Trop Anim Health Prod (2017) 49:1597–1605
DOI 10.1007/s11250-017-1364-y

REGULAR ARTICLES

Evaluation of anthelmintic antimicrobial and antioxidant activity of *Chenopodium album*

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Received: 15 February 2017 / Accepted: 16 July 2017 / Published online: 22 July 2017

Abstract The objective of this study was to evaluate the anthelmintic, antimicrobial and antioxidant activities of *Chenopodium album* against gastrointestinal nematodes of sheep and some pathogenic microbes. A worm motility inhibition assay was used for in vitro study, and a faecal egg count reduction assay was used for an in vivo study. Various concentrations ranging from 100 to 500 μg/ml of the extract were subjected to antimicrobial screening by disc diffusion method against four selected bacterial (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas multocida* and *Escherichia coli*) and two fungal (*Aspergillus flavus* and *Candida albicans*) strains in order to estimate the medicinal potential of the herb. DPPH (1,1-diphenyl-2-picrylhydrazyl), riboflavin photo-oxidation, deoxyribose, lipid peroxidation assays were used for antioxidant activity. The extracts exhibited dose- and time-dependent anthelmintic effects on the *Haemonchus contortus* as compared to levamisole. The extract showed maximum inhibitory effect against *S. aureus* (28 ± 0.14 mm), while as mild inhibitory effect was observed against *E. coli* among the selected microbial strains. The effect produced by the different extract concentrations was comparable with the standard antibacterial agent streptomycin sulphate and antifungal agent nystatin, which were used as effective positive control in the study. The antioxidant activity showed that the extracts exhibited scavenging effect in concentration-dependent manner on superoxide anion radicals and hydroxyl radicals leading to the conclusion that the plant has broad spectrum anthelmintic, antimicrobial and antioxidant activities and could be a potential alternative for treating various diseases.

Keywords Anthelmintic · Antimicrobial · Antioxidant · *Chenopodium album* · Aqueous extract · Metanolic extracts

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Abbreviations

CAECA	crude aqueous extract of *Chenopodium album*
CMECA	crude methanolic extract of *Chenopodium album*
PC	positive control
NC	negative control
PBS	phosphate buffer saline
BW	body weight
GI	gastrointestinal
SEM	standard error mean
Meth	methanol
Aq	aqueous
AA	ascorbic acid
BHT	butylated hydroxy toluene

Introduction

Plants have been used as healers and health-rejuvenators since time immemorial. The use of plant products in the form of local medicines to treat animal and human diseases has its roots in prehistoric times. Even today, plants play an important role in the health care of about 80% of the world population and is estimated that more than half of the drugs under clinical use at present owe their origin to plants (Sarin 1996). Plants are utilized as therapeutic agents since time immemorial in both organized (Ayurveda, Unani) and unorganized (folk, tribal, native) form Girach et al. (2003). Nowadays, multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious diseases—a situation that forced scientists to search for new antimicrobial substances (Khanahmadi et al. 2010). Reactive oxygen species (ROS) including free radicals, such as $O^{2-\bullet}$, $OH^{\bullet}$, and non-free radicals, H_2O_2, along with different forms of active oxygen are involved in diverse physico-chemical processes in the body (Qureshi et al. 2009) playing a critically important role in the pathogenesis of different diseases, such as neurodegenerative disorders (Valko et al. 2007), diabetes, cancer (Vijayakumar et al. 2006), cardiovascular diseases, atherosclerosis (Halliwell and Gutteridge 1985), liver cirrhosis (Lin et al. 2012), cataracts and inflammation (Turkoglu et al. 2007; Conforti et al. 2008).

Considering the vast potentiality of plants as sources of antimicrobial, anthelmintic and antioxidant agents, the present study was undertaken to screen the in vitro and in vivo anthelmintic activity of *Chenopodium album* commonly used in ethno-veterinary medicine by many tribes of Kashmir valley (Kaul 1997; Khan et al. 2004). There is, however, no published scientific evidence for the anthelmintic and/or antimicrobial effects of *C. album*.

Materials and methods

All animal proceedings were approved by the Ethical Committee of University of Kashmir (Number: F (Ethical Com. Animal) KU/2012/419.

Collection of plant materials

The present plant material *C. album* (local name *Ganhar*, *Lisa*) is frequently seen throughout the Kashmir valley in kitchen gardens, wastelands, agricultural lands and on open mountainous slopes. The plant material was collected from Ganderbal, Kashmir (34° 17′ 04″ N, 75° 13′ 46″ E altitude 10,068 ft) during May–August. The mature plant at peak of flowering was collected in polythene bags and was processed by standard technique adopted by KASH (Kashmir University Herbarium). The plant was identified and authenticated by Plant Taxonomist Prof. Irshad Ahmad Nawchoo, Department of Botany, University of Kashmir, Srinagar, India. A voucher specimen (voucher no. 1701) was deposited in KASH (Kashmir University Herbarium). The collected plants were processed for shade drying at the environmental temperatures (25–30 °C) in a well-ventilated room (drying room at the Centre of Research for Development, University of Kashmir, Srinagar). The dried plant parts were milled to a fine powder using an electric stainless steel blender. The powdered plant material was stored in an airtight container/cellophane bags at 4 °C until extraction.

Preparations of extracts

Methanolic extracts were prepared by dissolving 200 g of the powdered plant material in a conical glass percolator to which 1000 ml (Qualigens) of methanol was added. The plant material was allowed to macerate for 16 h at room temperature, and the percolate was collected by filtering through non-absorbent cotton wool. The process of maceration/percolation was repeated three times. The combined filtrate was evaporated in a vacuum rotary evaporator (R-201, Shanghai Shenshen) under reduced pressure of 22–26 mmHg at 40 °C. The final crude methanol extract (8.43 g) was scrapped off and transferred to a container and kept airtight for storage at 4 °C until further use.

Aqueous extracts were prepared by dissolving 200 g of the powdered plant material in 500 ml of distilled water in a glass percolator. It was allowed to macerate for 24 h at room temperature, and the brew was filtered using Whatman #1 filter paper. The process of percolation was repeated three times. The combined filtrate was evaporated in a vacuum rotary evaporator (R-201, Shanghai Shenshen) under reduced pressure of 22–26 mmHg at 40 °C. The final crude aqueous extract (6.5 g) was scrapped off and transferred to a container and kept airtight for storage at 4 °C until further use.

In vitro anthelmintic activity by adult motility assay

In vitro anthelmintic activity of the plant extracts was evaluated by exposing the adult *H. contortus* worms to aqueous and methanolic extracts of *C. album* as described in our earlier work (Lone et al. 2012, 2016). Adult live and motile *H. contortus* nematodes were collected from the gastrointestinal tract of slaughtered sheep at a local abattoir. Briefly, a minimum of 20 female *H. contortus* worms were exposed in three replicates to each of the treatments (12.5, 25, and 50 mg of aqueous and methanolic extracts) in separate petri dishes at room temperature (25–30 °C), and two petri dishes were also set for levamisole (0.55 mg ml^{-1} reference drug, Himedia Laboratories Pvt. Ltd., India) positive control and phosphate buffer saline (0.95%) as negative control. The inhibition of motility and/or mortality of the worms kept in the above treatments were used as a criterion for anthelmintic activity. The

motility was observed after 0, 1, 2, 5 and 8 h intervals, and post-treatment revival of motility (if any) was observed by keeping the treated worms in the lukewarm fresh PBS for 30 min. The number of worms found dead at 8 h post-treatment to aqueous and methanolic extracts of *C. album* were compared to the control group and evaluated statistically through a *z*-test using Statistical Package for the Social Sciences (SPSS) (17.0) for Windows.

In vivo experiment

A total of 18 Kashmir Marino sheep of both sexes (1 year of age) weighing 18–25 kg having naturally acquired gastrointestinal (GI) nematode infection were selected from the local sheep farm of Sindhbal, Ganderbal, Kashmir. The sheep were pre-adapted to the pen conditions for 18 days prior to the start of the study. Water, hay and feed were provided regularly to the study animals. The study continued for a period of 20 days post-treatments. Before the start of the study, the animals were confirmed positive with an infection of mixed GI nematodes by faecal examination using the standard parasitological procedures applicable to detection of nematode eggs in sheep faeces (Soulsby 1982). Faecal samples were cultured to cultivate the L_3 larvae and identified for dependable diagnosis of mixed GI nematode infection in sheep as per the methods of Coles et al. 2006. The sheep ($n = 18$) used for experiment were randomly divided into six treatment groups of three animals each on the basis of faecal egg counts (mean ± S.E. of eggs per gram of faeces) and assigned to different treatments as given below:

Group I: treated with single dose of CME @ 1.0 g kg^{-1} bw.
Group II: treated with CAE @ 1.0 g kg^{-1} bw.
Group III: treated with single dose of CME @ 2 g kg^{-1} bw.
Group IV: treated with single dose of CAE @ 2 g kg^{-1} bw.
Group V: treated with single dose of levamisole @ 7.5 mg kg^{-1} bw as positive control.
Group VI: untreated control.

Each group was isolated from other groups, and anthelmintic effectiveness was assessed as per the guidelines of World Association for the Advancement of Veterinary Parasitology (WAAVP) (Wood et al. 1995).

Faecal egg count reduction test

To determine the faecal egg count reductions of GI nematodes in sheep, faecal samples of each animal in the respective treatment groups were collected directly from the rectum in the morning, starting from day 0 and at days 5, 10 and 15 post-treatment (PT). The faecal samples were homogenized so that the eggs were uniformly distributed throughout the faeces prior to counting. The total number of nematode eggs (faecal egg counts) was determined using Mac Master Egg counting technique (Soulsby 1982); with each egg counted representing 50 eggs per gram of faeces. Faecal egg count percent reduction (FECR %) was calculated using the formula as described by Lone et al. (2012):

$$FECR\% = \frac{(\text{Pre}-\textit{treatment}\ \text{egg}\ \textit{count}\ \text{per}\ \textit{gram}) - (\textit{Post}-\textit{treatment}\ \text{egg}\ \textit{count}\ \text{per}\ \textit{gram})}{(\text{pre}-\textit{treatment}\ \text{egg}\ \textit{count}\ \text{per}\ \textit{gram})} \text{ x } 100$$

Antimicrobial activity

Different concentrations of the prepared *C. album* were tested by the disc diffusion method for the antibacterial activity against five strains of bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Pseudomonas multocida* and *Klebsiella pneumonia* (Bacteriological Section, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura Srinagar Kashmir). The test microorganisms were seeded into respective medium by spread plate method with the 24-h cultures of bacteria grown in nutrient broth. After solidification, the filter paper discs (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Streptomycin sulphate (200 μg/ml, Himedia Laboratories Pvt. Ltd.) was used as positive control, the antibacterial assay plates were incubated at 37 °C for 24 h and the diameters of the inhibition zones were measured in millimetre.

The antifungal activity was tested by disc diffusion method (Taylor et al. 1995) on Sabouraud's agar plates against two fungal strains *Aspergillus flavus* and *Candida albicans* (Mycological Section, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura Srinagar Kashmir). Filter paper discs (5 mm in diameter) impregnated with 100, 200, 300, 400 and 500 μg/ml concentrations of extracts and were placed on test organism-seeded plates. Nystatin (200 μg/ml, Himedia Laboratories Pvt. Ltd.) was used as positive control. The activity was determined after 72 h of incubation at 30 °C, and the diameters of the inhibition zones were measured in millimetre.

Antioxidant activity assays:

For evaluation of antioxidant activity of two alcoholic extracts following four methods were followed:

DPPH assay

The antioxidant activity of both the extracts of the plant was measured with 1,1-diphenyl 2-picryl hydrazyl radical (DPPH) spectrophotometrically at 517 nm (Blois 1958). The stock solution of both the plant extracts (5 mg/ml) was prepared by dissolving a known amount of dry extract in 10% aqueous DMSO. The working solutions (50, 100, 150, 200, 250 and 300 μg/ml) of all the extracts were prepared from the stock solution using suitable dilution. The scavenging activity was observed by bleaching of DPPH solution from violet colour to light yellow, and ascorbic acid was used as control.

Superoxide anion radical scavenging activity-riboflavin photo-oxidation method

Measurement of superoxide anion scavenging activity of both the extracts of the plant was calculated in accordance to the method described by Liu et al. (1997) spectrophotometrically at 590 nm using Phosphate buffer (also taken as control) as Blank after illumination for 5 min.

Hydroxyl scavenging activity—deoxyribose assay

The colorimetric deoxyribose (TBARS) method was applied as the reference method of comparison for determining the hydroxyl radical scavenging activity of both the extracts of the plant (Soobrattee et al. 2008) at 532 nm.

Lipid peroxidation method

A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the lipid peroxide formed using the egg yolk homogenate as lipid rich media (Padmaja et al. 2011) at 532 nm. The percentage of inhibition of the free radicals in the abovementioned methods was calculated by using the formula:

$$\textit{Percentage inhibition} = \frac{\mathrm{Ac}-\mathrm{As}}{\mathrm{Ac}} \times 100$$

where 'Ac' is the absorbance of the blank and 'As' is the absorbance of sample.

Statistical analyses

The data of adult motility assay (AMA), faecal egg counts and zone of inhibition was presented as mean ± standard error of mean (Lone et al. 2012). The FECR % was determined by the method described by Coles et al. (1992). The data from adult mortality test, FECRT and zone of inhibition from various treatments among different days were compared using the analysis of variance (ANOVA), Duncans test by using SPSS 17.0 program for windows.

Results

In vitro anthelmintic activity

The crude aqueous and methanolic extracts of *C. album* demonstrate the dose- and time-dependent anthelmintic activity against *H. contortus* as revealed from the inhibition of motility or death of worms after exposing to different extracts (Table 1). The methanolic extract of *C. album* exhibited highest worm mortality (100%) after 8 h exposure at 50 mg/ml concentration, while the aqueous extract at the same concentration resulted (70%) mortality. Methanolic extracts of *C. album* were more potent than the aqueous extracts. There was 100% mortality of worms in levamisole (used as a reference drug) within 4 h post exposure. There was no mortality of worms kept in PBS till 8 h post exposure.

In vivo anthelmintic activity by faecal egg count reduction test

The mean eggs per gram counts (EPG) and percentage reduction in faecal egg counts of sheep treated with different doses of the aqueous and methanolic extracts of *C. album* and commercial anthelmintic drug (levamisole) are presented in Table 2. The results revealed that a gradual reduction in percent faecal egg counts (% FEC) of naturally infected animals treated with both the aqueous and methanolic extracts and were significant ($p < 0.05$) on day 15 post treatment. The maximum reduction (86.07%) in faecal egg counts was recorded for methanolic extract followed by aqueous extract of *C. album* (65.35%) @ 2 mg/kg body weight at day 15 post-treatment.

Antimicrobial activity

The different extracts of *C. album* exhibit significant antimicrobial activity at higher concentrations (500 μg/ml) against *S. aureus*, *P. multocida* and *E. coli*. The overall highest antibacterial activity of 28 ± 0.14 mm recorded in *S. aureus* was observed at a concentration of 500 μg/ml, whereas least inhibition of 2 ± 0.50 mm was recorded for *E. coli* at 100 μg/ml concentration. Antifungal activity of the extract was observed at 200 μg/ml and above with maximum inhibition zone of 19 ± 0.88 mm against *A. flavus* at 500 μg/ml and minimum

Table 1 In vitro anthelmintic efficacy of crude methanolic and aqueous extracts of *Chenopodium album* on sheep parasite *H. contortus*

Treatment	Conc. mg/ml	Mean ± SEM of number of *Haemonchus contortus* worms showing motility (percent mortality)					
		0 h	1 h	2 h	5 h	8 h	Fresh PBS for 30 min[a]
Crude aqueous extract	50 mg/ml	20 ± 0.00	11 ± 0.0 (45.00)	8 ± 0.6 (60.00)	8 ± 0.5 (60.00)	6.00 ± 0.5 (70.00)	6.00 ± 0.5 (70.00)
	25 mg/ml	20 ± 0.00	14 ± 0.0 (30.00)	13 ± 0.7 (35.00)	10 ± 0.3 (50.00)	8 ± 0.0 (60.00)	8 ± 0.0 (60.00)
	12.5 mg/ml	20 ± 0.00	16 ± 0.0 (20.00)	16 ± 0.3 (20.00)	14 ± 0.0 (30.00)	10 ± 0.7 (50.00)	10 ± 0.7 (50.00)
Crude methanolic extract	50 mg/ml	20 ± 0.00	8 ± 0.0 (60.00)	5 ± 1.2 (75.00)	2 ± 0.9 (90.00)	0 ± 0.0 (100.00)	0 ± 0.0 (100.00)
	25 mg/ml	20 ± 0.00	12 ± 0.0 (40.00)	9 ± 0.6 (55.00)	6 ± 0.3 (70.00)	3 ± 0.0 (85.00)	3 ± 0.0 (85.00)
	12.5 mg/ml	20 ± 0.00	14 ± 0.0 (30.00)	12 ± 0.0 (40.00)	9 ± 1.2 (55.00)	7 ± 0.6 (65.00)	7 ± 0.6 (65.00)
Levamisole (positive control)	0.55 mg/ml	20 ± 0.00	3 ± 0.57 (64.00)	0.00 ± 0.0 (100.00)	0.00 ± 0.0 (100.00)	0.00 ± 0.0 (100.00)	0.00 ± 0.0 (100.00)
PBS (negative control)	0.9%	20 ± 0.00	20 ± 0.0	20 ± 0.0	20 ± 0.0	20 ± 0.00	20 ± 0.00

SEM standard error of mean, *PBS* phosphate-buffered saline, (0 h time of starting of experiment; 1 h, 2 h, 5 h and 8 h post-exposure hours)

[a] Worms were exposed to phosphate-buffered saline for 30 min after exposure to the different treatments to confirm their mortality

Table 2 Mean faecal egg counts and percentage reduction in egg counts for *Chenopodium album* extracts-treated sheep compared with untreated controls

Parasite	Fraction	Mean ± SEM of eggs per gram of faeces pre- and post-treatment (FECR %)			
		Pre-treatment	Post-treatment		
		Day 0	Day 5	Day 10	Day 15
Group I. *C. album* 1 g/kg bw	Aqueous	817.0 ± 20.59[a]	755.6 ± 18.22[b] (7.51)	605.0 ± 13.5[c] (25.94)	456.23 ± 5.05[d] (53.57)
	Methanol	1312.5 ± 23.9[a]	925.00 ± 59.5[b] (29.52)	717.5 ± 67.7[c] (45.33)	533.6 ± 65.7[d] (65.35)
Group II *C. album* 2 g/kg bw	Aqueous	1475.0 ± 9.4[a]	1201.5 ± 57.7[b] (18.54)	497.5 ± 42.7[c] (66.27)	439.6 ± 28.9[d] (70.19)
	Methanol	912.0 ± 6.04[a]	768.0 ± 12.17[b] (15.78)	378.0 ± 14.70[c] (58.55)	127.0 ± 1.94[d] (86.07)
Group III levamisole 7.5 mg/kg bw (Positive control)	–	880.4 ± 10.03[a]	60.8 ± 12.37[b] (93.09)	31.6 ± 20.46[c] (96.41)	12.6 ± 0.25[d] (98.56)
Group IV untreated (negative control)	–	682.6 ± 4.95[a]	697.6 ± 2.92[b]	705.0 ± 2.79[c]	742.6 ± 3.93[d]

Different letters indicate significantly different values ($P < 0.05$) comparing the sampling days

FECR % mean faecal egg count reduction percent, *SEM* standard error of mean, *bw* body weight; (day 0, day of starting the treatment; day 5, day 10, day 15 post-treatment days)

inhibition zone of 6 ± 0.01 mm against *C. albicans* at 300 μg/ml (Table 3).

Antioxidant activity

The antioxidant activity of both the extracts as measured by the ability to scavenge DPPH free radicals was compared with the standards/ascorbic acid and butylated hydroxyl toluene (BHT). Highest percentage inhibition was shown by aqueous extract with a maximum of 96% inhibition compared to the positive control (ascorbic acid 95%) at 300 μg/ml followed by methanol (73%). In riboflavin photo-oxidation method, the highest percentage inhibition was exhibited by methanol extract (85%) at 300 μg/ml concentration followed by aqueous (74%). However, the highest percentage of inhibition of 94% for hydroxyl scavenging activity was exhibited by methanol extract at 300 μg/ml followed by aqueous (83%). The effect of these plant extracts on in vitro inhibition of lipid peroxidation was recorded as aqueous (86%) and methanol (78%) (Table 4).

Discussion

Pharmaceutical and scientific communities have recently received the attention of the medicinal plants, as the herbal remedies prepared from the whole plant are generally safe with fewer side effects if used in the proper therapeutic dosages (Hanrahan 2001). In view of the apparently conspicuous effect of both the extracts of *C. album* on inhibition of motility of the *H. contortus* worms, the mortality of the worms was much faster in levamisole treatment than in crude aqueous extract and crude methanolic extract treatments. We found that methanolic extracts showed good in vitro and in vivo anthelmintic activity, and this could be due to the presence of a higher concentration of the alcohol-soluble active molecule(s) in the extract. The total action of the extracts is the sum of the activities of their constituents (Rates 2001). In vitro studies showed highest mortality (100%) of worms were found at 50 mg ml^{-1} concentration of methanolic extract than aqueous extracts of same concentration. These observations are in agreement with the findings of various earlier workers who tested anthelmintic efficacy of various plant species against *H. contortus* (Ketzis et al. 2002; Bizimenyera et al. 2006; Hordegen et al. 2006; Tariq et al. 2008). Similar results 98.00% mortality of worms have been observed in our previous findings on evaluating aqueous and methanolic extracts of *Euphorbia helioscopia* (Lone et al. 2012, 2013). Ketzis et al. (2002), for example, reported significant in vitro reduction in *H. contortus* egg-hatch with the plant *Chenopodium ambrosiodes* and its essential oils but no in vivo effects in reducing faecal egg counts in experimentally infected goats.

We found that methanolic extracts showed highest percent faecal egg count reduction (86.07%) in in vivo, and this could be due to the presence of a higher concentration of the alcohol-soluble active molecule(s) in the extract. A parallel in vivo study to ours though using a different *Chenopodium* species had demonstrated efficacy against *H. contortus* parasite (Eguale et al. 2007). Khare (2007) has demonstrated that dried *C. album* is effective against round and hookworms. Ascaridole, an active constituent of the oil of same plant, is highly active against roundworms, hookworms and amoebic dysentery and intestinal infections.

The antimicrobial activity of both the plant extracts against the different clinical strains of bacteria and fungi supported the scientific validity of the plant being used traditionally as a medicine Nisa et al. (2013). The inhibition of a maximum of four bacterial and two fungal strains by methanol extract may be attributed to the presence of soluble phenolic and polyphenolic compounds in the extract. The oil of this plant exhibits antimicrobial and strong antifungal activity against human pathogenic fungi. Plant contains 8% saponins, cryptomeridiol, isolated from the seeds and leaves showed significant growth promoting activity (Khare 2007). The results are in confirmation with a recent study of Bandh et al. (2011) in which it was shown that the methanol extract of *Nepeta cataria* inhibited the growth of all the bacterial and fungal test organisms, suggesting that the antimicrobial activity of the extract may be related to some phenolic components.

The mechanism of an antioxidant action in vitro involves direct inhibition of the generation of reactive oxygen species, or the scavenging of free radicals. Thus, it is clear that a single method cannot give a comprehensive prediction of antioxidant efficacy of the extracts. So, use of more than one method is recommended. Due to strong DPPH scavenging property of ascorbic acid, it is used as a standard antioxidant. The results show that aqueous extract of *C. album* may have hydrogen donors thus scavenging the free radical DPPH, with highest scavenging activity than the methanolic plant extract, which may be attributed to the total phenolic compounds. The extracts of this plant scavenged free radicals in a dose-dependent manner corresponding with the results (Siddhuraju et al. 2002; Sithisarn et al. 2005) showing that the plant metabolites like flavonoids, tannins, catechins and other phenolic compounds possess antioxidant activity. The highest superoxide anion radical scavenging activity of methanol extract of *C. album* corroborates with the results of Jayasri et al. (2009) who reported methanol to be the highest scavenger of superoxide radicals at higher concentration of plant extract. The different extracts of *C. album* when added to the reaction mixture removed the hydroxyl radicals from the sugar and prevented them from degradation. The methanol extract of *C. album* showed maximum inhibitory effect compared to the standard antioxidant, butylated hydroxy toluene. The lipid peroxidation inhibitory activity of the methanolic extracts of the plant are a

Table 3 Antimicrobial activity of methanolic extract of *Chenopodium album*

Test organism	Zone of inhibition in mm					Streptomycin sulphate (200 μg/ml)	Nystatin (200 μg/ml)
	Concentrations μg/ml						
	100	200	300	400	500		
E. coli	2 ± 0.50	7 ± 0.09	11 ± 0.35	16 ± 0.16	20 ± 1.20	22 ± 0.25	NC
S. aureus	3 ± 0.50	8 ± 0.06	16 ± 0.57	22 ± 0.10	28 ± 0.14	22 ± 0.57	NC
P. multocida	–	–	10 ± 0.33	16 ± 0.57	22 ± 0.25	28 ± 0.14	NC
K. pneumoniae	–	4 ± 0.16	8 ± 0.09	13 ± 0.57	16 ± 0.57	18 ± 1.20	NC
A. flavus	–	–	–	13 ± 0.33	19 ± 0.88	NC	22 ± 0.57
C. albicans	–	–	6 ± 0.01	11 ± 0.57	16 ± 0.66	NC	18 ± 0.16

Values are mean inhibition zone (mm) ± S.D. of three replicates

NC not taken as positive control

result of the effects of polyphenols (flavonoids) on lipid peroxidation at the stage of initiation and termination of peroxyl radicals in confirmation with the findings of Paramaguru et al. (2012). The same is also supported by the results of Prasad et al. (2005) and Nisa et al. (2013) showing that the lipid peroxidation inhibitory activity as an attribute of flavonoids. *C. album* has got a broad spectrum antimicrobial and antioxidant activity and could be a potential alternative for treating various diseases.

Conclusion

The discovery of a potent remedy from plant origin will be a great advancement in anthelmintic, antimicrobial infection therapies. The results of present investigation clearly indicate that the anthelmintic, antimicrobial and antioxidant activities of the plant used. These results suggest that the plant extracts possess compounds with anthelmintic, antimicrobial and antioxidant properties that can be further explored for antimicrobial activity. This anthelmintic, antimicrobial and antioxidant study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. Further work is needed to isolate the active principle from the plant extracts and to carry out pharmaceutical studies.

Acknowledgements We acknowledge the financial support from the Department of Science and Technology, Govt. of India, New Delhi. We also appreciate the technical assistances of the CORD technical staff for their valuable assistance with the initial *H. contortus* cultures (Microbiology Lab). The authors would like to express their sincere thanks to the Director of the Centre Prof. Azra N. Kamili for providing the necessary facilities.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Table 4 Antioxidant activity of different extracts of *Chenopodium album*

Conc. (μg/ml)	DPPH assay			Riboflavin photo-oxidation method			Hydroxyl scavenging activity			Lipid peroxidation method		
	Meth	Aq	AA	Meth	Aq	AA	Meth	Aq	BHT	Meth	Aq	AA
50	45 ± 2.0	84 ± 1.0	70 ± 1.0	57 ± 1.0	53 ± 1.52	65 ± 1.0	60 ± 1.52	55 ± 0.57	60 ± 1.0	55 ± 1.0	61 ± 1.52	59 ± 1.52
100	52 ± 1.52	86 ± 0.57	75 ± 1.52	64 ± 1.15	55 ± 1.0	71 ± 1.52	68 ± 1.0	64 ± 1.0	68 ± 1.0	61 ± 0.57	64 ± 2.08	64 ± 2.08
150	59 ± 1.0	88 ± 1.0	83 ± 1.15	68 ± 1.52	60 ± 2.08	80 ± 1.52	73 ± 1.0	69 ± 0.57	75 ± 1.52	62 ± 1.0	70 ± 0.57	70 ± 1.0
200	64 ± 1.52	91 ± 1.52	87 ± 1.52	73 ± 1.0	64 ± 0.57	87 ± 1.0	80 ± 0.57	74 ± 1.52	84 ± 0.57	69 ± 1.0	74 ± 1.0	75 ± 2.0
250	69 ± 0.57	94 ± 0.57	93 ± 0.57	79 ± 0.57	70 ± 1.52	94 ± 0.57	87 ± 1.15	78 ± 1.15	89 ± 1.0	73 ± 1.52	80 ± 1.52	81 ± 1.0
300	73 ± 1.0	96 ± 1.0	95 ± 1.15	85 ± 1.0	74 ± 1.52	97 ± 1.0	94 ± 1.0	83 ± 1.0	95 ± 1.52	78 ± 1.0	86 ± 0.57	89 ± 2.08

Data is represented as mean ± SD

Meth methanol, *Conc.* concentration, *Aq* aqueous, *AA* ascorbic acid, *BHT* butylated hydroxy toluene

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