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Screening of Anti-inflammatory and Antimicrobial activities of stem extract of *Capparis sepiaria* Linn

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ABSTRACT

Capparis sepiaria was used to treat diarrhoea and other infective disorders in folklore. The present study aims at evaluating the action of chloroform soluble fraction (CSCF), ethanol soluble fractions (CSEF) of *Capparis sepiaria* stem, against inflammatory diseases and also against some Gram positive (*Enterococcus fecalis, Staphylococcus aureus)* and Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli)* pathogenic bacteria of Gastrointestinal tract. The ethanol soluble fraction was found to be the most potent among them which shows good anti-inflammatory response with reference to standard drug indomethacin and also showed good antibacterial activity towards gastro intestinal pathogens with reference to standard drug penicillin.

Keywords: CSCF: *Capparis sepiaria* chloroform soluble fraction, CSEF: *Capparis sepiaria* ethanol soluble fraction

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INTRODUCTION

Capparis sepiaria Linn is a rigid, wiry and much branched shrub. Folklore usage reveals that it is used in treatment of inflammation, in wound healing, in jaundice treatment and in the treatment of dysentery [1-3]. Root bark, stem leaves and flowers contain alkaloids, glycosides, terpenes, sterols and flavonoids [4]. Many Phytochemical have proven to have action against infective diseases. Currently used synthetic anti-inflammatory agents cause side effects like gastric irritation, ulcer induction where as antimicrobial agents cause side effects like gastric irritation, hyper sensitive reactions, head ache, insomnia etc [5, 6]. Hence there is a need to discover some suitable alternatives for the synthetic drugs. The current study is an attempt in evaluating the potency of *Capparis sepiaria* stem towards inflammatory diseases and against some GIT pathogenic bacteria.

MATERIALS AND METHODS

The plant *Capparis sepiaria* Linn was collected from Regional Plant Resource Centre, Bhubaneswar in December 2006, and was identified by Dr.P.C.Panda, Senior Research Scientist, Regional Plant Resource Centre, and Bhubaneswar. A voucher specimen of (TSN-CS13/12/06) has been deposited in the Pharmacogonosy department, Andhra University.

Toxicity studies

The acute toxicity study is aimed to establish the therapeutic index i.e. the ratio between the pharmacologically effective dose and the lethal dose, and also to perform the primary screening. The test extracts were administered once orally at 3 dose levels (250, 500, 1000 mg/kg) to a group of 10 mice of both sexes about equal in no which have been fasting over night (about 18hours). The treated mice were observed continuously for 2 hours and then occasionally for further four hour and finally over night mortality was recorded.

Preparation of plant extracts

Fresh Stem of *Capparis sepiaria* Linn. were collected, dried under shade and powdered. The coarsely powdered (#20) material was extracted with ethanol continuously using Soxhlet apparatus for 72hrs at a temp of 80-85^{o}c. The extract obtained was made solvent free by concentrating at 30^{o}c under reduced pressure in a Rotary evaporator and the yield of crude ethanolic extract is 82%. The dried ethanolic extract was suspended in water and fractionated with chloroform in order to get chloroform soluble fraction and ethanol soluble fraction. Yield of chloroform fraction was 12.7% and yield of ethanol soluble fraction was 75%. These fractions were then subjected for qualitative Phytochemical analysis represented in Table: 1 and are screened for anti inflammatory activity and anti microbial activity.

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Experimental work

Determination of Anti-inflammatory activity

The experimental protocol was approved by the institutional animal ethics committee of Andhra university, Vishakhapatnam, which was registered with Committee for the purpose of control and supervision of experiments on animal (CPCSEA),Govt. off india (registration no. 516/01/A/CPCSEA). Adult albino rats (200-250 g) of either sexes were used. Animals were housed in polyacrylic cages under standard laboratory conditions (Temp: 25-30^{o}c, relative humidity 60-70% and 12 hr light and dark cycle) and fed with standard pellets and water *ad lbitum* two weeks before and during the experimental period

Carrageenan induced rat paw edema

Twenty five rats were divided into 5 groups of 5 rats in each group. Animals of all the groups were injected with 0.1 ml of 1% carrageenan solution in 0.9% saline in to the sub plantar region of the left hind paw. Group I animals were injected with 0.1ml of 0.9% saline solution into the sub plantar region of the left hind paw. group –II, the standard reference group was given P.O., an aqueous solution of indomethacin(10mg/kg). Group III and IV received acacia solution of chloroform soluble fraction and aqueous soluble fraction respectively for 7 consecutive days and subsequently 60 min after above treatment, 0.1ml of 1% carrageenan was injected subcutaneously into the planter region of right hind paw to induce edema on 8th day. The paw thickness was measured initially and at 1, 2, 3, 4, 5 and 6 h after carrageenan injection using Zetlin's apparatus [7, 8]. Values for paw thickness were given in Table: 2 and the values for % inhibition of edema were given in the Table: 3

Measurement of paw thickness ($Y_t - Y_O)/Y_O \times 100$

Y_t = paw thickness at the time't' hours (after injection)
Y_O = paw thickness at the time '0'hours

The percentage increase in paw thickness during 3 hours was determined. The percent inhibition of paw edema thickness is calculated using the formula

Percentage inhibition = $100[1-Y_t/Y_C]$

Y_t =average increase in paw thickness in groups tested with test compounds
Y_c =average increase in paw thickness in control

Determination of Antimicrobial activity

Organisms used for the study were *Enterococcus fecalis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* These organisms were identified by standard microbiological methods [9]. The test organisms used in this study were obtained from Post Graduate Department of Microbiology, Utkal University, Bhubaneswar, Orissa.

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MIC determination

The minimum inhibitory concentration (MIC) values for the microbes were determined to assess their sensitivity to CSCF and CSEF using agar diffusion method[10]. The results of MIC values are given in Table 4.

Determination of Zone Of Inhibition

The CSCF and CSEF were dissolved separately in DMSO to get 4 different concentrations of 62.5mg/ml, 125mg/ml, 250mg/ml and 500mg/ml, to study the antimicrobial activity by using Agar diffusion method [10,11]. Benzyl penicillin was used as a reference standard at a dose of 10units/ml. DMSO was used as negative control. 0.05ml each of the extracts and reference standard were added into the wells. The inoculated plates were then incubated at 37^0c for 24hours and the zone of inhibition was measured and recorded in millimeters. The results were presented in Table 5.

RESULTS AND DISCUSSION

In acute toxicity study no mortality was found up to 1000mg/kg p.o of *Capparis sepiaria* extract fractions. The LD_{50} was not determined and $1/10^{th}$ of the tested proven safe concentration is taken as our experimental dose.

Phytochemical analysis (Table:1) revealed the presence of steroids and triterpenoids in chloroform soluble fraction and glycosides, carbohydrates and alkaloids in ethanol soluble fraction.

Table:1 Phytochemical analysis of *Capparis sepiaria* stem extracts

Constituents	Ethanol extract	Chloroform soluble fraction	Ethanol soluble fraction
steroids:	+	+	-
Triterpenoids:	+	+	-
Glycosides:	+	+	+
saponins:	+	+	-
Carbohydrates:	+	+	+
Alkaloids:	+	+	+
Flavonoids:	-	-	-
Phenolic nucleus:	-	-	-

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The pharmacological screening of young stem extracts of *Capparis sepiaria* revealed that it possess potent anti-inflammatory effect in the carrageenan induced acute inflammation model. These extracts may have inhibited the release of proinflammatory mediators of acute inflammation such as histamine and prostaglandins. Inflammation induced by carrageenan involves 3 distinct phases of the release of the mediator including serotonin and histamine in the 1st phase (0-2nd hours), kinin in the 2nd phase (3rd hour) and prostaglandin in 3rd phase [12] (more than 4th hour). These fractions significantly inhibit the paw edema in 1st and 2nd phase suggesting on inhibitory effect on release of histamine and serotonin.

The perusal of (Table: 2) shows the percentage of edema in paw edema induced by carrageenan in rats. All the extract fractions of CS has got action against the acute anti-inflammation. CSEF has got more pronounce anti-inflammatory effect as compare to CSCF at a concentration of 100mg/kg at 3rd hour, CSEF inhibit the paw edema 70.14% which is comparable to standard Indomethacin 89%, CSCF decreases the paw edema 19.4% at the end of 3rd hour.

Table: 2 Anti inflammatory effect of fractions of C*apparis sepiaria* stem extract in carrageenan induced paw edema in rats

S. no	Group	Measurement of paw thickness in mm					
		1hr	2hr	3hr	4hr	5hr	6hr
1.	Control	33.02±0.22	40.74±0.33	47.86±0.46	46.42±0.45	34.26±0.45	24.44±0.21
2	Indomethacine (10mg/kg)	10.3±0.11[b]	15.5±0.45[a]	17.10±0.23[a]	18.3±0.34[a]	14±0.32[b]	10±0.44
3	CSCF (100mg/kg)	20±0.34	25.96±0.31[c]	40±0.31	38±0.22	19.02±0.21	17.14±0.44
4	CSEF (100mg/kg)	12.96±0.45[b]	16.94±0.32[a]	18±0.11[a]	21.1±0.34[a]	16.86±0.33[b]	11.5±0.23

CSCF: capparis sepiaria chloroform fraction
CSEF: capparis sepiaria ethanolic fraction

From the observed percentage inhibition of edema (Table:3) values it was found that CSCF showed only moderate anti-inflammatory activity at 2nd hour where as CSEF at dose of 100mg/kg showed significant activity from 1st hour onwards and highly significant activity from 2nd hr to 4th hr which was comparable to activity of standard drug Indomethacin at dose of 10mg/kg.

Table:3 Comparison of %inhibition of paw edema by C.sepiaria fraction with standard

S.no	Group	Percentage inhibition of paw edema					
		1hr	2hr	3hr	4hr	5hr	6hr
1	Standard	27%	47%	89%	72%	63%	42%
2	CSCF	10.5%	14%	19.4%	18.46%	15.08%	11.76%
3	CSEF	15%	38%	70.14%	65.3%	58.3%	35.88%

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From the observed MIC (Table-4) values it was found that CSEF was the most potent. The MIC for CSEF and CSCF against *P.aeruginosa* was found to be 31.25, 250 and 125mg/ml respectively. All the two fractions have showed MIC value of 62.5mg/ml against *S.aureaus.* MIC of CSCF against *E.coli* is 500mg/ml indicating least sensitivity compared to CSEF (62.5mg/ml). From the MIC values it was found that CSEF showed better activity with less concentration (31.25mg/ml) towards the gastro intestinal microbes as compared to that of CSCF.

Table: 4 Minimum inhibitory concentration of the plant extracts:

Pathogens/organisms	**MIC of *CAPPARIS SEPIARIA***	
	CSEF (mg/ml)	**CSCF** (mg/ml)
Enterococcus facalis	31.25	31.25
Staphylococcus aureus	62.5	62.5
Pseudomonas aeruginosa	31.25	250
Escherichia coli	62.5	500

From the observed Z.O.I (Table:5) values it was found that CSEF showed dose dependent antibacterial activity at the doses tested 62.5, 125, 250, 500 mg/ml against all microbes, whereas CSCF showed moderate activity against *E. fecalis, S. aureus*, less activity against *E.coli* and no activity against *P.aeruginosa.*

CSEF showed prominent antibacterial activity against all microbes which was comparable with standard drug penicillin (10units/ml).

Further studies

Further studies must be conducted in order to clarify the exact mechanism of anti-inflammatory and anti microbial activity and to isolate constituents of the extract responsible for this activity. Detailed chronic acute and sub acute toxicity studies will be carried out to find out the therapeutic index of this plant

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Table-5: **Anti microbial activity of stems of *Capparis sepiaria*.**

S.no	Extracts of *C.sepiaria*	Zone of inhibition (mm)			
		E.f	S.a	E.c	P.a
1 . Ethanol soluble fraction					
500mg/ml		20±0.33	14±0.56	21± 0.42	15± 0.22
250mg/ml		12±0.42	13 ±0.67	16± 0.23	13±0.43
125 mg/ml		10±0.23	10±0.52	14±0.32	9±0.43
62.5mg/ml		10±0.34	9± 0.44	11±0.44	8±0.23
2 . Chloroform soluble fraction					
500mg/ml		11±0.54	11±0.44	10±0.24	8±0.52
250mg/ml		10±0.23	9±0.32	9±0.21	0
125mg/ml		10±0.44	8±0.45	0	0
62.5mg/ml		9± 0.22	7±0.34	0	0
4. Penicillin (10units/ml)		20	22	20	21
5. DMSO		--	--	--	--

All the values are mean ± standard deviation of three determinations

E.f= enterococcus facalis; S.a=staphylococcus aureus; P.a=pseudomonas aeruginosa,; E.c=Escherichia coli

REFERENCES

[1] Matthew K M. ovary bome on gynophore-ovules parietal armed or unarmed of capparaceae family, in Excursion flora of central Tamilnadu, India, (Oxford & IBH Publishers, New Delhi) 1991, 14.

[2] "Indian Medicinal plants" (Orient Longman Publishers, CSIR, New Delhi) 1999, Vol: 1, 348.

[3] Mohammed Anis, Sharma M P & Muhammad Iqbal. J Pharma Biol 2000; 38(4): 241- 253.

[4] Ali Mohammed, Flavonoids in Text book of Pharmacognosy, (CBS Publishers and Distributors, Delhi) 1994, 233

[5] Tripathi KD. Essentials of Medical Pharmacology, (Jaypee Brothers Publications, Delhi) 2003, 629,648.

[6] Rath CC, Dash S K , Mishra RK, & Charyulu JK. J Essence 1999; (2):82.

[7] Winter CA, Risley EA, Nuss GW. Proc Soc Expt Biol Med 1962;111: 544-7.

[8] Battu GR, Zeitlin IJ, Gray A I, Waterman PG. Brit J Pharmacol 1999; 128:274-280.

[9] Collins CH & Lyne PM. Microbiological Methods, 6th Edn, (Butterworths, London) 1970, 66.

[10] Colins CH, Lyne PM & Grange JM. Microbiological methods, 7th Edn (Butterworth Heineman, Oxford, New delhi) 1995,178

[11] Bauer AW, Kirby WMM, Sherris JC, & Turk MAM. J Clin Pathol 1996; 45:493.

[12] Singh S, Majumdar DK, Rehan HMS. J Ethnopharmacol 1996;54:19-26.