100 µg/mL) were prepared by dissolving 0.001 mg in 10 mL of ethanol. This solution was two-fold diluted serially with ethanol to get the sample solution with the concentration of 6.25, 12.5, 25, 50, 100 ppm. Accurately weighed 0.001 mg of ascorbic acid was dissolved in ethanol and made up the volume to 10 mL. It was used as standard solution.

Procedure

DPPH radical scavenging activity was determined by UV visible spectrophotometric method. Control solution was prepared by mixing 0.002 % of DPPH solution (1.5 mL) and 70 % ethanol (1.5 mL). The sample solution was prepared by thoroughly mixing 0.002 % (w/v) DPPH solution (1.5 mL) and the test sample solution (1.5 mL). The solution was allowed to stand at room temperature for 30 minutes. Absorbance of the solution was measured at 517 nm by using spectrophotometer and % inhibition was calculated by using the equation (Bugchi & Puri, 1998). Antioxidant analysis was performed at Chemistry Department, Yadanabon University.

$$\% \text{ Inhibition} = \left[\frac{A_{DPPH} - (A_{Sample} - A_{Blank})}{A_{DPPH}} \right] \times 100$$

where % Inhibition = percent inhibition of test sample

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{Sample} = absorbance of sample + DPPH solution

A_{Blank} = absorbance of sample + EtOH solution

The IC_{50} (50 % inhibitory concentration) values were also calculated by linear regression excel program.

(f) Antibacterial activities of the leaves of *Chenopodium album* L.

The powdered of leaves was extracted with ether, acetone, methanol, ethyl acetate, ethanol and distilled water. For initial screening, nutrient agar was prepared according to the method described by Cruickshank (1975). Then, the extracts were tested with five pathogenic microbes.

Table 1. Test organisms utilized in the antimicrobial activities by Madigan, 2005.

No.	Test organisms	Source	Diseases
1.	*Aspergillus flavus*	-	Bronchitis
2.	*Bacillus subtilis*	JAP-0225215	Food spoilage and fever
3.	*Candida albicans*	IFO-1060	Skin infection, alimentary tract infection

No.	Test organisms	Source	Diseases
4.	*Escherichia coli*	ATCC-25922	Cholera, diarrhea and vomiting, urinary tract infections
5.	*Pseudomonas fluorescens*	-	Bacteria for leaf blight
6.	*Xanthomonas oryzae*	-	Bacteria for leaf blight

Paper Disc Diffusion Assay

Different solvent extracts of leaves of *Chenopodium album* L. were used to perform antimicrobial activities by paper diffusion method. In this method, nutrient agar was used as culture media. Two grams of nutrient agar were boiled with 75 ml of distilled water and isolated bacterial strains grown on nutrient agar were inoculated into 50 ml conical flasks containing 10 ml of sterile growth medium. Then, they were incubated at 30˚C for 72 hours on a reciprocal shaker at 200 rpm. Test organisms were *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans*, *Escherchia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. Paper disc having eight millimeter diameter were used for antibacterial assays. 0.3 ml of test organisms was added to assay medium, then poured into plates. After solidification, paper disc impregnated with broth samples were applied on the test plates and these plates were incubated for 24-36 hours at 30°C. After for 24-36 hours, clear zones (inhibitory zones) surrounding the test discs indicated the presence of bioactive compounds which inhibit the growth of test organisms.

Results

Scientific Name - *Chenopodium album* L.

Myanmar Name - Myu

English Name - Goose foot; Pig Weed

Family - Chenopodiaceae

Part used - Tender Leaves and stems

Medicinal Uses - Treating diarrhea and constipation

Uses - Making salads and soup

Flowering period - January to April

(a)Morphological characteristics of *Chenopodium album* L.

Annual erect herbs; stem and branches longitudinal ribbed. Leaves simple, alternate, exstipulate, petiolate, fleshy, cuneate at the base, irregularly dentate along the margin, acute at the apex, dark-green, with a whitish coat on the underside, mealy appearances. Inflorescences axillary or terminal cymose clusters forming paniculate spike, many flowered; peduncle stout. Flower bisexual, actinomorphic, hypogynous, apetalous, greenish-white; bract minute. Calyx cup-shaped, 5-lobed; tubes long, lobes ovate-oblong, green, glabrous, persistent. Stamens 5, free, filament filiform, anther dithecous, basifixed. Carpels two, fused, ovary superior, style very short; stigma bifid. Fruit utricles, ovoid, indehiscent, within the persistent calyx, black-brown.

Figure 1. A. Habit B. Leaves C. Inflorescence D. Stamens E. Carpel

(b)Nutritional values of the Leaves of *Chenopodium album* L.

The leaves of *Chenopodium album* L. show the presence of nutritional value. The leaves are rich carbohydrates, proteins and organic matter. The content of fat is low in leaves. The results of nutritional value were shown in table 2.

Table 2. Nutritional values in the leaves of *Chenopodium album* L.

No.	Test Parameter	Results
1.	Moisture	12.7%
2.	Ash	17.2%
3.	Crude Protein	30.4%
4.	Crude Fiber	12.1%
5.	Fat	1.8%
6.	Carbohydrate	20.0%
7.	Organic matter	82.8%

(c) Preliminary study on Phytochemical constituents in leaves of *Chenopodium album* L.

According to experiment**,** the leaves of *Chenopodium album* L. contains alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, starch, saponin and tannins respectively. This results are shown in table 3.

Table 3. Results of Phytochemical Test for Leave extract of *Chenopodium album* L.

No.	Test	Extract	Reagent Used	Observations	Results
1.	Alkaloids	1%HCl	Dragendroff's solution	Reddish brown ppt	+
2.	Flavonoids	EtOH	Conc HCl, Mg turning	Green color solution	+
3	Glycosides	H_2O	10% Lead acetate	White ppt	+
4	Phenolic compounds	H_2O	10% $FeCl_3$	Greenish-blue solution	+

No.	Test	Extract	Reagent Used	Observations	Results
5	Reducing sugars	H_2O	Benedict's solution	Reddish brown ppt	+
6	Saponins	H_2O	Distilled water	Froth	+
7	Tannins	H_2O	10%$FeCl_3$,dil H_2SO_4	Yellowish brown ppt	+
8	Carbohydrates	EtOH	Fehling solution (A+B)	Red ppt	+
9	Starch	EtOH	Iodine solution	Black color ppt	+

(+) presence, ppt = precipitate

(d)Antioxidant Activities of the leaves of *Chenopodium album* L.

The leaves of *Chenopodium album* L. samples were sequentially extracted with ethanol, methanol and water. Antioxidant activity of *Chenopodium album* L. was expressed as percentage of DPPH radical inhibition and IC_{50} values (ug/mL). IC_{50} value of the sample was calculated from the concentration and percent inhibition curve. The results of antioxidant activity using DPPH method in sample and using Ascorbic acids as a positive control were shown in figure 4.

Determination of absorbance was carried out at wavelength 517 nm using spectrophotometer. Decrease in absorbance indicates increase in radical scavenging activity. The absorbance value of extracts was described in (Table 4).

Table 4. Absorbance of Leave extracts from *Chenopodium album* L. (Myu)

Concentation / Extract	Absorbance at 517 nm				
	6.25	**12.5**	**25**	**50**	**100**
EtOH	0.001	0.011	0.012	0.013	0.014
	0.791	0.757	0.693	0.562	0.309
	0.762	0.751	0.699	0.569	0.319
	0.771	0.752	0.697	0.565	0.309
Watery	0.001	0.011	0.012	0.013	0.014
	0.805	0.751	0.695	0.601	0.431
	0.802	0.752	0.696	0.611	0.433
	0.802	0.741	0.697	0.601	0.435
MeOH	0.001	0.011	0.012	0.013	0.014
	0.841	0.781	0.705	0.579	0.228
	0.842	0.772	0.706	0.575	0.218
	0.851	0.771	0.707	0.577	0.227

From these results, it was found that as the concentration increased the absorbance decreased, i.e., increased in concentration increased the radical scavenging activities for all samples. Free radical scavenging activities of extracts usually in terms of % inhibition were calculated.

Table 5. Radical Scavenging Activity and IC_{50} of Ethanol, Methanol and Watery extracts of the *Chenopodium album* L. Leaves

Test Samples	% RSA (mean ± SD) in different concentration (µg/mL)					IC_{50} µg/mL
	6.25	12.5	25	50	100	
Ethanol	15.56 ± 0.1	18.98 ± 0.3	25.31 ± 0.3	39.72 ± 0.3	67.44 ± 0.6	68.64
Methanol	8.26 ± 0.5	16.96 ± 0.5	24.53 ± 0.1	38.67 ± 0.2	77.12 ± 0.5	62.63
Watery	12.79 ± 0.1	19.86 ± 0.6	25.62 ± 0.1	35.70 ± 0.6	54.44 ± 0.2	87.01
Ascorbic acid	5.74 ± 0.2	17.71 ± 0.1	32.41 ± 0.8	55.80 ± 0.1	99.45 ± 0.1	46.78

For the average values of radical scavenging activity, IC50 values in µg/mL was calculated by computer program called linear regressive excel program. These result were shown in (table 5) and concentration versus radical scavenging activity was plotted as shown in (figure 4, 5) and the comparison of IC50 values of crude extracts of Chenopodium album L. was shown in (figure 6).

According to antioxidant activity, the methanol extract was lower in IC50 value than ethanol and watery extracts. The methanol extract is higher in antioxidant activity than ethanol and watery extract. So, it was found that methanol, ethanol and watery extract of Chenopodium album were moderately good in antioxidant activities.

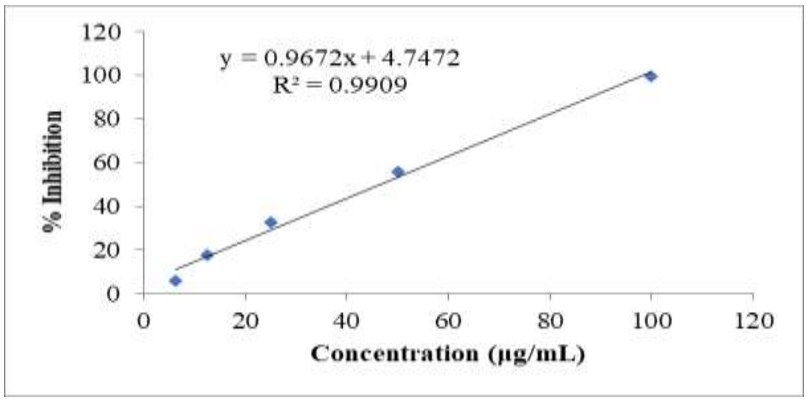

Figure 4. % RSA of standard Ascorbic acid

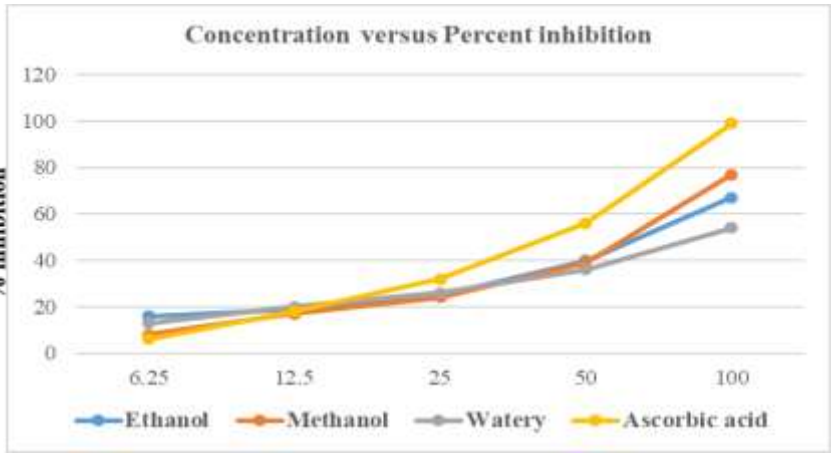

Figure 5. A plot of % inhibition Vs concentrations of extracts of *Chenopodium album* L.

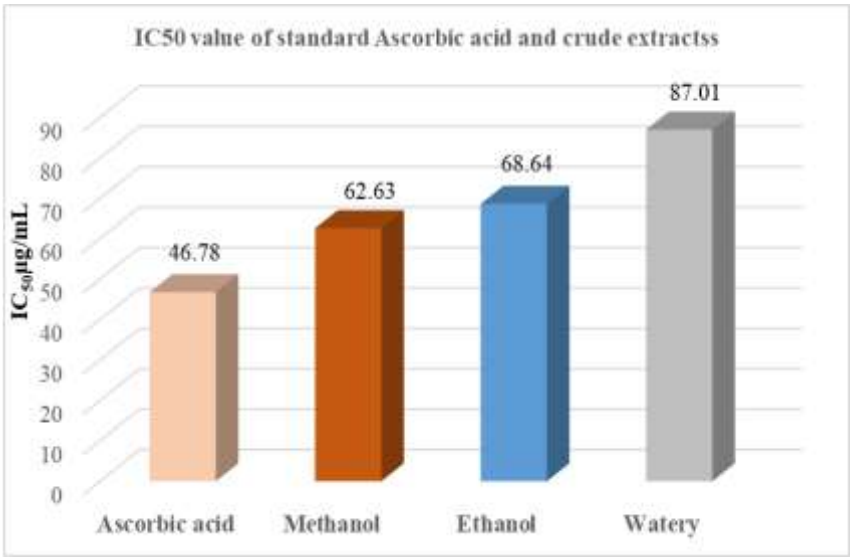

Figure 6. Comparison of IC_{50} values of crude extracts of *C. album* L. with standard

(e) Antimicrobial Activities of the leaves of *Chenopodium album* L.

The antimicrobial activities of the leaves of Chenopodium album L. were carried out by various solvent such as acetyl, ethyl acetate, ethanol, methanol and petroleum-ether and water. The results of antimicrobial activities of the leaves extracts against on pathogens as shown in table 6 and figure (7). Among these, the water extract has shown the highest inhibition zone against on Escherichia coli, Pseudomonas fluorescens and Xanthomonas oryzae (20mm). The inhibition zone (16mm) was found in the water extract against on Bacillus subtilis and Candida albicans and the inhibition zone (18mm) occurred against on Aspergillus flavous. The acetyl extract performed antibacterial activity against on Aspergillus flavous, Bacillus subtilis and Xanthomonas oryzae with (14mm) zone of inhibition. The Petroleum ether extract has shown the lowest inhibition zone (8mm) against on Aspergillus flavous, Bacillus subtilis, Candida albicans and Xanthomonas oryzae. According to results, the acetyl, methanol and water extract of the leaves of Chenopodium album L. was effective on the tested pathogens. The water extract has been found to have significantly antibacterial activities on Escherichia coli, Pseudomonas fluorescens and Xanthomonas oryzae. So. the leaves of Chenopodium album L. have the ability to protect against pathogenic infection.

Table 6. Antimicrobial activities on various extracts of leaves of *Chenopodium album* L.

Microorganisms	Diameter of Inhibition zone (mm)					
	Acetyl	EtOAc	Ethanol	Methanol	Pet Ether	Water
Aspergillus flavous	14	8	8	10	8	18
Bacillus subtilis	14	10	10	10	8	16
Candida albicans	12	12	12	14	8	16
Escherichia coli	10	10	10	10	10	**20**
Pseudomonas fluorescens	12	12	14	14	10	**20**
Xanthomonas oryzae	14	10	12	14	8	**20**

Paper disc- 8mm, 8mm~12mm (+), 13mm~17mm (++), 18mm~20mm (+++)

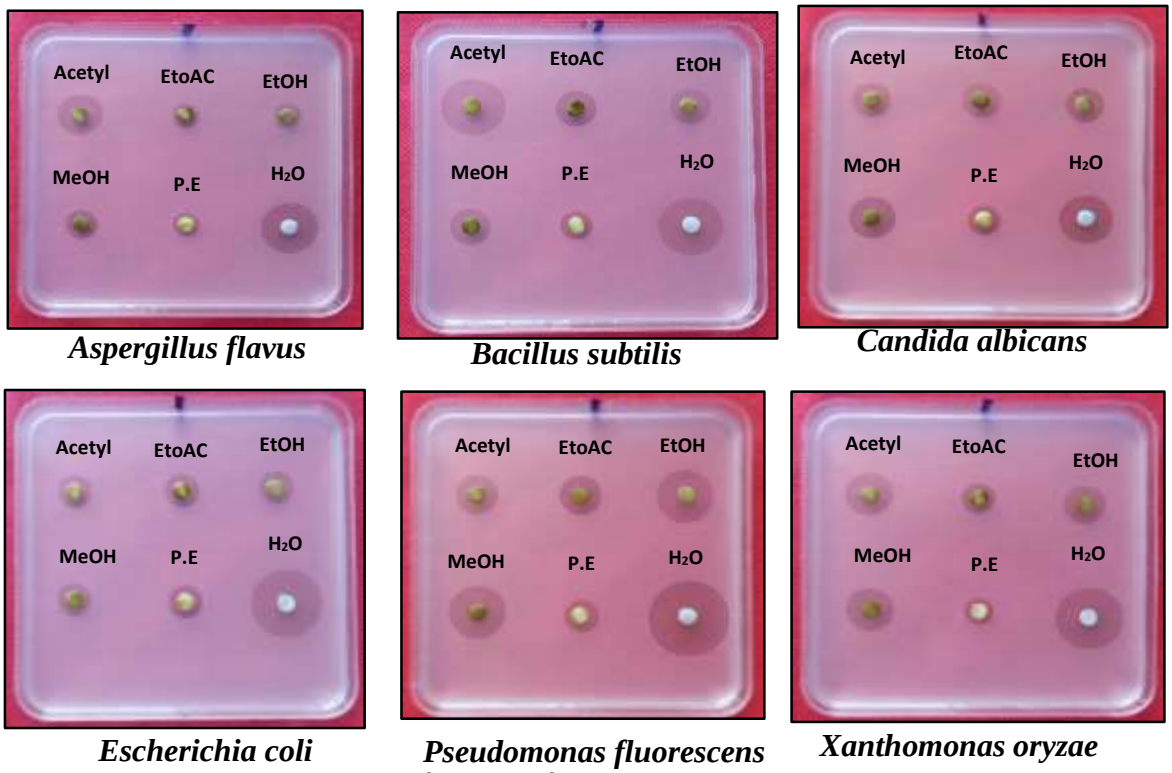

Figure 7. Antimicrobial activities of leaves of *Chenopodium album* L. on six pathogens.

Discussion

The plant of Chenopodium album L. belongs to the family Chenopodiaceae. The local name of this plant is Myu and the English name is goosefoot. The fresh leaves of Chenopodium have many beneficial medicinal properties. It is used to treat kidney stone, swelling, anemia, heart disease, jaundice and other diseases.

In the morphological study, Chenopodium album L. are annual erect herbs; stem and branches longitudinal ribbed. The leaves are simple, alternate, petiolate, fleshy, irregularly dentate along the margin, with whitish coat, mealy appearance on the under surface. Inflorescences axillary or terminal cymose clusters forming paniculate spike.

Pandey and Grupta (2014) reported that the leaves of Chenopodium album L. contain carbohydrates (40.84%), protein (28.69%), fat (4.41%), moisture 5.06%, ash 21% and dietary fiber 0.1%. In this result, the leaves contain the rich of carbohydrates (20.0%), crude proteins (30.4%) and organic matter (82.8%). The fat content is low amount (1.8%). The fiber, ash and moisture content are 12.1%, 17.2% and 12.7%. The fiber related to its indigestibility in the small intestine. The leaves should be cooked as diet for overweight people. Therefore, the leaves should be eaten raw in salads or cooked due to having nutritional values.

Arora et al., (2020) stated that the leaves of Chenopodium album L. possesses secondary metabolites. The different extracts of C. album contain steriods, triterpenoids, carbohydrates, flavonoids, tannins, saponins, proteins, alkaloids. In this study, the leaves contain alkaloids, phenolic compounds, flavonoids, saponin, tannin and etc. Alkaloids are good anesthetic agents while saponin helps the immune system and reducing the risk of intestinal cancer. The presence of flavonoids and phenolic compounds in the leaves could be responsible for their free radical scavenging activities.

Samanta and Somnath (2015) reported that revealed that ethanol extract is better than the aqueous extract for the antioxidant activity in Chenopodium album L.. In this study, the antioxidant activity of C. album L. indicated that the methanol extract exhibited the higher antioxidant potential than ethanol and watery extract. The leaves of Chenopodium. album L. should be consumed as antioxidant rich, vegetables for human health.

In antimicrobial activities, Saini et al., (2019) described the methanol leaf extract of Chenopodium album possess inhibitory activity against on E. coli, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans and C. glabrata. Al-Snafi (2019) represented that the aqueous extract performed highest antibacterial activity against on Staphylococcus aureus with (25 mm) zone of inhibition and the least antibacterial activity observed against on Salmonella typhimurium with 17.75 mm zone of inhibition. On the other hand, methanol extract displayed potential antibacterial activity all the test bacteria. In this study, the water leaf extract showed maximum activity against on Escherichia coli Pseudomonas fluorescens and Xanthomonas oryzae with inhibition zone of 20 mm. The methanol and acetyl extract showed that the inhibition zone (14 mm) was found the activity against on all test organisms except E. coli. the various extract of Chenopodium album L. exhibited significantly antibacterial activity against all test organisms. The Pet-ether extract was the lowest antibacterial activity was observed against on test organisms. Therefore, the antimicrobial activities of Chenopodium album L. showed significantly against on Escherichia coli Pseudomonas fluorescens, Xanthomonas oryzae, Bacillus subtilis and Candida albicans.

Conclusion

It was concluded that the leaves of Chenopodium album L. effects for human health. The leaves contain fibers, ash, proteins, flavonoids, carbohydrates, saponins, tannins, alkaloids. The nutritional constituents are used traditional medicines and the leaves should be eaten as curry and salad for human health. Addition the leaves of Chenopodium album L. have the potential antioxidant and then antimicrobial activities against on test organisms. Therefore, the leaves of Chenopodium album can be used to cure of various diseases. It was suggested that the leaves of Chenopodium album L. should be cooked and as salad because it contains beneficial nutritional contents, phytochemical constituents, antioxidant properties and against on test organisms.

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