Journal of Comprehensive Pharmacy

L&L Publications

Journal of Comprehensive Pharmacy

Research Article Available Online at: www.jcponline.in ISSN NO: 2349-5669

Comparative study of *Invitro* anti inflammatory activity of *capsicum annum* and *capsicum frutescense* fruits

Aruna Bizzam,* Madhuri Donakonda, A. Venkata Badrinath[1], Shaik Karimulla [2]

[1]*Department of Pharmacology, Dr.K.V. Subbareddy Institute Of Pharmacy, Dupadu, Kurnool, A.P., India.*
[2]*Department of Pharmacology, Narayana Pharmacy College, Nellore, A.P., India.*

ARTICLE INFO

Article history:
Received 28 October 2016
Accepted 18 December 2016
Available online 30 December 2016

***Corresponding author:**

Aruna Bizzam

Email:
sahitireddy.2007@gmail.com

Tel.:+91-9160450507.

ABSTRACT

Objective:

The present study was designed to evaluate the *invitro* anti inflammatory activity of various extracts of fruits of capsicum species.

Methods:

capsaicin was extracted from the fruits of C.annum and C.frutescence using different solvents like ethanol, acetone and acetonitrile. The anti inflammatory profile of these extracts was investigated by *invitro* methods like albumin denaturation assay, HRBC membrane stabilization assay, Diclofenac sodium was used as a reference standard.

Results:

Ethanolic, Acetone and acetonitrile extracts were subjected for the measurement of percentage inhibition of albumin denaturation and percentage protection against hypotonicity induced and heat induced haemolysis of RBC. Among the extracts only ethanolic extract showed significant anti inflammatory activity at the concentration of 400µg/ml., may be due to the presence of higher phenolic content.

Conclusion:

On the basis of these findings, it may be inferred that capsaicin present in the fruits of C. annum and frutescence posses anti inflammatory activity.

Key words: Invitro-anti inflammatory, albumin denaturation, HRBC membrane stabilization, capsicum frutescence, capsicum annum.

Introduction

Inflammation is a normal protective response to tissue injury, and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair. A variety of molecules are released from cells and plasma membrane during inflammation whose net overall effect is to increase vascular permeability, resulting in tissue edema, pain, redness. The released molecules include histamine, PGs, eicosanoids, PAF, bradykinin and serotonin. The most commonly used drugs for the management of inflammation are glucocorticoids and NSAIDs, these drugs have not been entirely successful in curing chronic inflammation and are accompanied by several adverse effects especially peptic ulcer, gastric bleeding ,inhibition of platelet function, asthma, cardiovascular adverse effects etc., for this reason , inrecent times more interest is seen in alternative and natural drugs.

The phytochemical constituents of capsicum are shown to produce an anti-inflammatory response that proves to alleviate peripheral neurogenic pain such as related to Crohn's disease, an inflammatory disease of the bowel. Another example of peripheral neurogenic inflammation is cutaneous pain of the skin. Its creams and balms are often massaged topically onto the skin with care taken to avoid open areas and mucous membranes. The burning effect of capsicum is felt

when applied to the skin. This is caused by an inflammatory response of the peripheral nerve endings, but regular application to dead ends of the sensory nerve endings, relieve chronic diabetic neurogenic pain. Some suggest that the anti-inflammatory effect of capsicum may also help to alleviate pain related to arthritis. Capsicum contains tannins. Tannins are astringents and often considered for their benefits when treating gastrointestinal disorders that produce diarrhoea such as dysentery and other microbial disorders. Gastric mucilage acts to protect the gastric lining. Another bioactive effect of tannin contained in capsicum molecule is in the prevention and treatment of cancer. Studies of flavonoids have suggested that they are beneficial towards preventing coronary heart disease. Capsicum is both warming and vasodilative. Many of the bioactive compounds of capsicum provide antioxidant effects. Improved vasodilatation allows for these antioxidant phytochemicals to circulate through regions of the body that may already been effected as well as healthy tissue. This allows capsicum to have a beneficial effect in repairing tissue proteins and possibly even DNA. As well, the healthy tissue receives protection from the antioxidant effect of the capsicum chili pepper. With the worldwide prevalence of diabetes, the consideration of capsicum as hypoglycemic medicinal has provoked research into this matter. Capsicum contains vitamin C is valued as an immune supportive bioactive phytochemical. Many clinical trial shown the effectiveness of Capsicum for treating symptoms of fibromyalgia when applied topically. Those using capsicum topically stated beneficial effects in reducing tenderness and improvement of sleep. A disease that often produces this type of neurogenic pain is diabetes. Capsicum is shown to have a beneficial effect on diabetic neuropathic pain when applied topically. Capsicum works to deaden cutaneous nerve endings and reducing the pain.

2. Materials and methods

Collection of plant material

Fruits of *capsicum frutescence* and *capsicum annum* were collected from the local market of Kurnool, collected plant material was dried in shade and powdered. Powederd crude drug was soaked with ethanol, acetone, and acetonitrile for seven days, after that solution was filtered and extracts were concentrated by vaccum evaporator, and stored in air tight container in a refrigerator. The fruits of *capsicum frutescence* and *capsicum annum* were analysed for the presence of flavanoids, alkaloids, glycosides, phenols, saponins and tannins etc. according to the standered methods.

Evaluation of invitro anti inflammatory activity

Inhibition of albumin denaturation:

Principle: The denaturation of proteins is defined as any non-covalent changes in the structure of the protein. The change alter the secondary, tertiary and quaternary structure of the molecules. The primary structure of the amino acid sequence remains the same after the denaturation process. The common factors that denatures proteins includes heat, alcohol, acids, bases, heavy metals, urea, UV, organic solvents, detergents etc. Denaturation of proteins is a well documented cause of inflammation. Most biological proteins lose their biological function when they denatured, so inhibition of albumin denaturation by plant extract is utilised for the evaluation of anti-inflammatory activity.

Procedure:

Method of Mizushima et al was followed with minor modifications. The reaction mixture was consisting of test extract at different concentrations and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of 1N Hcl. The samples were incubated at 37c for 20 min and then heated at 57c for 20 min. After cooling eg the samples, the turbidity was measured spectrophotometrically at 660nm.The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

$$\text{Percent protection} = [\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}] \text{ X } 100/\text{Abs}_{\text{control}}$$

Membrane stabilization test

Preparation of red blood cells [RBCs] suspension

Principle: The *in-vitro* erythrocyte haemolysis assay is generally used for screening anti-inflammatory activity of drugs. Majority of the anti-inflammatory drugs stabilise the plasma membrane of mammalian erythrocytes and thereby inhibit the Heat-induced and the hypotonicity-induced haemolysis. The plasma membrane of mammalian red blood cells (erythrocytes) has been particularly useful as a model for studies of membrane structure. Mammalian red blood cells do not contain nuclei or internal membranes, so they are used as a source from which pure plasma membrane can be easily isolated for biochemical analysis. The erythrocyte Plasma membrane resemblances to the lysosomal membrane and hence the stabilising effect of drugs on erythrocyte membrane may correlate with its lysosomal membrane stabilising effect. The lysosomal membrane stabilization leads to the inhibition of release of the inflammatory mediators and consequent inhibition of the process of inflammation. In the

membrane stabilization assay, the erythrocyte are challenged with different haemolytic stimuli like heat, osmotic shock and free radicals. The heat-induced and hypotonicity induced haemolysis of erythrocytes is extensively used as a rapid, simple, economic and sensitive tool in determining the anti-inflammatory property of drugs. Due to this simplicity and economy, the researchers prefer to use this model in the preliminary screening of extracts of medicinal plants.

Procedure:

Fresh whole human blood [10ml] was collected and transferred to the heparinised centrifuges tubes. The tubes were centrifuged at 3000rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

Percent inhibition = $[Abs_{control}---Abs_{sample}]$ x $100/Abs_{control}$

Heat induced haemolysis

The reaction mixture [2ml] consisted of 1ml of test drug solution and 1 ml of 10% RBCs suspension, instead of drug only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500rpm for 5min and the absorbance of the supernants was taken at 560nm. The experiment was performed in triplicates. Percent membrane stabilization activity was calculated by the formula

Percent inhibition = $[Abs_{control}---Abs_{sample}]$ x $100/Abs_{control}$

3. Results:

Preliminary phytochemical screening :

The extraction of powdered fruits of *capsicum annum* (50g) and *capsicum frutescense* (50g) were carried out by maceration using ethanol, acetone, acetonitrile as solvents.

Colour nature and yield of extract:

Qualitative phytochemical investigation of crude plant extract of *capsicum annum* and *capsicum frutescense* revealed the presence of alkaloids, carbohydrates, starch, proteins, aminoacids, and flavanoids etc.

Assessment of in-vitro anti-inflammatory activity

Inhibition of albumin denaturation

Method of Mizeshim et at was followed with minor modifications. The reaction mixture consisting of test extract at different concentrations and 1% aqueous solution of bovine albumin fraction PH of the reaction mixture was adjusted using small amount of 1N HCL. The sample were incubated at 37c for 20min and then heated 57c for 20min. After cooling the samples, turbidity was measured spectrophotometrically at 660nm.The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs control-Abssample)x100/Abscontrol

Membrane stabilization method

Preparation of Red Blood cells (RBCs) suspension:

Fresh whole human blood (10ml) was collected and transferred to the heparinized centrifuged tubes. The tubes were centrifuged at 3000rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline.

Heat induced haemolysis:

The reaction mixture (2ml) consisted of 1ml of test drug solution and 1ml of 10% of RBCs suspension, instead of drug only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56c for 30min.At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicates. Percent membrane stabilization activity was calculated by the formula mentioned above.

4. Discussion:

The present study demonstrates the comparative anti inflammatory activity of c.annum and c.frutescence with different extracts i.e., ethanol, acetone, acetonitrile. In the present study fruits of *c.annum* and *c.frutescence* were evaluated for the anti inflammatory activity by different invitro methods like albumin denaturation assay and HRBC membrane stabilization assay. Diclofenac was used as a reference standered. Anti inflammatory activity was assessed by percent protection against albumin denaturation and percent protection against heat induced and hypotonicity induced haemolysis.

Table 1: Preliminary Phytochemical Tests

SNO	Extract	Colour	Consistency	Yield(gm)
1	Ethanol	White	Watery	3.2
2	Acetone	White	Watery	2.8
3	Acetonitrile	Brown	Sticky	2.1
Preliminary Phytochemical Tests:				
S.NO	**Phytoconstituents**		**Test**	**Results**
1	Carbohydrates		Molish test	+ve
2	Starch		Iodine test	-ve
3	Protein		Millons test	+ve
4	Amino acid		Cysteine test	+ve
5	Steroids		Salkowski test	+ve
6	Flavanoids		Ferric chloride test	+ve
7	Alkaloids		Mayers test	+ve
8	Tannins and phenolic compounds		5%$fecl_3$ test	+ve
9	Oxalic acid		Calcium chloride	+ve
10	Inorganic acid		Sulphate test	+ve

5.1 Capsicum annum

S.NO	Conc.(µg/ml)	Standard diclofenac	Ethanolic extract	Acetone extract	Acetonitrile extract
1	100	45	23	20	22
2	200	54	27	25	26
3	400	63	32	29	30

5.2 Capsicum frutescense

S.NO	Conc(µg/ml)	Standard diclofenac	Ethanolic extract	Acetone extract	Acetonitrile extract
1	100	45	32	23	26
2	200	54	47	27	32
3	400	63	56	31	43

5.3 Capsicum annum

S.NO	Conc(µg/ml)	Standard aspirin	Ethanolic extract	Acetone extract	Acetonitrile extract
1	100	60	47	30	36
2	200	70	55	39	44
3	400	81	70	47	61

5.4 Capsicum frutescense

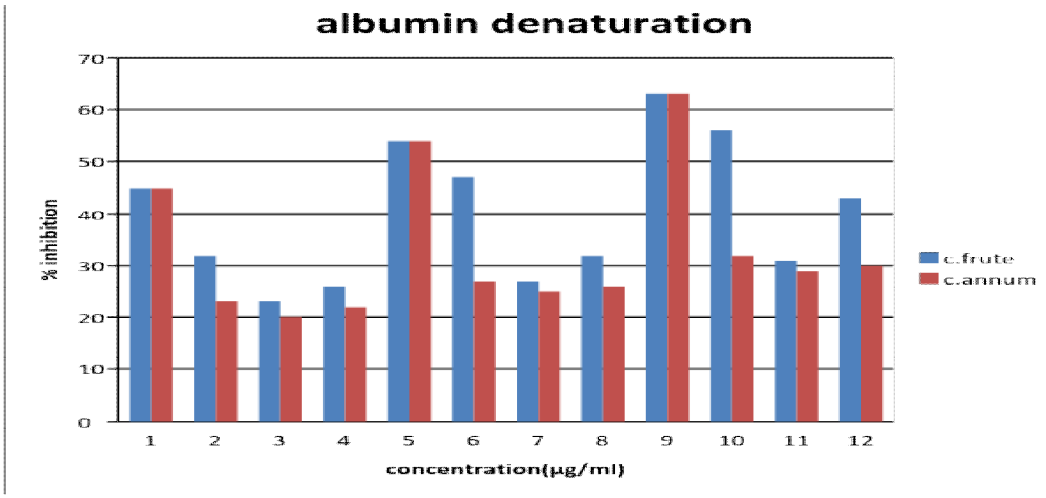
S.NO	Conc(µg/ml)	Standard aspirin	Ethanolic extract	Acetone extract	Acetonitrile extract
1	100	60	52	47	48
2	200	70	63	54	56
3	400	81	74	68	71

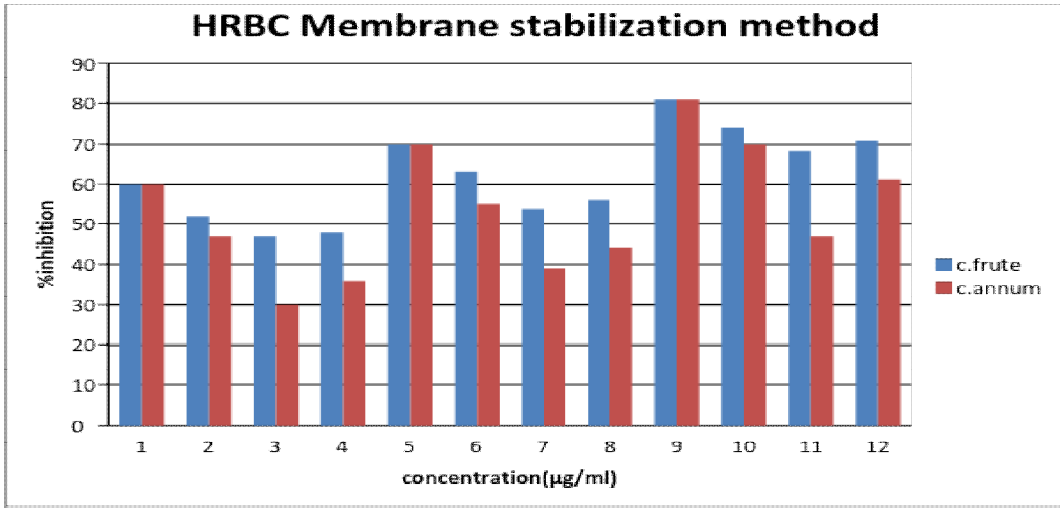
5.5 Capsicum annum

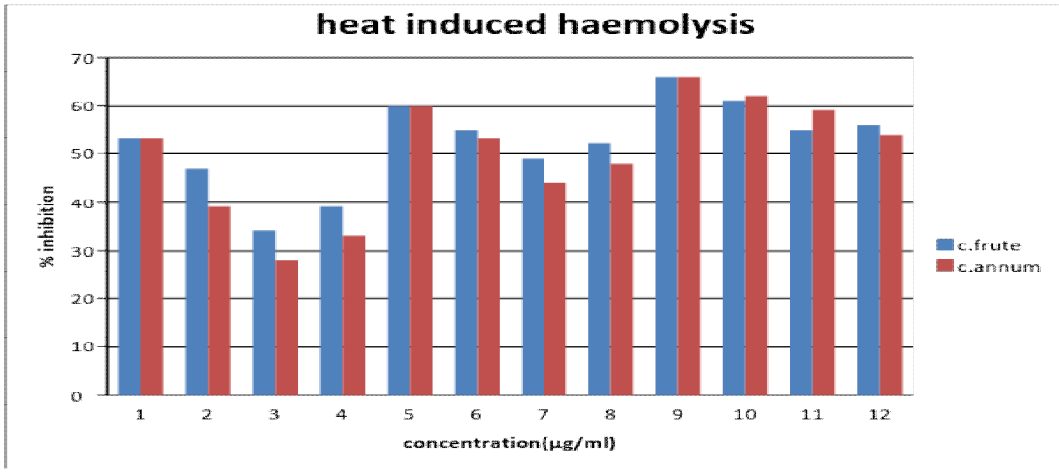
S.NO	Conc(µg/ml)	Standard aspirin	Ethanolic extract	Acetone extract	Acetonitrile extract
1	100	53	39	28	33
2	200	60	53	44	48
3	400	66	62	59	54

5.6 Capsicum frutescense

S.NO	Conc(µg/ml)	Standard aspirin	Ethanolic extract	Acetone extract	Acetonitrile extract
1	100	53	47	34	39
2	200	60	55	49	52
3	400	66	61	55	56


albumin denaturation
% inhibition
concentration(µg/ml)
c.frute
c.annum


HRBC Membrane stabilization method
%inhibition
concentration(µg/ml)
c.frute
c.annum


heat induced haemolysis
% inhibition
concentration(µg/ml)
c.frute
c.annum

5. Conclusion:

The present study has established the anti inflammatory activity of c.annum and c.frutescence by invitro methods. In this study the phytochemicals present in c.annum and c.frutescence fruits showed the anti inflammatory property comparable to diclofenac sodium among ethanolic, acetonitriole, acetone extracts, ethanolic extract showed highest anti inflammatory property and compared to c.annum , c.frutescence showed highest anti inflammatory property.

6. References:

1. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE; Nielsen; Andersen; Girardin (February 2007). "Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation". Clin. Exp. Immunol. 147 (2): 061127015327006—. doi:10.1111/j.1365-2249.2006.03261.x. PMC 1810472.PMID 17223962. CS1 maint: Multiple names: authors list (link)
2. Piira, Olli-Pekka; Miettinen, Johanna A.; Hautala, Arto J.; Huikuri, Heikki V.; Tulppo, Mikko P. (2013). "Physiological responses to emotional excitement in healthy subjects and patients with coronary artery disease". Autonomic Neuroscience. 177 (2): 280–285. doi:10.1016/j.autneu.2013.06.001. PMID 23916871.
3. Rather, L. J. (1971). "Disturbance of function (functio laesa): the legendary fifth cardinal sign of inflammation, added by Galen to the four cardinal signs of Celsus". Bull N Y Acad Med. 47 (3): 303–322. PMC 1749862. PMID 5276838
4. Kumar, Rukmini; Clermont, Gilles; Vodovotz, Yoram; Chow, Carson C. (2004-09-21). "The dynamics of acute inflammation". Journal of Theoretical Biology. 230 (2): 145–155. doi:10.1016/j.jtbi.2004.04.044.
5. Werner, Ruth (2009). A massage Therapist Guide to Pathology (4th ed.). Philadelphia, PA and Baltimore, MD: Wolters Kluwer.
6. Porth, Carol (2007). Essentials of pahtophysiology: concepts of altered health states. Hagerstown, MD: Lippincott Williams & Wilkins. p. 270. ISBN 0-7817-7087-4.
7. Dormandy, Thomas (2006). The worst of evils: man's fight against pain. New Haven, Conn: Yale University Press. p. 22. ISBN 0-300-11322-6.
8. Wiedermann U, et al. (1996). "Vitamin A deficiency increases inflammatory responses". Scand J Immunol. 44 (6): 578–584. doi:10.1046/j.1365-3083.1996.d01-351.x. PMID 8972739.
9. Montiel-Duarte, C.; Ansorena, E.; López-Zabalza, M. J.; Cenarruzabeitia, E.; Iraburu, M. J. (2004). "Role of reactive oxygen species, glutathione and NF-κB in apoptosis induced by 3,4-methylenedioxymethamphetamine ("Ecstasy") on hepatic stellate cells". Biochem Pharmacol. 67 (6): 1025–1033. doi:10.1016/j.bcp.2003.10.020. PMID 15006539.
10. Hendrik Ungefroren; Susanne Sebens; Daniel Seidl; Hendrik Lehnert; Ralf Haas (2011). "Interaction of tumor cells with the microenvironment". Cell Communication and Signaling. 9 (18). doi:10.1186/1478-811X-9-18.
11. Salazar-Gonzalez, J. F.; Martinez-Maza, O.; Nishanian, P.; Aziz, N.; Shen, L. P.; Grosser, S.; Taylor, J.; Detels, R.; Fahey, J. L. (1998-08-01). "Increased immune activation precedes the inflection point of CD4 T cells and the increased serum virus load in human immunodeficiency virus infection". The Journal of Infectious Diseases. 178 (2): 423–430. doi:10.1086/515629. PMID 9697722.
12. Ipp, Hayley; Zemlin, Annalise (2013-02-01). "The paradox of the immune response in HIV infection: when inflammation becomes harmful". Clinica Chimica Acta; International Journal of Clinical Chemistry. 416: 96–99. doi:10.1016/j.cca.2012.11.025.
13. Ashcroft, G. S.; Yang, X; Glick, A. B.; Weinstein, M; Letterio, J. L.; Mizel, D. E.; Anzano, M; Greenwell-Wild, T; Wahl, S. M.; Deng, C; Roberts, A. B. (1999). "Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response". Nat Cell Biol. 1 (5): 260–266. doi:10.1038/12971. PMID 10559937 .

Cite this article as: Bizzam A, Donakonda M, Badrinath AV, Shaik K. Comparative study of *Invitro* anti inflammatory activity of *capsicum annum* and *capsicum frutescense* fruits. J Compr Phar 2016;3(6):198-203.