Available online at www.pharmacie-globale.info ISSN 0976-8157

Research Article

PHARMACIE GLOBALE

INTERNATIONAL JOURNAL OF COMPREHENSIVE PHARMACY

HUMAN RED BLOOD CELL MEMBRANE STABILITY TESTING FOR THE ESTIMATION OF ANTI-INFLAMMATORY ACTIVITY AND ANTI BACTERIAL ACTIVITY OF THREE DIFFERENT OF EXTRACT OF *Sechium edule* FRUITS

Vaijayanthimala Palanisamy* and Sangameswaran Balakrishnan

SSM College of Pharmacy, Department of Pharmaceutical Chemistry, Erode, Tamilnadu, India.

Received: 18 May 2016; Revised: 9 June 2016; Accepted: 22 June 2016; Available online: 30 June 2016

ABSTRACT

Chronic inflammatory processes contribute to the pathogenesis of many age-related diseases. In search of anti-inflammatory foods, we have systematically screened three different extract of *Sechium edule* plant extract for anti-inflammatory activity and antimicrobial activity. The *in-vitro* anti-inflammatory activity was performed by human red blood cell membrane stability model and from the selected three extract aqueous extract shows more protection from inflammation by reducing the precipitation of proteins present in human cell membrane. For *in-vitro* antimicrobial activity the minimum inhibitory concentration method (MIC) was carried out and methanol extract exhibited the highest activity against *Bacillus subtilis* (MTCC 736) giving values of MIC = 125 µg/ml.

Keywords: *Sechium edule*; MIC; Anti-inflammatory, antimicrobial and *Bacillus subtitis*.

INTRODUCTION

Inflammation is one common and major cause of sufferings now and every time past. Those drugs that are available are known as NSAID, i.e. non-steroidal anti-inflammatory drugs, act by inhibiting the function of prostaglandin[1].

The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs[2]. Several anti-inflammatory, digestive, antinecrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an antioxidant or radical scavenging mechanism as part of their activity[3] The mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species from activated neutrophils and macrophages. This over production leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes[4,5]. Increasing evidence suggests that systemic low-grade inflammation is a contributing factor in these age-related diseases[6,7].

Herbal medicines derived from plants rich in the secondary metabolite salicylic acid, such as the bark of the willow tree (Salix alba), have been used for the treatment for diseases with a prominent inflammatory component for thousands of years. Many other medicinal plants are known to have anti-inflammatory activity[8] but neither the underlying mechanisms nor their potential for the development of new drugs has been fully explored. Several mechanisms are proposed to explain their anti-inflammatory action, including inhibition of cyclooxygenases and lipoxygenases or modulation of pro-inflammatory gene expression such as inducible nitric oxide synthase, and several pivotal cytokines including tumor necrosis factor-a (TNF-α)[9].

Sechium edule is an edible plant that belongs to the family *Cucurbitaceae* also known as sayote, choko, chocho, chow-chow, and vegetable pear. The chayote is a herbaceous, perennial, monoecious, vigorous creeper or climbing plant. The fruits grow either individually or in pairs on a shared peduncle. They are fleshy or fleshy-fibrous, may have longitudinal ridges or furrows, and come in many different shapes (globose, ovoid, subovoid, pyriform) and colours (dark or light green)[6]. The fruits and the seed especially, are rich in several important amino acids. A lectin from the exudate of *Sechium edule* was purified[10] Eight flavonoids, including three *C*-glycosyl and five *O*-glycosyl flavones, were detected[11] Twenty known Gibberellins' have been identified in extracts of the seeds of *Sechium edule*. The leaves and fruits have diuretic, cardiovascular and anti-inflammatory properties, the leaves have been used in the treatment of arteriosclerosis and hypertension, and to dissolve kidney stones[12].

MATERIALS & METHODS

Drugs and Chemicals

All reagents procured were analytical grade.

Plant collection

Fresh fruits of *S. edule* were collected from field of Jambai and authenticated by Dr P Satyanarayana, Scientist D & Head office in charge, Southern Regional Centre, TNAU campus, Coimbatore. Voucher specimen (No: SSMCP/ 0106) has been deposited in the Department of Pharmacognosy, SSM College of Pharmacy, Jambai, Bhavani, Tamilnadu, India.

The fruits of *S.edule* were dried and then crushed into fine powder by using laboratory Homogenizer then stored for further use.

***Corresponding Author:**
Vaijayanthimala Palanisamy
Department of Pharmaceutical Chemistry,
SSM College of Pharmacy, Jambai, Erode, Tamilnadu, India.
Email: vaijphd2014@gmail.com

Preparation of Plant Extracts

The dried fruits of *S. edule* were subjected to extract with petroleum ether, alcohol and water.

Procedure for extraction

Fine powerded fruits of *S. edule* were extracted with petroleum ether, methanol and water using soxhlet apparatus. The extract was filtered and evaporated to separate solvent and residue. The semisolid residue thus obtained was stored in desiccator until further use.

Anti-inflammatory activity

Preparation of solutions of the extracts: The suspension of all the three extracts and indomethacin, as standard were prepared using Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL). positive control is Indomethacine 100 µg /ml.

Procedure: The anti-inflammatory activity of various extracts of fruits of *S. edule* was assessed by *in vitro* HRBC membrane stabilization method. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) and centrifuged with isosaline. To 1 mL of HRBC suspension, equal volume of test drug in different concentrations 10, 20, 30 ,40 and 50 µg/ml was added. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm [13]. The percentage of protection can be hence calculated from the equation as given below:

$$\%\ Inhibition\ of\ haemolysis = \frac{A1 - A2}{A1} \times 100$$

Where,

A1 = Absorption of Control

A2 = Absorption of test sample

Here, the negative control used was Alsever's solution with blood in it and it contained no Indomethacine or extract of the plant material in it. The absorbance of the negative control was found to be 0.218.

Antimicrobial activity

Bacterial Strains

Authenticated cultures of five bacterias such as *Escherichiae coli* (MTCC 1687), *Proteus mirabilis* (MTCC 9242), Streptomyces *fulvissimus* (MTCC 7336), *Bacillus subtitis* (MTCC 736) and *Pseudomonus auruginosa* (MTCC 2488) were collected from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Sector 39-A, Chandigarh, India.

Fungal Strains

Two fungi *Aspergillus niger* and *Candida albicans* were selected for screening.

Preparation of solutions of the extracts: Before testing of these extracts for antimicrobial activity, they were completely dried at normal conditions. Dissolving them in same solvent and diluting it with sterilized water prepared the stock solutions of each extract of different concentrations. Positive control was Oxytetracycline and for fungal strain Ketaconazole.

Procedure: Solutions of all the three extracts (1 mg/ml) were prepared and used for screening their antimicrobial activity. The study involved a series of eight assay tubes for each title compound against each microorganism. To the first assay tube, 1.8 ml of seeded broth and 0.2 ml of title compound (10mg/ml) was added and mixed thoroughly and the two-fold serial dilution was done up to the sixth tube containing 1 ml of seeded broth. The addition of the drug solutions and serial dilutions were done under strict aseptic conditions. Solvent control and drug control were maintained during the experiment. The assay tubes were incubated at 37°C for 24 hours for bacteria and at 25°C for 48 hours for fungi. The lowest concentration, which apparently caused complete inhibition of growth of microorganisms was considered as the Minimum Inhibitory Concentration (MIC). The extracts were compared with standard drug OxytetraCycline and Ketaconazole for bacteria and fungi respectively[14].

RESULTS AND DISCUSSION

Anti-inflammatory Activity

De naturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of different solvent plant extract protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation (Table **1**).

Table 1. HRBC Membrane stabilizing activity

Effect of herbal extracts in erythrocyte haemolysis Concentration		Absorbance at 560nm Average ± SEM	% Inhibition of haemolysis
Control		0.218 ± 0.0014	----------
Indomethacin (100µg/ml)		0.020 ± 0.001	90.82%
PESE	10 µg /ml	0.199 ± 0.002	8.71%
	20 µg /ml	0.157 ± 0.001	27.98%
	30 µg /ml	0.128 ± 0.001	41.28%
	40 µg /ml	0.079 ± 0.002	63.76%
	50 µg /ml	0.080 ± 0.001	65.76%
MESE	10 µg /ml	0.213 ± 0.002	2.29%
	20 µg /ml	0.197 ± 0.001	9.63%
	30 µg /ml	0.188 ± 0.001	13.76%
	40 µg /ml	0.159 ± 0.002	27.06%
	50 µg /ml	0.127 ± 0.001	41.74%
AESE	10 µg /ml	0.193 ± 0.002	11.46%
	20 µg /ml	0.167 ± 0.001	23.39%
	30 µg /ml	0.108 ± 0.001	50.45%
	40 µg /ml	0.059 ± 0.002	72.93%
	50 µg /ml	0.027 ± 0.001	87.61%

Maximum percentage of inhibition 87.61% was observed from AESE followed by PESE (Table **1**). All the solvent extracts inhibited the albumin denaturation, the ethanol extract stood first compared to AESE, Indomethacin a standard anti-inflammatory drug showed the maximum inhibition 90.82% at the concentration of 100 µg/ml. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair. Denaturation of proteins is a well-

documented cause of inflammation and rheumatoid arthritis. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. Ability of *Sechium edule* extract to bring down thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity. The anti-inflammatory activity of *Sechium edule* extract found may be due to the presence of therapeutically active flavonoids. The therapeutic applications of flavonoids on inflammation have previously been reported. The data of our studies suggests that *Sechium edule* showed significant anti-inflammatory activity by *in vitro* method tested. Further studies involving the isolation and purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with a low toxicity and better therapeutic index.

Antimicrobial activity

In-vitro antimicrobial activity which may lead to the finding of more effective agent for the management of diseases and effective potential source of natural antimicrobials that may help in preventing various disease. In anti-inflammatory activity in membarane stabilizing method AESE shows more activity than rest of two extracts (PESE and MESE). But in the case of Antimicrobial activity MESE shows potent activity than other two extracts (Table **2** and **3**). Hence it was concluded that aqueous extract of *selenium edule* exhibits promising *in-vitro* anti-inflammatory activity and Methanol extract exhibit potent *in-vitro* antimicrobial activity on both bacteria and fungi strain.

Table 2. Minimum inhibitory concentration of extracts and standard drug on selected bacterial strain

Micro organisms	Extracts	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml
E. coli (MTCC 1687)	PESE	***	***	+	+	+
	MESE	***	***	***	+	+
	AESE	***	***	+	+	+
	Oxytetra Cycline	***	***	***	***	+
P.mirabilis (MTCC 9242)	PESE	***	***	***	+	+
	MESE	***	***	***	+	+
	AESE	***	***	+	+	+
	Oxytetra Cycline	***	***	***	***	***
S. fulvissimus (MTCC 7336)	PESE	***	+	+	+	+
	MESE	***	***	***	***	+
	AESE	***	***	+	+	+
	Oxytetra Cycline	***	***	***	***	+
B. subtitis (MTCC 736)	PESE	***	***	+	+	+
	MESE	***	***	***	***	+
	AESE	***	***	+	+	+
	Oxytetra Cycline	***	+	***	***	***
P.auruginosa (MTCC 2488)	PESE	***	***	***	+	+
	MESE	***	***	***	+	+
	AESE	***	***	+	+	+
	Oxytetra Cycline	***	***	***	***	+

*+ Indicates presence of cloudiness, *** indicates absence of cloudiness*

Table 3. Minimum inhibitory concentration of extracts and standard drug on selected fungal strain

Micro organisms	Extracts	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml
C. albicans (MTCC-282)	PESE	***	***	+	+	+
	MESE	***	***	***	+	+
	AESE	***	+	+	+	+
	Ketaconazole	***	***	***	***	***
A.niger (MTCC-183)	PESE	***	***	+	+	+
	MESE	***	***	***	+	+
	AESE	***	+	+	+	+
	Ketaconazole	***	***	***	***	+

*+ Indicates presence of cloudiness, *** indicates absence of cloudiness*

CONCLUSION

The results of the current study suggest that methanol extract and aqueous extract of ***Sechium edule*** has anti-inflammatory effects in HRBC membrane stabilizing activity model. But Aqueous extract of ***Sechium edule*** shows less antimicrobial activity than compared to other extracts*.* From current study it was concluded the aqueous extract of ***Sechium edule*** has anti-inflammatory activity and methanol extract has antimicrobial activity.

REFERENCES

1. Perry E K, Pickering A T, Wang W W, Houghton P J and Perru N S; Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. Journal of Pharmacy and Pharmacology. 1999; 51:527–534.
2. Lin C C and Huang P C; Antioxidant and hepatoprotective effects of *Acathopanax senticosus*. Phytotherapy Research. 2002; 14:489–494.
3. Kimura Y, Yasukawa K, Takido M, Akihisa T and Tamura T; Inhibitory effect of some oxygenated stigmastane-type sterols on 12- teradecanoylphorbol-13-acetate-induced inflammation in mice. Biological & Pharmaceutical Bulletin. 1995; 18:617–619.
4. Bjarnason I; Gastrointestinal safety of NSAIDs and overthe- counter analgesics. *Int J Clin Pract*. 2013; 178:37–42.
5. Wolfe M M, Lichtenstein D R and Singh G; Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *N Engl J Med*. 1999; 340:1888–

1899.

6. Kim H P, Son K H, Chang K H W and Kang S S; Anti-inflammatory plant flavonoids and cellular action mechani sms. *J Pharmacol Sci*. 2004; 96:229–245.
7. Rafel lira saade. CHAYOTE, sechium edule (jacq), IPGRI. 1996; 28-9.
8. Kim S; "*Sechium edule* (jacq)". Available at URL: http://en.wikipedia.org/wiki/Chayote. 2009.
9. Vozari-Hampe M M, Viegas C, Saucedo C, Rosseto S and Manica G G; A lectin from *Sechium edule* fruit exudates. Phytochemistry. 1992; 31(5):1477-80.
10. Siciliano T and De Tommasi N; Study of Flavonoids of *Sechium edule* (Jacq) swartz (Cucurbitaceae) different edible organs by liquid chromatography photodiode array mass spectrometry. *J Agric Food Chem*. 2004; 52(21):6510–15.
11. Albone K S, Gaskin P, MacMillan J and Sponsel V M; Identification and localization of gibberlins in maturing seed of cucurbit *sechium edule*, and a comparison between this cucurbit and the legume *Phaseolus coccineu"s*. Planta springer-verl. 1987; 16:560-65.
12. Kamble M B, Dumbre R K, Rangari V D; Hepatoprotective activity studies of herbal formulations. *Int J Green Pharm*. 2008; 2:147-51.
13. Jain R; Anti- inflammatory action of *Abutilon indicum* (L.) sweet leaves by HRBC membrane stabilization. Research Journal of Pharmacy and Technology. 2009; 2:415-416.
14. Zavala M A, Perez G S and Perez G R M; Antimicrobial screening of some medicinal plants. *Phytother Res*. 1997; 11:368–371.