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PHYTOCHEMICAL, ANTI-OXIDANT AND ANTHELMINTIC ACTIVITIES OF AERIAL PARTS OF *ERANTHEMUM CAPENSE LINN*

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ABSTRACT

The present study was carried out to investigate the phytochemical constituents, *in vitro* antioxidant potential and anthelmintic activities of *Eranthemum capense Linn*. The dried powdered leaves of *Eranthemum capense Linn* were extracted using petroleum ether, chloroform, ethyl acetate and methanol using a soxhlet extractor and preliminary phytochemical screening was performed using standard protocols. All the extract was evaluated for their potential antioxidant activities using test such as DPPH, hydroxyl radical and superoxide anion radical scavenging abilities. Anthelmintic activity of extract was screened in adult Indian earthworm model. Preliminary screening revealed the presence of bioactive compounds especially phenolics, tannins and steroids in all extracts. The paralytic (11.22±0.260) and death time (28.10±0.885) of methanolic extract was found to be significant ($P<0.05$) when compared with paralytic (5.30±0.134) and death time (11.35±0.431) of standard albendazole at 100 mg/ml concentration. The results of the present study indicate that the aerial parts of *Eranthemum capense Linn* exhibited strong anti-oxidant activity and possess significant anthelmintic activity and thus it is a good source of antioxidant and anthelmintic constituents.

KEYWORDS

Antioxidant, Anthelmintic, *Eranthemum capense Linn* and Albendazole.

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INTRODUCTON

In recent years herbal drugs are rapidly becoming popular as an alternative therapy. As plants produce huge amounts of antioxidants to control the oxidative stress due to sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity. Plants contain a wide variety of free radical scavenging molecules, such as phenolic compounds[1], nitrogen compounds, vitamins,

terpenoids (including carotenoids) and some other endogenous metabolites, which are rich in antioxidant activity[2]. Natural antioxidant have a wide range of biochemical activities including inhibition of reactive oxygen species (ROS) generation, direct or indirect scavenging of free radicals and alteration of intracellular redox potential[3].

The use of antioxidants found naturally has gained much attention from consumers because they are considered safe to use than synthesized antioxidants. Recently there has been a worldwide trend towards the usage and intake of natural antioxidants present in different parts of certain plants due to their phytochemical constituents[4,5]. The intake of natural antioxidants has been associated with the reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with ageing[6,7].

Helminthic infestations are now being recognized as a reason for chronic ill health and sluggishness amongst the children. More than half of the total population suffers from diseases of worm infestations of one or other. Helminthes also affects domestic animals and live stocks causing considerable economic loss. Various alternative and traditional systems of treatments reports the efficacy of several natural products eliminating helminthes[8]. Only synthetic drugs are frequently used in the treatment of human beings as per WHO, but these synthetic drugs are out of reach of millions of people and have a lot of side effects[9].

Eranthemum capense Linn belonging to the family acanthaceae is a sub shrub and is endemic to peninsular India widely distributed in Goa, Chickmaglur, Mangalore, Mysore, Uduppi. It is an endemic species usually found in selected sacred groves of kasaragod district in kerala. Various parts are widely used in folk medicine as anti-inflammatory, anthelmintics, antidiabetic, anti-dysenteric and woundhealing[10]. However no scientific report is available in the literature regarding anti-oxidant and anthelmintic activities of the *Eranthemum capense Linn*.

Thus in the light of knowledge that *Eranthemum capense Linn* is having wide folklore uses, we intend to evaluate the antioxidant and anthelmintic activities of the various extracts of *Eranthemum capense Linn* using *in vitro* models.

MATERIAL AND METHODS

Plant Material

The aerial parts of *Eranthemum capense Linn* were collected from Tirunalveli district, Tamilnadu, India during the month of March 2016. The plant was identified and authenticated by Mr. Chelladurai, Research Officer- Botany, Central Council for Research in Ayurveda and Siddha, Government of India (Ref No:- DCP/CH/AN02)

Chemicals and instruments

DPPH were purchased from Sigma – Aldrich, USA. 2-deoxy-2-ribose, gallic acid, curcumin, Ascorbic acid and quercetin were purchased from Himedia Labs., Pvt. Ltd, Mumbai, India. All other drugs and chemicals used for the work were purchased commercially and were of analytical grade. U-V spectrophotometer Shimadzu was used to measure the absorbance.

Extraction

The aerial parts of *Eranthemum capense Linn* were collected, shade dried, powdered mechanically and sieved through No. 20 mesh sieve. About 100g of the powdered aerial part is first extracted with petroleum ether (PEE, 60º-80ºC) and then consecutively with chloroform (CEE), ethyl acetate (EAEE) and methanol (MEE). The percentage yield of the extracts is listed in Table No.1.

Phytochemical screening of the extracts

Chemical tests were carried out for the all the extract of *Eranthemum capense Linn* for the presence of phytochemical constituents like phenols, tannins, saponins, flavonoids, terpenoids, alkaloids, glycosides and steroids[11,12].

Antioxidant activity

DPPH radical scavenging activity[13]

DPPH (1-diphenyl-2-picrylhydrazyl) assay gives an account on the free radical scavenging ability[13]. Briefly about 1ml (0.1mM) of DPPH solution prepared in methanol was added to 3 ml of test or standard (Gallic acid) solution at different concentration (0.25-64µg/ml). The mixture was incubated in dark at 30ºC for 30 min and the

absorbance measured at 517 nm and percentage inhibition calculated. A control reaction was carried out without the test sample.

Hydroxyl radical scavenging activity[14]

Hydroxyl radical scavenging activity of the extract is determined by its ability to scavenge the hydroxyl radicals produced by the EDTA-Fe^{3+}-H_2O_2-ascorbic acid system by a reaction known as Fenton reaction[14]. The reaction mixture amounts to a final volume of 1.0 ml which contains 100µl of 2-deoxy2-ribose (28mM) in phosphate buffer solution (20mM, pH 7.4), 500µl of the extracts at various concentrations (10-160µg/ml) in buffer solution, 200µl of 1.04mM EDTA and 200µM $FeCl_3$ (1:1v/v), 100µl of H_2O_2 (1.0mM) and 100µl of ascorbic acid (1.0mM). Test samples were incubated at 37°C for 1 h. Thiobarbituric acid test was used to assess the free radical damage inflicted on the substrate, deoxyribose. The positive control which was used for this assay was quercetin (10-160 µg/ml).The percentage inhibition of the extracts and standard were calculated.

Superoxide radical scavenging activity[15]

The superoxide radicals are generated in a phenazinemethosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by the reduction of nitrobluetetrazolium (NBT)[15]. The superoxide radicals were generated in this experiment in 3 ml of Tris–HCl buffer (16mM, pH 8.0) containing 78mM NADH, 50mM NBT, 10mM PMS and extracts to be tested at different concentrations (10-160µg/ml). The color reaction between superoxide radicals and NBT was detected at 560nm and the percentage inhibition calculated. Positive control used was Ascorbic acid (10-160µg/ml).

Calculation of 50% inhibitory concentration (IC_{50})

The concentration (µg/ml) of the extract required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the extracts. Percentage inhibition (I%) was calculated using the formula:

$$I\% = \frac{Ac - At}{Ac} x100$$

Where A_c is the absorbance of the control and A_t is the absorbance of the test sample.

Anthelmintic activity

Adult Indian earthworms, *Pheretima postuma* resembled the intestinal roundworm parasites of human beings both anatomically and physiologically[16,17] and hence where used to study the anthelmintic activity. Indian adult earthworm 6-8 cm length and 0.1-0.2cm in width were used for the *in vitro* anthelmintic bioassay of petroleum ether, chloroform, ethylacetate and methanol extracts. The earthworms were divided into the different group containing six earthworms in each group. All the prototypes were dissolved in minimum quantity of 2% v/v Tween80 and then the volume was adjusted to 10 ml with normal saline for producing the concentrations of 25, 50 and 100µg/ml. All the prototypes and standard drug solution were freshly prepared before the starting of the experiments. All the earthworms were washed in normal saline solution before they were released into 10ml of respective formulation as follows,- vehicle (2% v/v Tween 80 in normal saline), standard albendazole (25,50 and 100 mg/ml) and prototypes (25,50 and 100 mg/ml) the anthelmintic activity was determined.

Paralysis was said to occur when the worms do not revive activities even in normal saline. Death was concluded when the worms lost their movements followed with fading away or discoloration of their body. They were observed for their spontaneous motility. Observations were made and recorded for time taken to paralysis and death of individual worms.

Statistical analysis

All the experiments were carried out in triplicate and results expressed as mean ± SEM. Significant differences among means of samples were evaluated by one-way analysis of variance (ANOVA).

RESULTS

Phytochemical screening of the extract

Phytochemical analysis showed the presence of phenolics, terpenoids, tannins, flavanoids and steroids in the extract (Table No.2).

DPPH radical scavenging activity

DPPH radical scavenging of various extracts of the leaves of *Eranthemum capense Linn* was investigated and results were shown (Table No.3). All the extracts showed a dose dependent scavenging activity, of which the methanolic extract showed the highest activity. The gallic acid which was used as standard had higher scavenging activity than any of the extracts. The highest activity was shown by MEE (IC_{50} = 2.28±0.132) and the order of decreasing scavenging ability is MEE> EAEE (17.08±0.186) > CEE (31.28±0.096). All extracts showed significant (P<0.05) scavenging ability when compare with standard gallic acid (IC_{50} = 1.83±0.116).

Hydroxyl radical scavenging activity

The extracts and the standard (quercetin) inhibited the formation of hydroxyl radical in a dose dependent manner (Table No.5). The MEE (IC_{50} = 55.37±0.085) showed the maximum quenching ability followed by EAEE (IC_{50} = 69.48±0.184) and CEE (IC_{50} = 112.06±0.163). The *in vitro* radical scavenging ability of the extracts were found to be significant (p<0.05) when compared with the standard quercetin (IC_{50} = 34.79±0.139).

Superoxide radical scavenging activity

The superoxide radical scavenging ability was found to increase with increase in concentration of the extract. The MEE (IC_{50} = 43.12±0.193) was found to be an efficient scavenger of superoxide anion radical generated from PMS-NADH system *in vitro* and the activity was significant (P<0.05) when compared to that of standard ascorbic acid (IC_{50} = 35.63±0.094). The scavenging effects of extracts on the superoxide anion radical decreased in order MEE> EAEE (IC_{50} = 75.23±0.216) > CEE (IC_{50} = 128.58±0.086) (Table No.4).

Anthelmintic activity

The extracts exhibited more potent activity at a higher concentration (100mg/ml) against *Pheretima posthuma* (earthworm). When observed the response of worms in case of paralysis and death, there was significant variations among the results produced by the different extracts at different concentrations (25, 50 and 100mg/ml) (Table No.8). All the extracts exhibited anthelmintic activity in dose dependent manner varying from loss of motility (paralysis) to loss of response to external stimuli, which eventually advanced into death. MEE, EAEE and CEE exhibited significant anthelmintic activity in dose dependent manner when compared with reference standard albendazole. The methanolic extract showed less time to cause paralysis(11.22±0.260) and death(28.10±0.885) of the earthworms and thus it was found to be more potent than other extracts (MEE> EAEE> CEE> PEE) at 100 mg/ml concentration.

DISCUSSION

Phytochemical screening

Various bioactive components such as flavanoids, tannins, phenolics, terpenoids and steroids were prominently revealed during the preliminary phytochemical screening. Phenolics, tannins and steroids were present in all the extracts whereas glycosides were absent in all the extracts. Alkaloids, terpenoids and proteins were absent in petroleum ether extract. Flavonoids were absent in petroleum ether and chloroform extract.

Antioxidant assay

Radical scavenging activities have huge importance due to the deleterious role of free radicals in biological systems. In certain conditions, over production of oxidants can cause imbalance leading to oxidative damage to large biomolecules such as lipids, DNA and proteins. Adverse side effects are present for many synthetic drugs even thou they protect against oxidative damage. Data from both scientific reports and laboratory studies show that the plant contain a large variety of substance called “plant chemicals” or “phytochemicals” that possess antioxidant activity[18,19]. Studies have attributed that antioxidant properties are due to the presence of phenols and flavanoids[20]. Thus the presence of these components would have contributed to significant antioxidant activity of plant extracts. Antioxidant of phenolic compounds is based on their ability to donate hydrogen atom to free radicals[21]. The scavenging activity of a stable radical is considered

a valid and easy assay to evaluate scavenging activity of natural compounds[22].

DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. From the present result it may be postulated that *Eranthemum capense Linn* reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles. The methanolic extracts exhibited high DPPH radical scavenging activity compared to other extracts in the present study. Superoxide anion is oxygen centered radical with certain selective reactivity. This species is created by a number of enzyme systems in auto-oxidation reactions and by non enzymatic electron transfers that univalently reduce molecular oxygen. It can also reduce certain iron complexes such as cytochrome[23]. The present study showed potent superoxide radical scavenging activity for *Eranthemum capense Linn.* Methanol extract showed potent superoxide radical scavenging activity with IC_{50} value compared to standard ascorbic acid.

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity[24]. One of the potent reactive oxygen species in the biological system is hydroxyl radical. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and cause damage to cell[25]. The present study shows that the extracts had significant scavenging effects on hydroxyl radical, which increased with the increase in concentration from 10-160µg/ml.

Anthelmintic activity

Helminthic infections of the gastrointestinal tract of human beings and animals have been acknowledged to have adverse effects on the health standards with a consequent lowering of resistance to other diseases. Now a days resistance to the available synthetic drugs is a major problem. A search for plant derived drugs is the primary choice of researchers in recent years as they are believed to have lesser side effects and are more compatible with the physiological flora[26,27]. Phytochemical analysis of the crude extract revealed the presence of flavanoids, phenolics, tannins and alkaloids which are known to exhibit anthelmintic property. Tannins and Phenolics are known to interfere with the energy generation in helminth parasites by uncoupling oxidative phosphorylation[28] and also bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite, causing it's death. Based on these we can assume that tannins, phenolic compounds and flavanoids present in the leaf extract of *Eranthemum capense Linn* may be responsible for the anthelmintic activity.

Table No.1: Percentage yield of various extracts

S.No	Extracts	% Yield (w/w)
1	PEE	7.1
2	CEE	1.3
3	EAEE	5.2
4	MEE	6.8

PEE:- Pet Ether Extract of *Eranthemum capense Linn*; CEE:- Chloroform E*x*tract of *Eranthemum capense Linn* EAEE:- Ethyl Acetate Extract of *Eranthemum capense Linn*; MEE:-Metanolic Exract of *Eranthemum capense Linn.*

Table No.2: Phytochemical screening of various extracts

S.No	Phytochemicals	PEE	CEE	EAEE	MEE
1	Tannins and phenolics	+	+	+	+
2	Saponins	+	-	-	+
3	Flavonoids	-	-	+	+
4	Terpenoids	-	+	+	+
5	Alkaloids	-	+	+	+
6	Glycosides	-	-	-	-
7	Steroids	+	+	+	+
8	Proteins	-	+	+	+

PEE:- Pet Ether Extract of *Eranthemum capense Linn*; CEE:- Chloroform Extract of *Eranthemum capense Linn* EAEE:-Ethyl Acetate Extract of *Eranthemum capense Linn*, MEE:-Metanolic Extract of *Eranthemum capense Linn.*

Table No.3: DPPH Radical scavenging activity of *Eranthemum capense Linn*

S.No	CONC µg/ml	PERCENTAGE INHIBITION (%)				
		PEE	CEE	EAEE	MEE	GALLIC ACID
1	0.25	-	-	-	5.44±0.342	08.34±0.214
	0.5	-	-	-	18.64±0.942	19.25±0.441
	1	-	-	-	36.17±0.452	30.74±0.302
	2	-	-	04.28±0.131	48.94±0.056	52.23±0.283
	4	06.45±0.328	09.32±0.233	15.05±0.152	64.12±0.155	70.18±0.102
	8	14.40±0.311	20.16±0.110	34.38±0.225	80.22±0.072	87.87±0.046
	16	19.88±0.436	35.45±0.195	49.93±0.252	87.36±0.194	93.54±0.018
	32	24.69±0.922	51.74±0.039	69.62±0.211	91.57±0.154	97.38±0.178
	64	29.01±0.062	58.53±0.138	86.34±0.156	93.30±0.038	100
2	IC_{50}µg/ml	#	31.28±096	17.08±0.186	02.28.±0.132	01.83±0.116

PEE:- Pet Ether Extract of *Eranthemum capense Linn*; CEE:- Chloroform Extract of *Eranthemum capense Linn* EAEE:-Ethyl Acetate Extract of *Eranthemum capense Linn*, MEE:-Metanolic Exract of *Eranthemum capense Linn.* All values determined were mean ± SEM; n= 3. *P < 0.05 when compared with standard

Table No.4: Hydroxyl radical scavenging activity of *Eranthemum capense Linn*

S.No	CONC µg/ml	PERCENTAGE INHIBITION (%)				
		PEE	CEE	EAEE	MEE	QUERCETIN
1	10	04.12± 0.128	08.87±0.054	11.61±0.064	13.24±0.134	18.71±0.112
	20	12.31±0.232	18.59±0.198	21.08±0.088	28.64±0.055	38.31±0.258
	40	19.45±0.085	36.43±0.102	39.85±0.125	43.93±0.094	54.43±0.251
	80	25.56±0.164	46.95±0.168	54.59±0.081	59.04±0.262	67.44±0.044
	160	30.64±0.123	55.73±0.108	64.76±0.118	73.52±0.113	82.37±0.197
2	IC_{50}µg/ml	#	112.06±0.163	69.48±0.184	55.37±0.085	34.79±0.139

PEE:- Pet Ether Extract of *Eranthemum capense Linn*; CEE:- Chloroform Extract of *Eranthemum capense Linn* EAEE:-Ethyl Acetate Extract of *Eranthemum capense Linn*; MEE:-Metanolic Extract of *Eranthemum capense Linn.* All values determined were mean ± SEM; n= 3. *P < 0.05 when compared with standard

Table No.5: Super oxide radical scavenging activity of *Eranthemum capense Linn*

S.No	CONC µg/ml	PERCENTAGE INHIBITION (%)				
		PEE	CEE	EAEE	MEE	ASCORBIC ACID
1	10	06.30± 0.321	08.36±0.292	10.92±0.138	14.24±0.231	23.45±0.174
	20	13.63±0.402	18.27±0.046	22.78±0.296	29.90±0.157	40.78±0.131
	40	22.37±0.089	30.18±0.178	38.61±0.152	48.77±0.188	52.50±0.102
	80	31.33±0.053	44.59±0.045	51.48±0.040	65.86±0.083	67.42±0.155
	160	39.27±0.188	55.63±0.136	63.29±0.118	80.06±0.055	82.93±0.086
2	IC_{50} (µg/ml)	#	128.58±0.086	75.23±0.216	43.12±0.193	35.63±0.094

PEE:- Pet Ether Extract of *Eranthemum capense Linn*; CEE:- Chloroform Extract of *Eranthemum capense Linn* EAEE:-Ethyl Acetate Extract of *Eranthemum capense Linn*; MEE:-Methanolic Extract of *Eranthemum capense Linn*. All values determined were mean ± SEM; n= 3. *P < 0.05 when compared with standard

Table No.6: Anthelmintic activities of *Eranthemum capense Linn* leaves

S.No	Test Sample	Concentration (mg/ml)	Time Taken for Paralysis (Minutes)	Time Taken for Death (Minutes)
1	Control (0.1% Tween in normal saline)	--	--	--
2	PEE	25	164.57±0.635*	--
		50	81.75±0.218*	--
		100	47.52±0.905*	185.07±1.012*
3	CEE	25	76.33±0.815*	164.14±0.104*
		50	53.29±0.078*	98.55±0.942*
		100	33.42±0.229*	76.42±0.612*
4	EAEE	25	39.37±0.174*	72.27±0.493*
		50	25.18±0.625*	48.11±0.855*
		100	14.22±0.815*	27.47±217*
5	MEE	25	31.48±0.294*	68.19±0.712*
		50	18.16±0.774*	41.54±0.461*
		100	11.22±0.260*	28.10±0.885*
6	Albendazole	25	12.13±0.108*	19.33±0.931*
		50	5.30±0.134*	11.35±0.431*
		100	2.33±0.238*	6.30±0.756*

PEE:- Pet Ether Extract of *Eranthemum capense Linn*; CEE:- Chloroform Extract of *Eranthemum capense Linn*; EAEE:-Ethyl Acetate Extract of *Eranthemum capense Linn*; MEE:-Methanolic Extract of *Eranthemum capense Linn*.

All values determined were mean ± SEM; n= 6. *P < 0.05 when compared with standard

CONCLUSION

The present study reveals that the leaf extract of *Eranthemum capense Linn* has significant antioxidant and anthelmintic activity. But further investigations on the isolation of active compounds present in the extracts and *in vivo* studies are necessary to identify a potential chemical entity for clinical use.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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