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Research Article

IN VITRO ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITY OF METHANOL EXTRACT OF *ERANTHEMUM CAPENSE*

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ABSTRACT

Objective: The present study was aimed to rationalize the scientific basis in traditional use of *Eranthemum capense* as an antibacterial, antifungal, and anti-inflammatory agent.

Methods: Agar well diffusion method is widely used to evaluate the antimicrobial activity of the *E. capense* aerial part of methanolic and ethyl acetate plant extracts. The same amount (15–20 mL) of Mueller-Hinton agar was poured on glass Petri plates of same size and allowed to solidify. *E. capense* aerial part of methanolic and ethyl acetate extracts was evaluated *in vitro* for their anti-inflammatory activities using the bovine serum albumin protein denaturation assay.

Results: The result of the study shows that methanolic exract (T3) of the plant, *E. capense* shows 16 mm zone of inhibition against *Pseudomonas fluorescens*, while the ethyl acetate extract of the same plant shows 14 mm zone of inhibition against *P. fluorescens* and *E. coli*. Hence the methanolic extract of T3 sample shows the antibacterial activity against gram negative bacteria, where as the ethyl acetate extract of T3 shows antibacterial activity against both gram positive and gram negative bacteria. The experimental report revealed that, the methanolic and ethyl acetate extract of the same plant produces zero percentage zone of inhibition against *Aspergillus niger* and Mucor, hence it does not show any antifungal activity.

Conclusion: It is observed that the EA and methanolic extract of *E. capense* can be used in the treatment of inflammation due to the significant percentage of inhibition of protein denaturation as well as its prove the good antimicrobial agent.

Keywords: *Eranthemum capense,* Antibacterial, Antifungal, Anti-inflammatory, Denaturation, Inhibition.

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INTRODUCTION

Natures fauna and flora gave us a complete store of remedies to treat the ailments of suffering humankind [1,2]; hence, these natural drugs have been used since ages as cure for diseases as they contain natural chemical compounds of the therapeutic value of *Eranthemum capense*. Inflammation is generally referred to as a complex biological response of vascular tissues to harmful stimuli [3]. As well, inflammation is associated with pain, and it involves in an increase of protein denaturation, protein denaturation has been identified as the cause of inflammation [4]. Indications are that when living tissues are injured, inflammation results [5,6]. This is characterized by redness, pain, heat, swelling, as well as loss of function in the affected area [7,8]. Disruption of the electrostatic, hydrogen, hydrophobic, and disulfide bonds in the protein structure occurs [9]. In addition, a complex array of enzyme activation, mediator release, cell migration, tissue breakdown, and repair occurs, causing the protein to lose its molecular conformation and functions or becomes denatured. Recently, traditional medicine worldwide is being reevaluated by extensive research on different plant species and their active therapeutic principles [10]. Natural products have contributed significantly towards the development of modern medicines. The rich wealth of the plant kingdom can represent a novel source of newer compounds with significant anti inflammatory activities [11].

E. capense (L.) Willd. Family: Acanthaceae. Conventionally, the plant has been used for some medicinal purposes in the Indian subcontinent [12]. The aerial part plant has been used in folk medicine to treat stomach ulcers. The plant is thought to be efficacious in the treatment of gastric problems. Conventionally, its leaf juice is applied to the affected area of the body in the treatment of wounds and bruises [13]. The plant is regarded as a rich source of bioactive compounds, especially flavonoids, tannins, phenolic, terpenoids, and steroids which were prominently revealed during the preliminary phytochemical screening. Phenolics, tannins, and steroids were present in the extract, whereas glycosides were absent in the extracts. Alkaloids, terpenoids, and proteins were absent in the extract [14]. The novelty work is to know *in vitro* antimicrobial and anti-inflammatory activity of methanol (MeOH) extract of *E. capense* and optimize the concentration which can be more effective.

METHODS

Plant material

The aerial parts of *E. capense* Linn. were collected from Tirunelveli district, Tami Nadu, 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).

Extraction

The aerial parts of *E. capense* Linn. were collected, shade dried, powdered mechanically, and sieved through No. 40 mesh sieve [15]. About 100 g of the powdered aerial part is first extracted with petroleum ether (PEE, 60–80°C) and then consecutively with chloroform (CEE), ethyl acetate (EA), and MeOH. The study continued to the plant EA and MeOH extract based on the already reported phytochemical study literature.

Table 1: Antibacterial activity of *Eranthemum capense* plant extracts

Microorganisms	Zone of inhibition (mm)						
	Positive control (gentamicin)	MeOH			EA		
		T1	T2	T3	T1	T2	T3
Staphylococcus aureus	28	-	-		-	-	
Bacillus subtilis	28	-	-		-	10	12
Pseudomonas fluorescens	19	9	14	16	10	12	14
Escherichia coli	27	11	12		-	12	14

Table 2: Antifungal activity of *Eranthemum capense* plant extracts

Microorganisms	Zone of inhibition (mm)				
	Positive control (fluconazole)	MeOH		EA	
		T1	T2	T1	T2
Aspergillus niger	25	-	-	-	-
Mucor	35	-	-	-	-

Table 3: IC_{50} value for standard (diclofenac) in protein denaturation inhibitory assay

Standard (diclofenac sodium)	Concentration (µg/ml)	% of inhibition	IC_{50}
Control	-	-	281.85±1.26
S_1	100	23.28±1.08	
S_2	200	47.46±1.74	
S_3	300	70.63±1.52	
S_4	400	89.52±0.98	

All values determined were mean±standard error of the mean; n=3. *p<0.05 when compared with standard

Table 4: IC_{50} value for ethyl acetate and extract of *Eranthemum capense* in protein denaturation inhibitory assay

Sample name	Marked as control	Concentration (µg/ml)	% inhibition (µg/ml)	IC_{50} (µg/ml)
EA	T1	250	19.6±0.68	1394.58±
(1 mg/ml)	T2	500	29.3±1.02	1.52
	T3	750	31.7±1.34	
	T4	1000	38.6±0.92	
MeOH	T1	250	31.2±1.34	802.63±
(1 mg/ml)	T2	500	40.4±0.74	1.28
	T3	750	57.3±1.22	
	T4	1000	64.1±1.08	

All values determined were mean±standard error of the mean; n=3. *p<0.05 when compared with standard

Procedure

Well diffusion assay antibacterial

Agar well diffusion method is widely used to evaluate the antimicrobial activity of the plant extracts. The same amount (15–20 mL) of Mueller-Hinton agar was poured on glass Petri plates of same size and allowed to solidify and followed by standardized inoculum of the test organism [16]. Four wells with a diameter of 8 mm (20 mm apart from one another) were punched aseptically with a sterile cork borer in each plate. Extract solution (20 and 40 µL) at the desired concentration was added to two of the wells and one well with gentamycin as positive and extract solvent as a negative control. Then, the agar plates were incubated under suitable conditions depending on the test microorganism [17]. After incubation, a clear zone was observed. Inhibition of the bacterial growth was measured in mm.

Well diffusion assay antifungal

Well diffusion assay antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of antifungal activities of the plant samples [18]. A sterile swab was used to evenly distribute fungal culture over the potato dextrose agar medium. The plates were allowed to dry for 15 min before use in the test [19]. Wells were then created and a pipette was used to place 20–40 µl of the sample into each well. The same extract was used on each plate, with a total of two plates used for each extract [20] including two wells for the positive and negative controls. The plates were incubated at room temperature for 3 days, after which they were examined for inhibition zones. A caliper was used to measure the inhibition zones.

Procedure for inhibition of protein denaturation assay

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (3% aqueous solution) and 0.05 ml isolated fraction (250 µg/ml of final volume); pH was adjusted to 6.3 using a small amount of 1 N hydrochloric acid. The samples were incubated at 37°C for 20 min and then heated at 80°C for 2 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube [20]. The absorbance was measured using a spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition=(Abs control−Abs sample)×100/Abs control

RESULTS AND DISCUSSION

Antibacterial activity of *E. capense* plant aerial part of EA and methanolic extracts shows a significant role in the *Staphylococcus*, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Escherichia coli*. Table 1 intimates the highest zone of the inhibition of methanolic extract of *E. capense* aerial part test sample MeOH (T3) against *Pseudomonas fluorescens* 16 mm and ethyl acetate extract test sample (T3) of *E. capense* aerial part against *P. fluorescens* and *E. coli* zone of inhibition 14 mm; hereby, both extracts have significant antibacterial activity compared with standard drug which is proved. Table 2 intimates the antifungal activity 0% zone of inhibition of MeOH and ethyl acetate extract test sample of *E. capense* aerial part test sample against *Aspergillus niger* and *Mucor*. The results of testing the diameter of the inhibition zone growth of microorganisms using EA and MeOH extract of *E. capense* showed that the MeOH fraction had more significant antibacterial effect than EA.

It was effective in inhibiting heat-induced albumin denaturation at different concentrations, as shown in Table 4. Maximum inhibition of EA extract (38.6%) was observed at 1000 µg/ml and maximum inhibition of MeOH extract (64.1%) was observed at 1000 µg/ml. IC_{50} value of ethyl acetate extract was found to be 1394.58 µg/ml and the IC_{50} value of MeOH extract was found to be 802.63 µg/ml. In Table 3, diclofenac sodium, a standard anti inflammatory drug, showed the inhibitions for different concentrations of 100, 200, 300 and 400 µg/ml. It showed the maximum inhibition 89.52% at the concentration of 400 µg/ml.

CONCLUSION

The result of the study shows that the EA and methanolic extract of *E. capense* can be used in the treatment of inflammation due to the significant percentage of inhibition of protein denaturation as well as its prove the good antimicrobial agent. Further investigations are required to find an active component of the extract and to confirm the mechanism of action.

AUTHORS' CONTRIBUTIONS

All authors have equally contributed for making this case report to be successful.

CONFLICTS OF INTEREST

None.

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