Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2017, Volume 5, Issue 9
ISSN: 2321–3647(online)

In Vitro Evaluation of Anti-Inflammatory and Antiarthritic Activity of *Citrus Limetta* Peel

Desai S*[1], Desai A[1], Kanzaria S[1], Mishra P[1], Meshram DB[1]
1. Pioneer Pharmacy Degree College, Sayajipura, Vadodara-390019

ABSTRACT

The present study aims on assessing the anti arthritic activity using bovine serum albumin and anti-inflammatory activity using human red blood cell membrane stabilization model. *Citrus limetta*, commonly known as sweet lime, belongs to family Rutaceae. It is a rich source of flavanoid, d-limonene, alkaloids, steroids, volatile oils and polyphenols. Various extracts of sweet lime peel at different concentrations (50, 100, 200, 400 and 800µg/ml) showed the significant ameliorating effect of hypotonic saline induced RBC membrane damage when compared against standard drug aspirin whereas ameliorating effect by inhibiting protein denaturation on bovine serum albumin *in vitro* model against standard drug diclofenac sodium. Protective effect on heat induced protein denaturation can be used as a tool to study the *in vitro* anti-arthritic activity whereas protective effect on heat and hypotonic saline-induced erythrocyte lysis is known to be a very good index of anti-inflammatory activity of any agent. The results of the present study revealed that at various concentrations of *Citrus limetta* peel extracts when subjected to *in vitro* anti-arthritic and anti-inflammatory activity showed % inhibition to be significant when compared with standard drug. Thus *Citrus limetta* fruit peel demonstrates a potential anti-inflammatory and anti-arthritic effect which may be attributed to its anti-oxidant property.

Keywords: Citrus limetta, anti-arthritic, anti-inflammatory, sweet lime

*Corresponding Author Email: sapna_peer@rediffmail.com
Received 23 August 2017, Accepted 03 September 2017

Please cite this article as: Desai S *et al*., In Vitro Evaluation of Anti-Inflammatory and Antiarthritic Activity of Citrus Limetta Peel. American Journal of Pharmacy & Health Research 2017.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by tenacious symmetric inflammation of multiple peripheral joints. It is one of the most common inflammatory diseases and is characterized by the development of a chronic inflammatory proliferation of the synovial linings of diarthrodial joints, which leads to truculent cartilage destruction and progressive bony erosions[1]. The mechanism of inflammatory injury is ascribing, in part, to release of Reactive Oxygen Species (ROS) from activated neutrophils and macrophages. Free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can impair inflammation. Tumour necrosis factor alpha (TNF-alpha) is the product of macrophages that has been demonstrated to play an important role in the pathogenesis of RA[2]**.** The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to gastric ulcers. Herbal drugs constitute a major part in all the traditional system of medicine. The screening and development of drugs for their anti-inflammatory activity is still in progress and there is hope for finding anti-inflammatory drugs from indigenous medicinal plants. Herbal medicine is a conquest of popular therapeutic diversity. Medicinal plants have been used from an era as remedies for human diseases because they contain chemical components of therapeutic value[3, 4]**.** Citrus genus belongs to the large family Rutaceae, containing 130 genera in the seven subfamilies with many important fruits and essential oil producers. It is extremely priceless throughout the world for its exceptional nutritional and medicinal properties[5]**.** *Citrus limetta* Risso (Rutaceae), commonly known as sweet lime in English and Mousambi in India, has been traditionally used for several medicinal purpose[6]**.** It is a popular indigenous citrus fruit relished for its cooling and therapeutic effects and also as a comestible delight in some parts of the subcontinent[7]**.** It is believed to possess bioactivities such as antioxidant, anti-inflammatory, antimicrobial, and is suggested to be responsible for the prevention of cancer and degenerative diseases. These bioactivities of Citrus are due to the presence of bioactive compound such as phenolics, flavonoids, essential oil and vitamins[8]. The present study was carried out to evaluate the anti-inflammatory and anti-arthritic activity of various extracts of citrus limetta peel[9] (Figure 1).

Figure 1: *Citrus limetta* peel

MATERIALS AND METHOD

Plant collection:

The peels of *Citrus limetta* were obtained from the local market during March 2017.

Preparation of the extract

Fresh peels of the above plant were collected, dried in shade under room temperature, powdered mechanically and sieved through No.20 mesh sieve. The finely powdered peels were kept in an airtight container until the time of use. About 5g of the dried powder was soaked with 100 ml of ethanol, water, ether, and methanol in different flasks for 24 h and then macerated at room temperature for 2 hours.

***Phytochemical screening*[10, 11]**

Phytochemical screening was carried out on the extracts using established protocols as described by Trease and Evans (1989), Sofowora (1993), and Harborne (1998). Stock solutions of each extract with a concentration of 10 mg extract/mL was prepared and used for the phytochemical screening **(Table 1)**.

Table 1: Phytochemical analysis of *Citrus limetta* for various extracts

Sr no.	Phytochemical constituents	Aqueous	Methanol	Ethanol	Ether
1	Tannins	+	+	+	-
2	Phlobatannins	+	+	+	-
3	Saponins	+	-	-	+
4	Terpenoids	-	+	+	+
5	Alkaloids	-	-	++	+
6	Phenols	+	+	+	+
7	Flavanoids	++	+++	+	+
8	Reducing sugar	+	-	-	-

***Assessment of in vitro anti-arthritic activity* [12, 13, 14]**

Inhibition of Protein Denaturation

The reaction mixture (5 ml) consisted of 0.2 ml of bovine serum albumin, 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of extract so that final concentrations become 50, 100, 200, 400, 800 µg/ml. Then the mixtures were incubated at 37 ± 2ºC in a BOD incubator for 15 minutes and then heated at 70ºC for 5 minutes. After cooling, absorbance was measured at 340 nm (SHIMADZU, UV 1800) by using vehicle as blank. In control 0.05 ml distilled water was used instead of test extract and Diclofenac sodium was used as the standard.

The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\text{Percentage inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs control}} \times 100$$

The control represents 100% protein denaturation. All the determinations were done in triplicate. The results are shown in Table 2.

Table 2: Percent Protection of Protein Denaturation

Sr. No.	Concentration (µg/ml)	Percent Protection				
		Water extract	**Ether extract**	**Methanolic extract**	**Ethanolic extract**	**Standard (Diclofenac Sodium)**
1	50	4.59	2.61	8.48	6.5	18.45
2	100	25.77	19.61	25.42	26.5	45.43
3	200	39.89	34.81	47.23	41.76	58.14
4	400	59.93	50.50	64.59	61.76	76.90
5	800	80.16	68.80	91.12	84.22	92.72

In vitro anti-inflammatory activity

Preparation of Red Blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the heparinised centrifuge tubes. The tubes were centrifuged at 3000 rpm 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.

Heat induced Haemolysis

The reaction mixture (2 ml) consisted of 1 ml 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 60°C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates. Percent

membrane stabilization activity was calculated by the following mentioned formula. The results are shown in Table 3.

Table 3: Percent Stabilization on HRBC Membrane

Sr. No.	Concentration (µg/ml)	Percent Stabilization				
		Water extract	Ether extract	Methanolic extract	Ethanolic extract	Standard (Aspirin)
1	50	3.01	3.12	6.28	16.06	23.22
2	100	31.58	28.90	23.24	33.50	41.46
3	200	41.24	47.91	38.59	41.02	50.59
4	400	67.0	76.82	47.51	82.73	83.35
5	800	79.47	81.25	70.76	88.7	91.82

Calculation:

% of Inhibition = 100 X [Vt / Vc – 1]

Where, Vt = absorbance of test sample, Vc = absorbance of control.

RESULTS AND DISCUSSION

Inflammation is a reaction of living tissue towards injury. Phytochemical screening of Citrus limetta indicates the presence of polyphenols, flavonoids, tannins, saponins, glycosides etc. In the present research study, *in vitro* anti-inflammatory and antiarthritic activity was performed using HRBC membrane stabilization method and protein denaturation method. The *in vitro* anti-inflammatory method involves the stabilization of HRBC Membrane by hypotonicity induced membrane lysis whereas denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritic diseases. From the results of the present study it can be stated that *Citrus limetta* is capable of controlling the production of autoantigens due to *in vivo* denaturation of proteins in rheumatic diseases. Hence, from the obtained results, it can be concluded that Citrus limetta peel extracts possesses anti-inflammatory and anti arthritic activity from the obtained results, it can be concluded that *Citrus limetta* peel extracts possesses anti-inflammatory activity. However, the methanolic and ethanolic extracts showed significant activity when compared with the standard drug **(Figure 2 and 3)**.

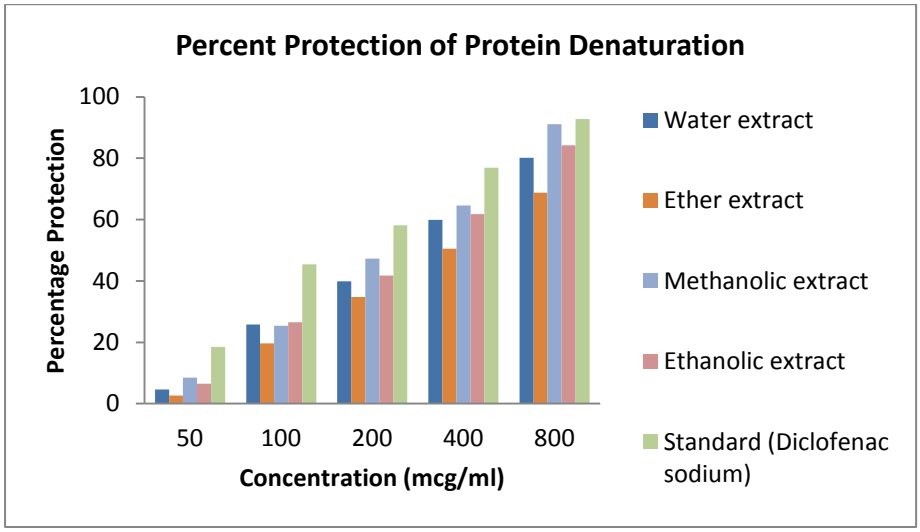

Figure 2: Percent Protection of Protein Denaturation using different extracts of *Citrus limetta*

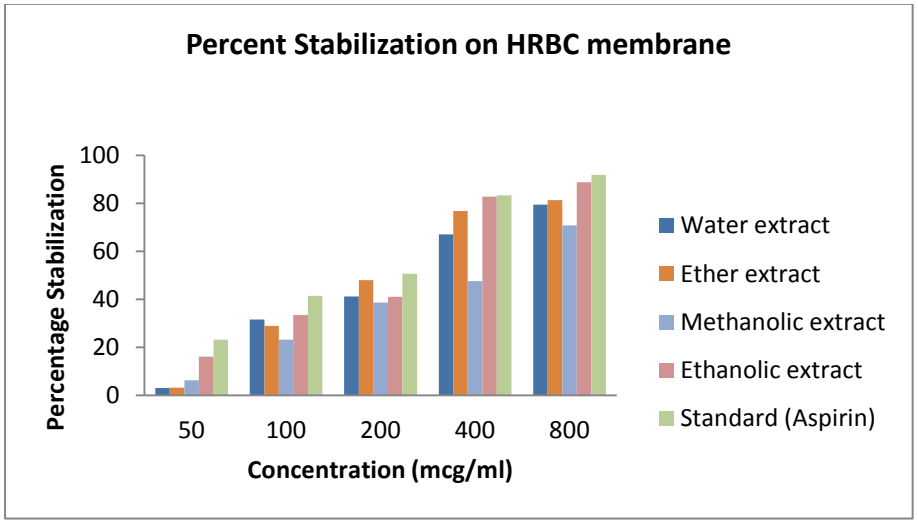

Figure 3: Percent Stabilization on HRBC Membrane using different extracts of *Citrus limetta*

ACKNOWLEDGEMENT

The authors are heartily indebted to Shri D.D Patel, President, Om Gayatri Education and Charitable Trust and Pioneer Pharmacy Degree College for their valuable guidance and support. Authors are grateful to the Management of Pioneer Pharmacy Degree College for providing necessary research facilities.

REFERENCES

1. McPhee SJ, Hammer GD. Pathophysiology of Disease: An Introduction to Clinical Medicine, 6th ed. Mc Graw Hill; 2006: 655.

2. Arya D, Meena M, Grover N, Patni V. *In vitro* anti-inflammatory and antiarthritic activity in methanolic extract of Cocculus Hirsutus (L.) Diels. International Journal of Pharmaceutical Sciences and Research. 2014; 5(5): 1957-62.
3. Gupta SK, Gupta A, Gupta AK, Pakash D, Vedpal. *In vitro* Anti-arthritic activity of ethanolic extract of Callicarpa macrophylla flower. International Research Journal of Pharmacy. 2013; 4(3):160-62.
4. Javed S, Ahmad R, Shahzad K, Nawaz S, Saeed S, Yasar Saleem.. Chemical constituents, antimicrobial and antioxidant activity of essential oil of Citrus limetta var. Mitha (sweet lime) peel in Pakistan. African Journal of Microbiology Research. 2013; 7(24): 3071-3077.
5. Younis K, Islam RU, Jahan K, Yousuf B, Ray A. Effect of addition of mosambi (Citrus limetta) peel powder on textural and sensory properties of papaya jam. Cogent Food & Agriculture. 2015; 1: 1023675.
6. Kundusen S, Saha P, Bhattacharya S, Bala A ,Mazumder U, Gupta M, Haldar P. Evaluation of *in vitro* antioxidant activity of citrus limetta and citrus maxima on reactive oxygen and nitrogen species. Pharmacologyonline. 2010; 3: 850-857.
7. Sidana J, Saini V, Dahiya S, Nain P, Bala S. International Journal of Pharmaceutical Sciences Review and Research. 2013; 18(2): 20-27.
8. Kumar SN, Kishor G, Kumar SG, Sindhu ES. *In vitro* anti-inflammatory and antiarthiritic activity of leaves of Physalis Angulata L. International Journal of Pharmacy and Industrial Research. 2011; 1(3): 211-213.
9. Leelaprakash G and Mohan Dass S. *In vitro* anti-inflammatory activity of methanol extract of enicostemma axillare. International Journal of Drug Development and Research. 2011; 3(3): 189-196.
10. Trease GE, Evans WC. Pharmacognosy. London: W.B Scandars Company Ltd; 1989:14: 269- 300.
11. Harborne JB. Phytochemical methods guide to modern technique of plant analysis. 3rd ed., London: Chapmen all Hall; 1998.
12. Govindappa M , Naga Sravya S, Poojashri M N, Sadananda TS, Chandrappa C P. Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. J Pharmacognosy Phytother. 2011; 3(3): 43-51.

13. Chandra S, Dey P, Bhattacharya S. Preliminary *in vitro* assessment of anti-inflammatory property of Mikania scandens flower extract. J Adv Pharm Edu Res. 2012; 2(1):25-31.
14. Jayaprakasam R, Ravi T K. Evaluation of anti arthritic activity of the root extract of acalypha indica linn. using *in vitro* techniques. International Journal of Phytopharmacy. 2012; 2 (6): 169-173.