In-Vitro EVALUATION OF PHYTOCHEMICALS, HEAVY METALS AND ANTIMICROBIAL ACTIVITIES OF LEAF, STEM AND ROOTS EXTRACTS OF *Caltha palustris var. alba*

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ABSTRACT

Plants are a rich source of secondary metabolites that have been found to have medicinal properties. The present study was conducted to evaluate the phytochemical screening, antimicrobial activities, and heavy metal analysis in different parts (leaves, stem and roots) of *Caltha palustris var. alba*. The phytochemical analysis for the plant was carried out in ethanol, acetone, ethyl acetate, *n*-hexane and deionized water extracts. The phytochemical screening confirmed the presence of several bioactive compounds like, alkaloids, flavonoids, tannin, phenols, and saponin. The antimicrobial activity was determined by the agar disc diffusion method using five different extracts. The antimicrobial study revealed that the *C. palustris var. alba* has the ability to fight against the selected microorganism. The phytochemical and biological screenings were correlated with the presence of heavy metals in selected plant *C. palustris var. alba*. The concentrations of heavy metals were determined by atomic absorption spectrophotometer and revealed the presence of the metals like cadmium, manganese, zinc, copper, lead, and chromium. The metal concentration was compared with the permissible limit set by World Health Organization (WHO) and the result was discussed. From these studies, it is clear that plant *C. palustris var. alba* can be used for medicinal purposes.

Keywords: Phytochemicals, antimicrobial activity, heavy metals, *Caltha palustris var. alba*.

INTRODUCTION

Medicinal plants are essential components of the earth since they are used to treat a variety of infectious diseases around the world and provide a source of numerous effective and potent medicines [1]. Alkaloids, flavonoids, saponins, tannins, and glycosides are secondary metabolites found in medicinal plants that are used to heal illnesses and relieve pain [2-3]. Numerous studies have shown that secondary metabolites produced from medicinal plants have therapeutic potential [4-5]. The ethnomedicinal qualities of the plants have been reported in numerous manuals [6-7]. Alkaloids derived from medicinal plants have been found to be particularly effective against *Staphylococcus aureus* in recent studies [8]. As antimicrobial resistance research advances, medicinal plant phytochemicals are building the framework for novel therapeutics [9-10]. Traditional remedies derived from plants or herbs are widely used in underdeveloped nations since they are easily accessible, have low toxicity, are inexpensive, and have minimal side effects. Physicians are frequently hesitant to give them advice due to a lack of understanding and a fear of liability [11-12].

Pakistan flora boasts a significant number of medicinal plants due to its different ecological areas and diverse climatic and soil conditions [13]. According to a survey, Pakistan has about 6000 plant species, with 600 of them having medicinal value, the bulk of which are found in the Himalayan region [14]. Around 300 medicinal plants are traded, with 12 % of Pakistan's flora being utilized in these medications. More than 75% of the population is treated with traditional medicines prescribed by more than 50,000 hakims (traditional herbal practitioners). In the last two decades, the ten most major Davakhanas, or herbal producers, have ingested 200 therapeutic plants and their use [14-15]. As a result of rising industry, urbanization, human population, and the release of organic hydrocarbons and inorganic heavy metals into the atmosphere, our country's natural resources and wealth are becoming diseased [16]. In Pakistan, where infectious diseases are frequent, it is critical to promote and discover plant-derived medications. These medicinal herbs can be used to kill or control microbes [17].

Different heavy metals accumulate in different concentrations in plants. Excessive consumption of herbal remedies can lead to an increase in heavy metal intake, which can lead to major problems such as accumulative poisoning, nerve damage, cancer, and death [18-20]. Heavy metal contamination, such as cadmium, copper, lead, and nickel, causes environmental pollution and can have major health consequences when it accumulates in plants above permitted levels. As a result, consumers should be informed of the heavy metal's permitted limitations [21]. Animals are susceptible to heavy metal concentrations, which results in a variety of ailments. Plants, like animals, are vulnerable to heavy metal concentrations [22]. The increase in heavy metal concentration in the environment is largely due to human activities. Heavy metal levels in the environment have risen dramatically in recent decades, owing primarily to human activity. As a result, it is critical to accurately and quickly assess heavy metal pollution in the environment, particularly toxic heavy metals [23].

Caltha palustris var. alba commonly known as White marsh marigold is a member of the family *Ranunculaceae*. It is a species of rhizomatous perennial plants. It is inhabitant to moist areas in cold and moderate regions of northern and southern hemispheres [24-25]. The flowering parts of this plant that grow above the ground are used by the people to make medicine. The whole plant is analgesic, diaphoretic, antispasmodic, expectorant, diuretic and rubefacient [26-27]. It has been used in the treatment of fits and anemia and is also used to remove warts [27-28]. The root is antirheumatic, diaphoretic, emetic and expectorant [29]. A decoction is used in the treatment of colds [30]. A poultice of the boiled and mashed roots has been applied to sores [31].A tea made from the leaves is diuretic and laxative [30-31]. Hence, the present study aims to investigate the phytochemicals, heavy metals concentrations and antimicrobial activities of *C. palustris var. alba* [32]. The current study was conducted in Pakistan Council of Scientific and Industrial research (PCSIR), laboratories complex, Peshawar, Pakistan.

MATERIALS AND METHODS

Plant collection and identification

The *C. palustris var. alba* plant was collected during the month of September from the mountains of District Dir (lower) Maidan Valley in Khyber Pakhtunkhwa, Pakistan. The plant species was identified and confirmed by Muhammad Siddique Afridi Senior Scientific officer in the Medicinal Botanic Center, PCSIR, laboratories complex, Peshawar, Pakistan.

Drying and grinding of plant material

The plant samples were thoroughly rinsed with distilled water to remove dirt, dust, and other unwanted materials. The plant samples were shaded dried at room temperature by keeping over a large piece of paper. The dry parts (root, stem, and leaves) were ground separately into powder form using an electric grinder machine and stored in clean polyethylene bags for further processing.

Extraction process

The dried powder from the plant sample (roots, stem and leaves) of weight 200 g each were taken in 1-liter conical flask and soaked separately in 500 ml of

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ethanol, acetone, ethyl acetate, hexane and distilled water for 24 h at room temperature and then filtered. All the flasks were periodically shaken to enhance extraction. The cotton swabs were plugged onto each flask to prevent the organic solvent from evaporation. The filtrates were collected in separate flasks using the previously described methods, and the process was repeated three times [33]. In a rotary evaporator, the filtrates, or crude extracts, were concentrated. The obtained crude extracts were resuspended in a minimum needed volume of corresponding solvents and placed on a water bath at 60 ℃ for the evaporation of excess solvents in order to isolate pure extracts. Following prior protocols, these extracts were stored in separate containers at 4 ℃ for subsequent experiments [34].

Extraction yield measurement

Each plant samples dried extract was weighed, and the yield % was estimated using the following formula:

$$\text{Extract yield (\%)} = \frac{\text{weight of dried extract}}{\text{weight of dried plant sample}} \times 100. \qquad (1)$$

Tested microorganisms

The *C. palustris var. alba* plant extracts were screened against nine bacterial strains and one fungal strain. Gram-positive and Gram-negative bacterial strains as well as a single fungal strain were obtained from American Type Culture Collection (ATCC), Microbiology Laboratory, PCSIR Peshawar. The nine Gram-positive and Gram-negative bacterial strains *Salmonella typhi* (ATCC # 14028), *Erwinia carotovora* (ATCC No. 672), *Escherichia coli* (ATCC No. 29922), *Pseudomonas aeruginosa* (ATCC No. 9721), *Bacillus subtilis* (ATCC No. 6051), *Bacillus atrophaeus* (ATCC No. 9372), *Staphylococcus aureus* (ATCC 6538), *Agrobacterium tumefaciens* (ATCC No.33970), *Klebsiella Pneumonia* (ATCC No.1705) and a single fungal strain *Candida albicans* (ATCC No.10231) were obtained from the American Type Culture Collection (ATCC), Microbiology Laboratory, PCSIR Peshawar.

Preparation of the test compound

All the extracts were prepared in dimethyl sulfoxide oxide (DMSO) solutions at a concentration of 1mg/1ml. DMSO was used as a solvent since it has no inhibitory action against bacteria and fungi [35-37]. A nutrient agar solution with a concentration of 28 g/L was prepared in separate conical flasks. In an autoclave, the media solution and petri plates were sterilized for 15 minutes at 1.5-pound pressure at 121°C. In a laminar flow, nutrient agar medium was poured into Petri plates and allowed to solidify for 20 minutes.

Antimicrobial activity

The antibacterial activity was determined by measuring the zone of inhibition against the test microorganisms using the agar disc diffusion method [38].

Disc diffusion method

The agar disc diffusion method was used to conduct antimicrobial activity of various extracts of *C. palustris var. alba* on nutrient agar media. The microbial cultures were uniformly swabbed throughout the agar surface. After the streaking was finished, the inoculums were allowed to dry for at least 5 minutes. The Petri plates were then accurately labelled, with a double sterile disc on top and a single sterile disc on the lower side of the Petri plates. After the discs were placed on the agar plates, 6 µl of extract was poured on the single disc and 12 µl on the double disc using a sterile micropipette. All of the Petri plates were incubated at 37°C for 24 h. The antimicrobial activity was assessed after the incubation period by measuring the width of the inhibition zones. Dimethyl sulfoxide oxide was used for negative control for all tested microorganisms. As a positive control, Ciprofloxacin was utilized for Gram negative bacteria and Azithromycin for Gram positive bacteria, while Clotrimazole was used for fungal strain (*C. albican*) as a positive control. The activity of various extracts against *C. albican* was tested using the same method as described earlier. The procedure was carried out for three times.

Phytochemical analysis

The following tests were carried out using standard procedures to determine the presence of active constituents in various solvent extracts.

Alkaloid test

The extracts were thoroughly mixed with a % HCl solution before being filtered. The filtrate was treated with Hager's reagent (picric acid saturated solution). The presence of alkaloids was indicated by the yellow color of the precipitate [39].

Carbohydrate test

One liter of Benedict's solution can be made with 100 g of anhydrous sodium carbonate, 173 g of sodium citrate, and 17.3 g of copper (II) sulphate pentahydrate. The presence of carbohydrate was confirmed using this reagent. In the plant extract, 2 ml Benedict solution was added. For a few minutes, the mixture was placed on a water bath. The formation of a reddish-brown color proved the presence of carbohydrate [40].

Flavonoid test

Crude extract of plant was mixed with 3 ml ammonia solution, then sulfuric acid was carefully added. The presence of flavonoids in the sample was revealed by the appearance of a yellow color [41].

Glycoside test

In the crude extract of the plant, acetic acid (2 ml) and sulfuric acid (2 ml) were added. The presence of glycoside in the sample was revealed by the reddish color appearance [42].

Phenol test

Add 2-10 drops of ferric chloride solution to the plant crude extract. The presence of phenol was visible as a greenish color [43].

Protein Test

Add 40% sodium hydroxide solution and two drops of % copper sulphate solution were added to 0.5 mg of extract. The presence of protein is indicated by the appearance of violet color [44].

Saponins test

20 ml water was added to the 0.5 ml extract of the plant. For 15 minutes, the mixture was vigorously shaking. The presence of Saponins was revealed by the appearance of a foam layer [39].

Starch test

The presence of starch was confirmed by adding 2-3 drops of Iodine-Potassium Iodide reagent to the plant extract. A blue-black color indicated the presence of starch [45].

Steroids test

A few drops of acetic anhydride, 2 ml chloroform, and 2 ml sulfuric acid were added to the plant crude extract. The presence of the color red resulted in a positive test [46].

Tannins test

1-2 drops of ferric chloride solution were added to methanol crude extracts. The resultant blue color suggested a positive result [39].

Evaluation of moisture and ash content

To determine the moisture content in each of the plant sample, 1 g of fresh plant sample was taken in already weighted small Petri plates. The Petri plates were kept in oven at 110^{o} C for hours. After 3 hours, the Petri plates were kept out and re-weighted. Ash content of plant sample was evaluated by the same method as described in the heavy metal analysis section. Moisture as well as ash content of plant sample is given with the relation as follows:

$$\text{Content (\%)} = \frac{A-B}{A} \times 100. \qquad (2)$$

In the case of moisture content, (A) represents the fresh weight of the plant sample, whereas in the case of ash content, it indicates plant sample before ignition and (B) represents the dry weight of the sample (moisture content) and plant sample after ignition (ash content).

Heavy metal analysis

The method described earlier was used to perform dry digestion [47]. The ash method was used to determine heavy metals in plant sample. At every step, necessary precautions were taken to avoid metallic contamination in any way. Pre-cleaned silica crucibles were placed in a muffle furnace set to 600 °C. The crucible was left in the furnace until the weight of the crucible reaches a steady level. Powdered plant material (5gm) was placed in a silica crucible and kept at 600 °C for 6 hours in a muffle furnace. The crucible was then kept out and placed in desiccators to cool to room temperature before the ash values were calculated. After that, the ash was dissolved in 100 ml of 5% HCl. The dissolved ash solutions were filtered through Whatman filter paper (No. 40) and stored in tightly closed plastic bottles. Using Atomic Absorption Spectrophotometer (AAS), the prepared solutions were directly used to determine various heavy metals [47].

RESULTS AND DISCUSSION

The increased emergence of antibiotic resistance has diverted researchers' attention towards medicinal plants in search of new, less toxic, and beneficial medications. Phytochemicals are found in abundance in plants. Many plants have been studied for their antimicrobial and phytochemical properties all around the world. As a result, the phytochemicals and antimicrobial activities of water, acetone, ethanol, ethyl acetate, and n-hexane extracts of leaves, stems, and roots of *C. palustris var. alba* were investigated.

Evaluation of Ash, Moisture, and Yield Value

Roots had the highest moisture content and ash values of 80 % and 76 %, respectively, when compared to the leaves and stem of the plant sample, while extracted values were evaluated separately for each of the 15 extracts. The **Table 1** shows that leaves ethanol extract had a higher percentage of extractive value (21%) which is followed by roots ethyl acetate (17%), root ethanol (16%), leaves ethanol (14.5%), stem aqueous (14%), stem ethyl acetate (12.5%), leaves acetone (12%), leaves aqueous (11.2%), stem acetone (10%), leaves ethyl acetate (8.7%), roots acetone (6.7%) leaves hexane (5.7), leaves aqueous (5%), roots hexane (3.93%), and stem hexane (2.05%). Ash, moisture, and extractive values of all fifteen extracts of *C. palustris var. alba* was determined and all these values are in accordance with the study conducted earlier [48-49].

Table 1: Ash, moisture, and extracted values of fifteen extracts of *C. palustris var. alba***.**

Plant part	Solvent	Extract value (%)	Moisture value (%)	Ash value (%)
	Water	11		
	Ethanol	21		
Leaves	Acetone	12	70	67
	Ethyl acetate	8.7		
	n-Hexane	5.7		
	Water	5.0		
	Ethanol	14.5		
Stems	Acetone	10	65	60
	Ethyl acetate	12.5		
	n-Hexane	2.05		
	Water	14		
	Ethanol	16		
Roots	Acetone	6.25	80	76
	Ethyl acetate	17		
	n-Hexane	3.93		

Antimicrobial activity

Crude extracts of *C. palustris var. alba* was tested in vitro for antimicrobial activity, and the results were compared to standard antimicrobial agents (ciprofloxacin and clotrimazole).

All of the root extracts had showed antimicrobial activity and were found active against all the tested microorganisms except *S. typhi* and *E. coli*, which were found sensitive to aqueous and ethyl acetate extracts respectively. The ethanolic extract showed excellent inhibitory action against many bacterial strains in roots extracts with the highest inhibition against *S. aureus* (22 mm), *P. aeruginosa* (18 mm), and *B. subtilis* (17 mm). Acetone extract showed best inhibitory activity against *S. aureus* (20 mm). Aqueous extract showed promising activity against all bacterial strains and has showed maximum inhibition against *S. aureus* (18 mm). Ethyl acetate extract showed maximum inhibitory activity against *S. aureus* (17mm). Hexane extract showed maximum inhibitory activity against *K. pneumonia* (17mm). Moreover, all of the root extracts had the same inhibitory effect against the fungal strain *C. albican* in the 10-14 mm range. The antimicrobial activity of *C. palustris var. alba* root extracts against several bacterial strains and a single fungus strain is shown in **Table 2.**

Table 2. Antimicrobial activity of *C. palustris var. alba* root.

Tested microorganisms	Zone of inhibition (mm)											
	Water Extract		Ethanol Extract		Acetone Extract		E. Acetate Extract		*n*-Hexane Extract		CIP	DMSO
Extract (μl)	6	12	6	12	6	12	6	12	6	12	Positive	Negative
B. subtilis	14	15	12	17	10	14	0	0	14	15	34	-
B. atrophoeus	09	15	0	0	10	12	0	0	10	12	33	-
K. pneumonia	12	16	15	20	12	15	10	14	13	17	32	-
E. coli	0	0	0	0	0	0	10	13	0	0	31	-
S. typhi	10	15	0	0	0	0	0	0	0	0	36	-
E. carotovora	14	17	10	13	11	14	09	12	09	13	27	-
S. aureus	18	18	16	22	17	20	12	17	10	08	32	-
P. aeruginosa,	13	18	13	18	12	15	13	15	11	10	31	-
A. tumefacieous	0	0	0	0	10	14	10	12	10	12	32	-
C. albican	10	12	10	14	10	13	10	14	10	12	33	-

Similarly, all the stem extracts had also showed antimicrobial activity and were found active against all the tested microorganisms except *S. typhi* and *E. coli*, out of which only *E. coli* was found sensitive to aqueous extract. The ethanolic extract showed excellent inhibitory action against several bacterial strains in stem extracts with the highest inhibition against *S. aureus* (22 mm) and *P. aeruginosa* (18 mm). Acetone extract showed best inhibitory activity against *S. aureus* (19 mm). Both aqueous and ethyl acetate extract had showed promising activity against *S. aureus* and has showed maximum inhibition (20 mm). Hexane extract showed maximum inhibitory activity against *S. aureus* (16 mm). Moreover, all of the stem extracts showed no inhibitory action against the fungal strain *C. albican* except aqueous and ethanolic extract, which showed inhibition in the 10-14 mm range. The antimicrobial activity of *C. palustris var. alba* stem extracts against several bacterial strains and a single fungus strain is shown in **Table 3**.

In leaves extracts, the ethanolic and aqueous extracts showed maximum inhibitory actions against many bacterial strains with highest inhibition against *S. aureus* 22 and 25 mm respectively. All the leaves extracts were found inactive against bacterial strains *S. typhi* and *E. carotovora*. The antimicrobial activity of *C. palustris var. alba* leaves extracts against several bacterial strains and a single fungus strain is shown in **Table 4**. Moreover, all the leaves extracts showed no inhibitory action against the fungal strain *C. albican* except aqueous and ethanolic extract, which showed inhibition in the 13-15 mm range.

Table 3. Antimicrobial activity of *C. palustris var. alba* stem

Tested microorganisms	Zone of inhibition (mm)											
	Water Extract		Ethanol Extract		Acetone Extract		E. Acetate Extract		*n*-Hexane Extract		CIP	DMSO
Extract (μl)	6	12	6	12	6	12	6	12	6	12	Positive	Negative
B. subtilis	10	15	0	0	11	14	10	12	10	13	34	-
B. atrophoeus	11	14	0	0	10	12	11	14	10	12	33	-
K. pneumonia	10	12	12	10	12	15	0	0	12	14	32	-
E. coli	08	12	0	0	0	0	0	0	10	12	31	-
S. typhi	0	0	0	0	0	0	0	0	0	0	36	-
E. carotovora	0	0	12	15	0	0	0	0	0	0	27	-
S. aureus	17	20	18	22	15	19	14	20	13	16	32	-
P. aeruginosa,	12	14	13	18	12	16	12	15	12	15	31	-
A. tumefacieous	10	13	14	12	0	0	10	13	0	0	32	-
C. albican	12	14	10	12	0	0	0	0	0	0	33	-

Table 4. Antimicrobial activity of *C. palustris var. alba* leaves

Tested microorganisms	Zone of inhibition (mm)											
	Water Extract		Ethanol Extract		Acetone Extract		E. Acetate Extract		*n*-Hexane Extract		CIP	DMSO
Extract (μl)	6	12	6	12	6	12	6	12	6	12	Positive	Negative
B. subtilis	12	16	0	0	11	14	11	14	10	14	34	-
B. atrophoeus	13	14	0	0	10	12	10	12	10	12	33	-
K. pneumonia	10	13	14	11	14	18	0	0	11	14	32	-
E. coli	08	12	0	0	0	0	0	0	10	12	31	-
S. typhi	0	0	0	0	0	0	0	0	0	0	36	-
E. carotovora	0	0	14	17	0	0	0	0	0	0	27	-
S. aureus	18	22	20	25	18	20	18	20	15	18	32	-
P. aeruginosa,	12	15	15	18	12	15	12	15	12	15	31	-
A. tumefacieous	10	14	14	12	0	0	10	13	0	0	32	-
C. albican	12	15	09	13	0	0	0	0	0	0	33	-

Previous research on the antimicrobial activity of *C. palustris* has revealed a wide range of antimicrobial activity against many microbes supporting our findings in the current study [25]. The broad spectrum of antibacterial activity of the methanolic ex tract of *C. palustris* may be due to the presences of diverse classes of compounds, such as lactones, terpenoids, etc.

Phytochemical screening

Plants have long been a good source of medications, and many of the drugs that are currently available are produced directly or indirectly from them. Phytochemical screenings were used to evaluate secondary constituents that are responsible for curing diseases. The presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins, phenols, saponins, steroids, starch and tannins were found in all extracts of *C. palustris var. alba* leaves, stems, and roots (**Table 5**), which is similar with many other studies conducted across the world [35, 49-51]. These compounds have both preventive and therapeutic potential in the fight against malignant proliferation, opening the way for new disease research [52-55]. Flavonoids play a significant role in human nutrition. Flavonoids have recently gained popularity as a result of the finding of their pharmacological properties. Saponins are a class of secondary metabolites with bioactive features such as antibacterial action. They also have features including cholesterol binding, red blood cell precipitation and coagulation, and hemolytic action [51, 56]. Tannin and steroids are commonly used in veterinary vaccinations due to their ability to function as adjuvants and aid in the development of resistance responses. Many of them can be used for intracellular histochemistry labelling, which allows antibodies to access intracellular protein molecules [57]. Mubashir et al. investigated the methanolic extract of *C. palustris var. alba* that showed marked anthelmintic, antimicrobial and antioxidative activity which supports the therapeutic effects claimed by traditional practitioners [25]. Moreover, the findings of our study are frequently in agreement with studies conducted by other researchers in the same field [58-60].

Evaluation of heavy metals

The results obtained from atomic absorption spectrophotometer were then converted to meaningful data by the following formula, which gives us actual metal concentration in different parts of selected plants *C. palustris var. alba*:

$$\text{Conc. of metals (mg/kg)} = \frac{\text{Observed Conc. (ppm)} \times \text{Vol. of Sample prepared (ml)}}{\text{Wt. of Plant Sample (g)}}$$

The concentration level of heavy metals in *C. palustris var. alba* leaves was found to decrease in the order of Manganese > Chromium > Zinc > Cadmium > Lead > Copper. The concentration level of heavy metals in *C. palustris var. alba* stem was found to decrease in the order of Manganese > Zinc > Lead > Cadmium > Chromium > Copper. The concentration level of heavy metals in *C. palustris var. alba* roots was found to decrease in the order of Manganese > Cadmium > Lead > Zinc > Chromium > Copper (**Table 6**).

Table 4 shows that Cadmium was above permissible value in all parts of *C. palustris var. alba*, whereas the World Health Organization maximum permissible limit (MPL) of value is 0.3 mg/kg [58]. Chromium was above permissible value in leaves of *C. palustris var. alba* and its MPL value is 1.5 mg/kg [61].

Table 5. Phytochemicals in the roots, stem and leaves of *C. palustris var. alba***.**

Phytochemicals	Water			Ethanol			Acetone			Ethyl acetate			*n*-Hexane		
	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves
Alkaloids	-	-	-	+ +	-	-	-	+	++	++	-	-	-	+	++
Carbohydrate	++	+	++	-	++	++	+	-	-	+	+	++	-	-	-
Flavonoids	++	++	++	++	++	+	-	-	-	-	-	-	-	+	++
Glycosides	++	+	++	++	++	++	++	+	++	++	++	++	-	+	+
Proteins	++	+	++	++	++	+	++	-	-	+	+	++	-	-	-
Steroids	++	++	++	+	++	++	+	-	-	+	-	+	+	-	+
Phenols	-	++	++	++	+	+	-	-	+	++	-	-	-	-	-
Saponins	++	+	++	+++	+++	+++	+++	+	+	++	+	++	+	+	+
Starch	++	++	++	++	+	+	-	-	-	+	+	+	-	-	-
Tannins	++	+	++	++	++	+	-	-	-	-	-	-	-	-	-

S-L for stem leaves; Symbol + indicates presence, ++ indicates moderately presence, +++ indicates appreciable presence and – indicates absence.

Table 6. Concentration of various heavy metals and trace elements in *C. palustris var. alba.*

Heavy metals	Concentration (mg/kg)		
	Leaves	Stem	Roots
Cadmium	2.65	1.56	2.10
Manganese	7.63	14.37	3.63
Zinc	3.07	6.04	1.07
Copper	0.43	0.45	0.43
Lead	1.37	2.56	1.37
Chromium	4.5	1.2	05.02

CONCLUSIONS

The aqueous extract, ethanol, and ethyl acetate extract were the most active extracts in an antimicrobial investigation of different parts of *C. palustris var. alba*. The results show that the plant *C. palustris var. alba* accumulates many phytochemicals in its various parts. These compounds have a variety of biological effects by disrupting the life cycle of certain bacteria and killing them. The results showed that the plants acquire various metals in different concentration in their various parts (root, stem, and leaves). Only a few metals (Cr, Mn, and Cd,) were found in excess above WHO guidelines in different parts of *C. palustris var. alba*. As a result, before processing a medicinal plant for further pharmaceutical applications or for local human consumption, it should be examined for pollutant load or heavy metals.

It is proposed that *C. palustris var. alba* is an important medicinal plant that could be a promising candidate for further in vivo bioassays leading to the development of safe herbal medicines with few or no negative effects of worldwide interest.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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