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Antimicrobial Evaluation and Physicochemical Study of *Chenopodium album* against some common Human Pathogens

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Abstract : In the present study, we have done antimicrobial study, physicochemical evaluation of the *Chenopodium album*(*C.album*) leaves and roots. Antimicrobial activity was performed by agar well diffusion with six different strains i.e. *Klebsicella*, *P.acne*, *E.coli*, *P.aeruginosa*, *C.albicans*, *S.cerevisiae*. Preliminary phytochemical screening of the plant was done according to WHO parameters for standardization. Physicochemical parameters such as ash values, foreign matter, loss on drying etc. were also determined. The methanolic roots and leaves showed significant antimicrobial activity against *P.acne, S.cerevisiae*. Whereas ethyl acetate extract showed mild antimicrobial activity. From microscopy, it contains epidermis, endodermis, collenchymas, mesenchyma, xylem, phloem, and from powdered study it was found that calcium oxalate crystal, stone cells. The microscopic and physicochemical analysis of *C. album* leaf and root is useful in standization for quality, purity and sample identification. From the study, it was found that plant is having significant antimicrobial activity.
Keywords : *Chenopodium albumum,* antimicrobial activity, physicochemical parameters.

Introduction

Chenopodium album Linn, commonly known as 'bathua' belongs to family Chenopodiaceae. Traditionally, *C.album* is used as a curative medicine for various diseases including antimicrobial agent, hepatoprotective, asthma, urinal disorder, cuts, wounds, haematuria, gynecological disease, arthritis, cardiac problem, seminal weakness, colitis and piles[1-9]. A polymorphous, nearly- white, erect herb, up to 3.5m in height, found wild up to an altitude of 4,700 m, and cultivated throughout India. Stems rarely slender, angled, often striped green, red or purple; leaves rhomboid, deltoid to lanceolate, upper entire, lower toothed or irregularly lobed, extremely variable in cultivated forms, 10-15 cm long, petioles often as long as thick panicle, shining black seeds, possessing sharp margin[10]. The herb is laxative, anthelmintic and cardio tonic. The leaves are anti-scorbutic; they yield ascaridole, which can be used for treating burns. The juice is used to treat burns. The powdered plant,when mixed withnormal food, was reported to spress oestrus cycle; a decoction of aerial parts, mixed with alcohol, is rubbed on the body affected by arthritis and rheumatism[11].

Materials and Methods

Chemicals

Phloroglucinol, HCl, Glycerin, potassium hydrate, petroleum ether,ethyl acetate, methanol, chloroform,H_2SO_4, $FeCl_3$, HNO_3,picric acid, glacial acetic acid, ammonia, *Klebsicella*, *P.acne*, *E.coli*, *P.aeruginosa*, *C.albicans*, *S.cerevisiae* microbial strains and all other chemicals used in this study were of analytical grade.

Plant Material

The plant material (leaves and root) was collected from the Kaithal and was authenticated by Dr.Sunita Garg Chief Scientist Raw material Herbarium and Museum with reference No.NISCAIR/ RHMD/consult/2523/102-1 and was identified as *Chenopodium album* L. (Family-Chenopodiaceae) sample no.1.

Antimicrobial Study

Microorgnisms

Klebsicella, *P.acne*, *E.coli*, *P.aeruginosa*, *C.albicans*, *S.cerevisiae* six microbial strains were selected for antimicrobial activity of different extracts of the *C. album*.

Antimicrobial Assay

Agar well diffusion method was used for screening of antimicrobial activity[12]. In assay for antibacterial activity, the agar plates of microbial strain treated with plant extracts (20 µL) of different concentrations were incubated at (37 ± 2) °C for 24 h. Antibacterial activity of the plant extracts was determined by measuring the zone of inhibition (mm) against all bacterial strains. Ciprofloxacin was used as standard for the assay.

Macroscopic and Microscopic Study

Macroscopic and microscopic study of the *C.album* was studied according to Brain and turner 1975[13]. For the microscopic studies, transverse section of the leaves and bark were cut and mounted in glycerine as well as stained with phloroglucinol, HCl(1:1) and studied according to standard procedures. Coarse powder was used for microscopic study of plant stained with phloroglucinol HCl (1:1)[14].

Physicochemical Analysis

Physicochemical parameter such as ash, extractive values, and loss on drying were performed according to WHO guidelines on quality control methods medicinal plant material[15-17].

Preliminary Phytochemical Screening

Each of the extract was taken separately in 5ml of 1.5% v/v HCl and filtered. The filtrate was then tested with the following reagents [15,18].

Fluorescence Study

Fluorescence analysis of powder of root and bark was done by standard[19]. In this analysis the root bark and its powder were treated with various acidic and basic solvents and were then observed in UV/visible chamber under visible, short wave and long wave regions simultaneously[20]. The changes in appearance and colour were observed and recorded.

Results and Discussion

The *in vitro* antibacterial activity of *C. album* was assayed by using agar well diffusion method against six different microbial strains. All the extract of the plants is having anti microbial activity. As is evident from table 1, all the extracts of *C. album* were strongly effective against *C. albicans and S. cervevisiae.* Methanolic

extracts of *C. album* inhibited all six pathogens while ethyl acetate extract shows mild activity. Both extract of methanol leaves and root showed effective results. Nowadays, due to globalization and huge marketing competition the quality and purity of the crude drug is deteriorating by rough handling or by mixing adulteration. The plants have been traditionally used as antimicrobial agents. The extracts from different plants have been used by researchers to carry out antimicrobial activity[21-23]. So, natural products have been used by researchers earlier in search of new antimicrobial agents. It is because natural products are safe and cheaper than synthetic products. *C. album* is traditionally used as antimicrobial[23]. Earlier study, authors had selected 78 plants for the screening of antimicrobial potential by using disc diffusion method. And 11 different microbial strains (6 Gram positive bacteria species, 1 Gram negative bacteria species, 2 yeast species and 2 mould species) have been used for the study. From our finding, *C. album* is having significant antimicrobial activity from methanol root extracts (MRE) against *P.acne, S.cerevisae, e.coli* and *P.aeruginosa*. The methanol leaves extract (MLE) is having significant activity against *C.albicans* and *P.acne* microbial strain. Parkash et al 2014 states that methanolic extract concentration shows significant antimicrobial activity five microbial strain [24].In both of the studies, it was found that methanolic extract is having significant results against *P.aeruginosa* bacterial strain. Xu et al showed that the ethanolic extract of *C.album* is having significant activity against *E.coli* and *S.aureus*[25].There is no conflict because different plant extracts used in the study. Pathak et al 2010, from the study on microscopy observed that it contains xylem, phloem, collenchymas, parenchyma and epidermis[26].*C. album* is almost evergreen shrub, 6-8 feet in height. Stems rarely slender, angled, often striped green, red or purple; leaves rhomboid, deltoid to lanceolate, upper entire, lower toothed or irregularly lobed, extremely variable in cultivated forms, 10-15 cm long, petioles often as long as thick panicle, shining black seeds, possessing sharp margins(Figure 1).Transverse section of leaf shows that it contains epidermis, endodermis, collenchymas, mesenchyma, vascular bundles (xylem and phloem).In the 1st picture we can see that upper epidermis, parenchyma and Collenchymas and vascular bundle. In the picture 2nd we can see that outer cork cells, cambium than phloem. From the root transverse section we have found seven lining called as rings(Figure 2). Coarse powder was mounted on the glass slide, and then extra powder was removed from the slide. The fine powder was mounted with the addition of glycerine and staining reagent (Phloroglucinol+HCl; 1:1).After observation under microscope, we have found calcium oxalate crystals, stone cells, cork cells(Figure 3).The physicochemical parameters such as ash values, extractive values, and loss on drying were evaluated by using WHO guidelines. Physicochemical parameters also states about the adulteration. Ash value determine about the calcium oxalate, silicates, carbon(Table 2).Preliminary phytochemical screening revealed that presence of carbohydrate, glycosides, alkaloids, flavonoids, saponin, and tannin from the extracts showed in table 3.The fluorescence study was done by using short range and long range ultra violate-visible cabinet instrument. From the study, it was observed that the plant is having no adulteration (Table 4). Earlier finding from Pathak et al 2010 states that plant contains flavonoids, alkaloids, anthraquinone, saponins, and essential oil from the thin layer chromatography(TLC).Previously, authors by using analytical techniques chemical constituents have isolated such as flavonoids, lignans, glucosides, alkaloids, saponins, phenols,ecdysteroids[27-31].Leaves and roots contain flavonoids such as beta sitosterol, ferulic acid, sinapic acid, oleanolic acid. These flavonoids are having antimicrobial activity [32-36].Antimicrobial activity may be due to the presence of flavonoids present in the leaves and root of the plant(previous study data not shown). From the study, *C. album*is a probableresource of antimicrobial drug against the skin disease, Urinary tract infection (UTIs), gastrointestinal tract (GITs) and microbial infection related to immune disease. The *C.album* extracts is predominantly active in the fight against various class of organisms which are resistant to presently available drugs. From the study, it is found that plant is having significant antimicrobial activity; in addition further *in vivo* study should be conducted to explore mechanism of action of the plant as antimicrobial agent.

Table 1Antimicrobial activity of *C.album*

Plant Extracts			Organisms					
			Klebsicella	***P. acne***	***E. coli***	***P. aeruginosa***	***C. albicans***	***S. cerevisiae***
Diameter of inhibition zone (mm*)	***C. album***	MLE	11.00±0.10	18.1±0.17	10.13±0.15	13.16±0.20	22.09±0.10	16.06±0.57
		ELE	09.96±0.15	11.9±0.05	09.00±0.00	11.03±0.15	18.16±0.15	16.13±0.11
		MRE	12.03±0.05	28.1±1.70	19.06±0.11	18.80±0.34	14.13±0.23	23.03±0.15
		ERE	10.06±0.11	20.01±0.02	09.1±0.10	11.04±0.06	15.00±0.00	15.20±0.21

MLE=methanol leaves; ELE= Ethyl acetate leaves extract; MRE=methanol root extracts; ERE= Ethyl acetate root extracts; mean±SD($p<0.005$)

Table 2Physicochemical characteristics of *C. album* leaves and root

Parameter	Value obtained (%w/w)	
	Leaves	**Root**
Total ash value	10.72	9.5
Acid insoluble ash value	08.2	7.5
Water soluble ash value	4.8	2.9
Sulphated ash value	11.2	10.7
Methanol extractive value	6.0	12.2
Ethyl acetate	7.5	3.0
Foreign organic matter	0.51	0.39
Loss on drying	5.0	2.5

Table 3Phytochemical screening of *Chenopodium album*

Test		Methanol leaves	Ethyl acetate Leaves	Methanol Root	Ethyl acetate roots
Carbohydrates	Molisch's Test	+	+	+	+
	Fehling's Test	-	+	-	-
	Benedict's Test	+	+	-	-
Glycosides	Libbermann-Burchard Test	+	-	-	-
	Borntrager's Test	-	-	-	-
Alkaloids	Dragendroff's Test	+	-	-	+
	Mayer's Test	-	-	-	-
	Hager's Test	+	+	-	+
	Wagner's Test	-	-	-	-
Saponins	Foam Test	+	+	+	+
Flavonoids	Shinoda Test	-	+	-	-
	Lead acetate Test	+	+	-	-
Steroids and Triterpenoids	Salkowaski reaction	-	-	-	-
	Liebermann-Burchard reaction	-	-	-	-
Tannins	Ferric chloride Test	-	-	+	+
	Lead acetate Test	+	-	+	+

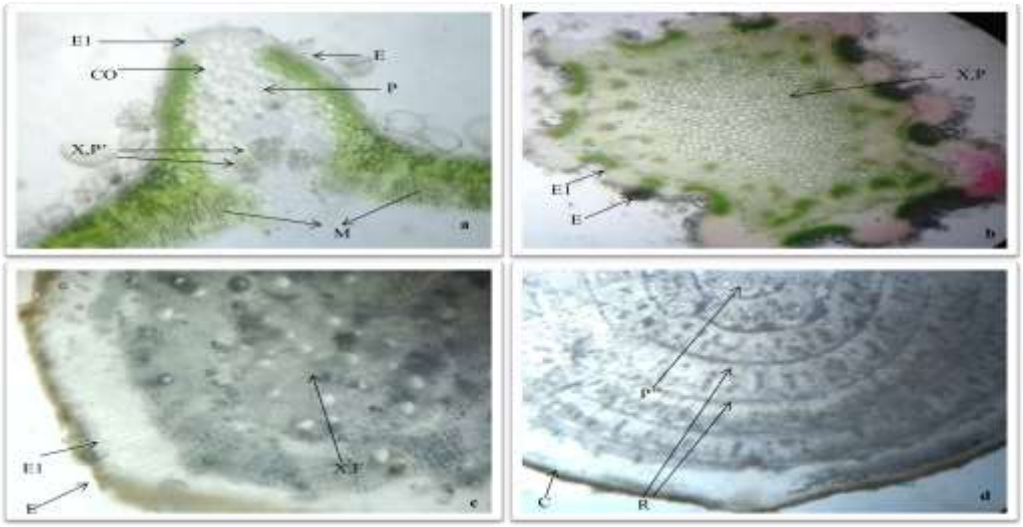
+=Present, - =Absent

Table 4Flourescence microscopy of the *C. album*

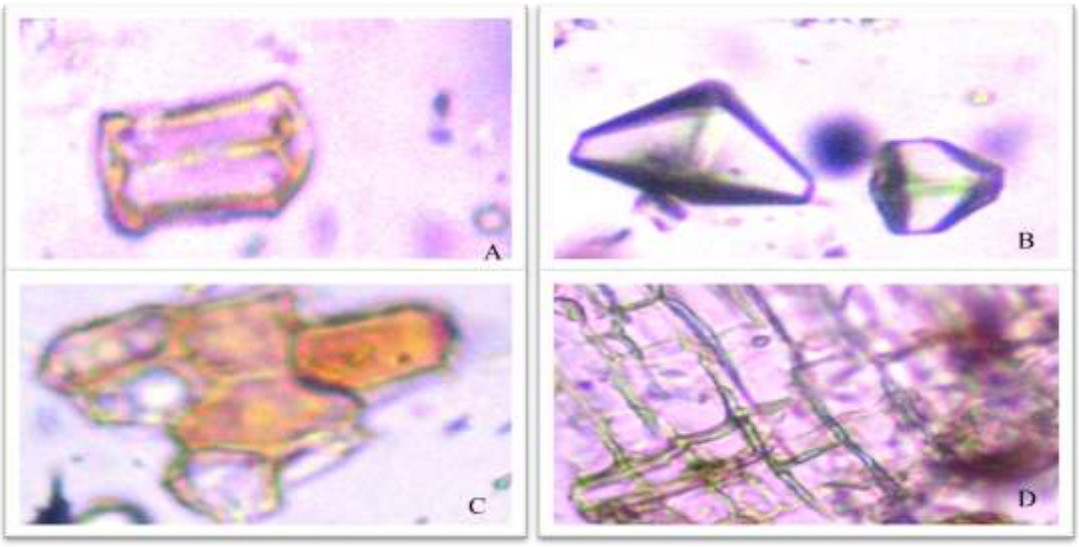
Chemical treatment	Visible	Short range	Long range
Powder(P)	Brown	Light brown	Dark brown
P+picric acid	Yallow	Green	Brownish black
P+chloroform	Light brown	Brown	Brown
P+methanol	Brown	Blackish brown	Brown
P+HCl	Brown	Yellowish brown	Dark brown
P+pet ether	Brown	Brown	Brown
P+Ammonia+Nitric acid	Yallowish brown	Green	Black
P+H_2So_4(50%)	Brown	Brown	Dark Brown
P+HNO_3(50%)	Dark Brown	Yallowish Brown	Brownish black
P+Glacial acetic acid	Brown	Brown	Yallow Brown
P+HCl(50%)	Brown	Dark Brown	Dark Brown
P+Ammonia	Dark Brown	Brown	Dark Brown

A, B= *Chenopodium album*; C= dried whole plant; D=bark of the plant

Figure 1 Macroscopical characters of leaf and root.



a= Leaf, b=stem, c=Rootlets, d=Root, E= epidermis, E1= Endodermis, C=Cork cell, CO= collenchymas; P=parenchyma, X= xylum,
P'=Phloem, M=mesonchyma, R=Rings, P''=Pith.
Figure 2.Microscopy of leaf, root and stem (100X)



A, B=Calcium oxlate crystals; C= Cork cells; D= Stone cells
Figure 3.Powder microscopy of *C. album* (X400)

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Conflict of Interest

We declare that we have no conflict of interest.

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