Int.J.Curr.Microbiol.App.Sci (2019) *8*(5): 1686-1698

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 05 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.805.195

Comparative Evaluation of *in vitro* Anti-Inflammatory Activity of Different Extracts of Selected Medicinal Plants from Saurashtra Region, Gujarat, India

Chirag M. Modi*, Punit R. Bhatt, Kajal B. Pandya, Harshad B. Patel and Urvesh D. Patel

Department of Veterinary Pharmacology & Toxicology, College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh (Gujarat) India

**Corresponding author*

Keywords

Medicinal plants, Saurashtra region, *Adansonia digitata*, Methanol and water extracts

Article Info

Accepted:
15 April 2019
Available Online:
10 May 2019

A B S T R A C T

In the present research work, an *in vitro* anti-inflammatory activity of twenty-five different medicinal plants growing around Junagadh region of Gujarat was evaluated. Phytochemical screening of each plant extracts was performed. Anti-inflammatory activity was evaluated using two different methods: 1. Inhibition of albumin denaturation and 2. Protease inhibition assay. In case of inhibition of albumin denaturation assay, water extracts of *Adansonia digitata* L. leaves, *Flueggea leucopyrus* Willd. leaves and *Solanum xanthocarpum* Schrad. & H. Wendl. aerial part showed an inhibition of 87.54, 80.23 and 80.38 %, respectively. While methanol extracts of *Adansonia digitata* L. leaves and *Solanum xanthocarpum* aerial part exhibited 87.54 and 81.79 % inhibition at 500 µg/ml concentration. In the case of protease inhibition assay, methanol and water extracts of *Adansonia digitata* leaves, *Flueggea leucopyrus* leaves and *Punica granatum* L. epicarp showed the higher inhibition at 500 µg/mL. The methanol extract of *Flueggea leucopyrus* leaves and water extract of *Peltophorum pterocarpum* (DC.) K. Heynebark exhibited protease inhibition of 91.94 % and 89.06 %, respectively at higher concentration. The observations from the present study may be useful for bioprospecting in the field of ethnopharmacology.

Introduction

Inflammation is a complex process associated with pain, an increase in vascular permeability and an increase in protein denaturation. Inflammation occurs in response to damage occurred to body cells either due to microbes or due to physical or chemical agents. In response to inflammation, the body produces various responses like pain, redness, swelling, heat and lack of function in the injured area (Tortora and Sandra, 1993). A number of biological proteins lose their biological functions when it becomes denatured due to inflammation.

Therefore, protein denaturation is a well-documented process in inflammation and substance that can inhibit the denaturation of protein can be a good candidate for anti-inflammatory action (Ingle and Patel, 2011; Leelaprakash and Dass, 2010).

To study this complex process, a large number of animals may be required. It is for the above reason Roach and Sufka (2003) have proposed the chick carrageenan response assay for the discovery of molecules with anti-inflammatory nociception properties. However, the Bovine Serum Albumin (BSA) assay seeks to eliminate the use of live specimens as far as possible in the drug development process. Grant *et al.,* (1970) have reported that one of the features of several non-steroidal anti-inflammatory drugs e.g. indomethacin, ibufenac, flufenamic acid and salicylic acid is their ability to stabilize (prevent denaturation) heat treated BSA at pathological pH [pH 6.2 – 6.5] (Williams *et al.,* 2008).

Various protease enzymes are involved in many essential intra and extracellular physiological processes but their role in the development of the disease is not well established. Recent reports in the field of proteinase have attracted researchers to study them closely related to biological systems. Significant evidence is available that indicates proteases can regulate its target cells by activating and breaking a family of G-protein coupled, Proteases activated receptors (PARs). Potential roles for PARs in inflammation have also been proposed. For example, because platelets can produce inflammatory mediators, such as serotonin and chemokines, platelet activation by thrombin through PAR1 might amplify inflammatory responses or recruitment of inflammatory cells (Coughlin, 2000). Recent reports have demonstrated that protease inhibitors may have anti-inflammatory roles other than mere suppressive effects on protease actions during inflammation (Dharmalingam *et al.,* 2014). Though a number of anti-inflammatory drugs are available in the market i.e. steroidal drugs like corticosteroids and non-steroidal like aspirin. NSAIDs are one of the best classes of the drug to prevent and treat postoperative pain orthopaedic conditions such as osteoarthritis, soft-tissue injuries and fractures *etc* (Boursinos *et al.,* 2009). The use of NSAIDs is associated with many side effects, but their unwanted effects on the gastrointestinal tract, the kidney and the cardiovascular system are considered as major issues with the use of these drugs (Alexandrina, 2010). Apart from this, rural and tribal people are largely depending on medicinal plants for their healthcare and as well as livestock. This attracted several researchers to evaluate medicinal plants as a secondary source of anti-inflammatory drugs (Sengupta *et al.,* 2012). Saurashtra region is a rich in plant flora. Many medicinal plants are naturally growing in this region and used in traditional remedies since old time. *A. aspera* is traditionally used in skin disorders and anal fistula. *A. squamosa* leaves are commonly employed for the treatment of infection of skin wounds and maggots in animals. *B. variegata* bark is traditionally used as astringent and also employed in various skin disorders. *C. amada* rhizome is used for inflammation of the liver and in rheumatism. *M. oleifera* leaves are also used to treat various inflammations for a long time (Khare, 2007). Therefore, the screening and development of drugs for their anti-inflammatory activity is the need of today's era and many studies all over the world have been carried out to evaluate anti-inflammatory drugs from indigenous medicinal plants (Srinivasan *et al.,* 2001). The present study was also done to screen the various plants for having active photochemicals and evaluate the anti-inflammatory activity of extracts of twenty five plants.

Materials and Methods

Collection and processing of plant material

All the plant materials listed in Table 1 were collected from surrounding regions of Junagadh district, Gujarat (India). Plant

materials were identified and authenticated. A voucher specimen of each plant was deposited in the department. Plant materials were washed with tap water and dried in an oven at 45°C for seven days. The material was ground; fine powder was made and stored in an air-tight container until use.

Preparation of extracts

Fine powders of plant material were defatted using n-hexane by soxhlet apparatus to remove chlorophyll and other non-polar debris. Defatted plant material was dried in the oven. About 50 g of plant material was extracted with 500 mL of chloroform, methanol and water separately at least two times.

The hydro-alcoholic extract was prepared by extracting 50 g of plant material with 500 mL of 60% methanol. The content was filtered off and solvents were evaporated under reduced pressure using rotary vacuum evaporator below 50°C. The extracted were collected; the yield was calculated and stored at 4°C for further use.

Phytochemical screening

Qualitative phytochemical screening was performed for each extract as per standard procedures (Table 2) (Harborne, 1998).

In vitro anti-inflammatory activity

The extract solutions were prepared by all three extracts in water/DMSO at a concentration 1mg/mL and suitable dilutions were made to get the test solutions.

Inhibition of albumin denaturation method

Inhibition protein denaturation method was followed with minor modifications (Alhakmani *et al.,* 2014; Williams, *et al.,* 2008). The reaction mixture (5ml) was consisting of 1mL (0.1%) of bovine albumin fraction, 1 mL Tris-HCl buffer pH 7.8 solution and 1 mL of test solutions. The mixtures were incubated at 37ºC for 20 min., followed by heating at 72ºC for 2-4 minutes in the water bath for denaturation.

After cooling the samples at room temperature, the turbidity was recorded by spectrophotometrically at 660nm. Aspirin and buffer were taken as a positive control and blank solution, respectively. Control solution contained 1 mL distilled water with 1mL (0.1%) bovine albumin fraction and 1 mL buffer solution. The experiment was carried out in triplicates and per cent inhibition for protein denaturation was calculated using:

Protease inhibition assay

The test was performed according to the modified method of Dharmalingam *et al.*, (2014). The reaction mixture (2 ml) was made with containing 0.06 ml trypsin, 1ml of 20mM Tris HCl buffer (pH 7.4) and 1ml test sample of different concentrations. The reaction mixture was incubated for 10 minutes at 37ºC.

Then, 1ml of 0.65% (W/V) casein was added. The mixture was re-incubated for 20 min. After incubation, 2 ml of 2M $HClO_4$ was added to terminate the reaction. The cloudy suspension was centrifuged at 7830 rpm for 15 minutes. The absorbance of the supernatant was measured at 280 nm against. Tris-HCl buffer was used as blank. The experiment was performed in triplicate. Anti-inflammatory activity was measured by calculating % inhibition against a range of concentrations. % inhibition can be calculated as follow: % inhibition= (1-Ac/At) ×100; where Ac is absorbance of control; At is absorbance of the test.

Thin-layer chromatography of various extracts showed an anti-inflammatory activity

Preparation of plant extracts and reference standard

The plant extracts for the detection of phenolic compounds were prepared by extracting 2g of each plant material in 10 mL of methanol on ultrasonic bath for 10 minutes. Then the extracts were centrifuged to 2500 rpm for 10 minutes, supernatants were collected and used as sample. Gallic acid (SD Fine Ltd, India) was dissolved in methanol at a concentration (0.5 mg/mL).

Thin-layer chromatographic analysis

All the plant extracts and gallic acid were applied as a band on 10 × 10 cm pre-coated aluminum-backed silica gel plates GF_{254} (Merck, Germany) using Linomate 5 applicator (Camag, Germany). The plate was developed in a mixture of solvents consist of Toluene: ethyl acetate: formic acid: water (6:6:1.2:0.25) (Shah *et al.*, 2016). The plate was allowed to run for 8 cm. Upon development, the plate was sprayed with natural product reagent (1% diphenyl boryloxyethylamine in methanol followed by 5% polyethylene glycol-4000 in methanol). The plates were then observed in UV cabinet (Camag, Germany) at 366 nm. The R_f values (Retention factor) of each separated bands and standard compound were calculated using dividing distance travelled by each solute to total solvent front (8 cm).

$$Rf \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

Results and Discussion

The results of *in vitro* anti-inflammatory activity of various medicinal plants for protease inhibition assay and inhibition of protein denaturation method are shown in table 9 and 10. Denaturation of proteins occurs in inflammatory conditions like rheumatoid arthritis, diabetes, cancer *etc.* Inflammatory conditions can be reduced by prevention of protein denaturation. The present study showed the *in vitro* anti-inflammatory activity of different extract of different parts of the plant by inhibiting protein denaturation. The extracts were effective in inhibiting heat induced albumin denaturation.

Mechanism of denaturation is a process in which proteins lose their tertiary structure and secondary structure due to in alteration of electrostatic force, hydrogen, hydrophobic and disulphide bonds by a large variety of chemical and physical agents, including acids, alkalies, alcohol, acetone, salts of heavy metals, dyes (Mann, 1906), heat, light, and pressure (Robertson, 1918). Vane and Botting, (1910) considered heat denaturation as a reaction between protein and water which implies in all probability hydrolysis. Some literature have reported that denaturation of protein is one of the cause of certain rheumatic diseases (Mizushima, 1966 and Grant *et al.*, 1970) due to the production of auto-antigens. Anti-inflammatory drugs have shown dose-dependent ability to inhibit the thermally induced protein denaturation by thermal (Grant *et al.*, 1970). Similarly, plant extract having pharmacologically active principles with anti-inflammatory activity can result in decrease protein denaturation (Sakat *et al.*, 2010). The plant extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon stimulation extracellularly released. The extracts having anti-inflammatory activity may release the lysosomal substance from neutrophils at the site of inflammation, which might be responsible for inhibition of heat

induced albumin denaturation at different concentrations.

In the present study, aspirin showed the maximum inhibition of 90.17 % at the concentration of 500 µg/ml which was used as a standard anti-inflammation drug. Maximum inhibition of 89.71% was observed by water extract of *Adansonia digitata* leaves followed by *Flueggea leucopyrus leaves* (80.23 %) and *Punica granatum* fruit epicarp (71.37 %). This inhibition was might be due to the presence of flavonoids and phenolic compounds in the leaf extracts. In the methanolic extract, maximum albumin denaturation inhibition of 87.54 % was observed by *Adansonia digitata* leaves followed by *Solanum xanthocarpum* aerial part (81.79%) and *Vitex negundo* leaves (71.47 %). The alkaloids are present in methanol and water extract may due to high polarity with high molecular weight. The chloroform extract of *Solanum xanthocarpum* has shown the highest percentage of inhibition of albumin denaturation (71.24 %); while *Flueggea leucopyrus leaves* water extract has shown 65.55 % inhibition of albumin denaturation (Table 3–8).

Proteinases have an important role in arthritic reactions. Neutrophils are known to be a rich source of serine proteinase which localized to carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das and Chatterjee, 1995). Different plant extracts exhibited significant anti-protease activity at different concentrations in the present study is shown in Table 10. The few extract effectively inhibited the proteinase activity. The standard aspirin drug showed the maximum inhibition of 84.79 % at 500µg/ml.

Out of tested extracts of medicinal plants, *Adansonia digitata, Flueggea leucopyrus, Peltophorum pterocarpum* bark, *Punica granatum* fruit epicarp, *Solanum xanthocarpum* and *Vitex negundo* exhibited good *in vitro* anti-inflammatory activity at 500 µg/mL concentration. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the antioxidant and anti-inflammatory activities. Higher inhibition (85.47%) was observed by the water extract of *Adansonia digitata* leaves at 500 µg/mL concentration. While methanolic extract of the same plant showed 84.10% inhibition of protease at 500 µg/mL concentration. The higher inhibition was might be due to the presence of flavonoids, phenolic and saponin compounds in the leaf extracts. Aqueous and methanolic extract of *Punica granatum* fruit epicarp also showed good inhibition of protease (81.07% and 82.76%, respectively) at 500 µg/mL concentration.

Methanolic extract of *F. leucopyrus* showed 91.94 % inhibition of protease at 500 µg/mL concentration, however, water extract of *F. leucopyrus* leaves has also shown the good percentage of protease inhibition (85.65%) was observed at 500 µg/mL concentration. This inhibition was might be due to the presence of flavonoids and phenolic compounds in the leaf extracts. The methanol extract of *Vitex negundo* leaves has shown 78.55% inhibition of protease while, water extract of *Solanum xanthocarpum* aerial part showed 67% at 500 µg/mL concentration. Presence of glyco-alkaloid named solasodine and solasonine might be responsible for the strong anti-inflammatory action of the plant. These alkaloids are present in methanol and water extract may due to high polarity with high molecular weight.

Thin-Layer Chromatography (TLC) of various plant extracts exhibited the presence

of gallic acid in *Peltophorum pterocarpum* bark, *Punica granatum* leaves and *Solanum xanthocarpum* leaves (Plate 1). All these plants are indigenous to the region from plants are collected and commonly used for various remedies like skin diseases, stomach disorders and respiratory disorders (Khare, 1996). Gallic acid is a colorless or slightly yellow crystalline compound used in pharmaceuticals and as an analytical reagent.

Table.1 List of medicinal plants used to evaluate an *in vitro* anti-inflammatory activity

Sr. No.	Plant species	Family	Local Name (Gujarati)	Part of plant used
1	*Aloe barbadensis*Mill.	Liliaceae	Kuwar pathu	Fresh Gel
2	*Achyranthes aspera* L.	Amaranthaceae	Aghedo	Seed
3	*Adansonia digitata* L.	Bombacaceae	Gorakh ambali	Leaves
4	*Annona squamosa* L.	Annonaceae	Sitaphal	Leaves
5	*Argyreia speciosa* (L. f.) Sweet	Convolvulaceae	Avli-Savli	Leaves
6	*Aristolochia longa* L.	Aristolochiaceae	Kidamari	Leaves
7	*Bauhinia variegata* L.	Caesalpiniaceae	Kachnar	Leaves
8	*Bryophyllum pinnatum* (Lam.) Oken	Crassulaceae	Paanfuti	Leaves
9	*Centratherum anthelminticum* (L.) Gamble	Asteraceae	Kalijiri	Seed
10	*Curcuma amada* Roxb.	Zingiberaceae	Amba haldar	Rhizome
11	*Derris indica* (Lam.) Bennet	Fabaceae	Karanj	Seed
12	*Euphorbia nivulia* Buch.-Ham.	Euphorbiaceae	Dandaliyo thor	Stem
13	*Ficus racemosa* L.	Moraceae	Umbaro	Bark
14	*Flueggea leucopyrus* Willd.	Euphorbiaceae	Chinvi	Leaves
15	*Jasminum arborescens* Roxb.	Oleaceae	Chameli	Leaves
16	*Leptadenia reticulata* (Retz.) Wight & Arn.	Asclepiadaceae	Dodi	Root
17	*Moringa oleifera* Lam.	Moringaceae	Saragvo	Leaves
18	*Peltophorum pterocarpum* (DC.) K.Heyne	Caesalpiniaceae	Pilo gulmohar	Bark, Leaves
19	*Prosopis juliflora* (Sw.) DC.	Mimosaceae	Gando baval	Leaves
20	*Psoralea corylifolia* L.	Fabaceae	Bavchi	Seed
21	*Punica granatum* L.	Punicaceae	Dadam	Fruit Epicarp
22	*Solanum xanthocarpum* Schrad. & H. Wendl.	Solanaceae	Bhoi ringani	Aerial Part
23	*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.	Combretaceae	Arjun	Bark
24	*Tridax procumbens* L.	Compositae	Gha buri	Aerial Part
25	*Vitex negundo* L.	Verbenaceae	Nagod	Leaves

Table.2 Phytochemical screening of different extract of leaves of plants

Plants	*A. digitata*			*A. squamosa*			*A. longa*			*B. variegata*			*B. pinnatum*			*F. leucopyrus*			*M. oleifera*			*P. pterocarpum*			*V. negundo*		
Extracts	CE	ME	WE	CE	ME	WE	CE	ME	WE	CE	ME	WE	CE	ME	WE	CE	ME	WE	CE	ME	WE	CE	ME	WE	CE	ME	WE
Alkaloid	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-
Glycoside	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	-	+
Saponin	-	+	+	-	+	-	+	-	+	-	-	+	-	+	+	-	+	+	-	-	+	-	-	-	-	-	+
Flavonoid	+	-	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	-	+	+	-	+	+	-	+	+
Steroid	+	-	+	+	+	+	+	-	-	+	+		+	+	-	+	+	-	+	+	-	-	-	-	+	-	-
Sugar	-	-	+	-	+	+	-	+	-	-	+	+	-	+	+	-	+	+	-	+	+	-	-	-	-	+	+
Tannin	-	-	+	-	+	+	-	-	+	-	-	+	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-
Phenolic	-	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	-	+

Note: +, indicates presence of phytoconstituents; -, indicates absence of phytoconstituents

Table.3 Phytochemical screening of different extract of various plants seed

Plants	*A. aspera*			*C. anthelminticum*			*D. indica*			*P. corylifolia*		
Extracts	CE	ME	WE	CE	ME	WE	CE	ME	WE	CE	ME	WE
Alkaloid	-	-	-	+	-	-	-	-	-	-	-	-
Glycoside	+	+	+	+	-	+	-	-	+		+	+
Saponin	-	-	+	-	+	+	-	+	+	-	-	-
Flavonoid	-	+	+	+	+	+	-	+	+	+	+	+
Steroid	+	-	-	+	-	-	-	+	-	+	-	-
Sugar	-	+	+	+	-	+	-	+	+	-	+	+
Tannin	-	-	-	-	+	-	-	-	+	-	-	+
Phenolic	-	+	+	+	+	+	-	-	+	-	+	+

Table.4 Phytochemical screening of different extract of aerial part of plants

Plants	***S. xanthocarpum***			***T. procumbens***		
Extracts	CE	ME	WE	CE	ME	WE
Alkaloid	+	+	-	-	-	-
Glycoside	-	+	+	-	+	+
Saponin	-	-	-	+	+	-
Flavonoid	-	-	-	-	-	+
Steroid	+	+	-	+	-	-
Sugar	-	-	-	+	+	-
Tannin	-	-	-	-	-	-
Phenolic	-	+	+	-	+	+

Table.5 Phytochemical screening of different extract of rhizomes of plant

Plant	***C. amada***		
Extracts	CE	ME	WE
Alkaloid	-	-	-
Glycoside	-	-	+
Saponin	-	+	+
Flavonoid	+	-	+
Steroid	+	+	-
Sugar	-	+	+
Tannin	+	-	-
Phenolic	-	-	+

Table.6 Phytochemical screening of different extract of root of plant

Plant	***L. reticulata***		
Extracts	CE	ME	WE
Alkaloid	+	+	+
Glycoside	-	-	+
Saponin	-	-	+
Flavonoid	-	+	+
Steroid	+	-	-
Sugar	-	+	+
Tannin	-	-	+
Phenolic	-	+	+

Table.7 Phytochemical screening of different extract of bark of plants

Plants	*P. pterocarpum*			*T. arjuna*		
Extracts	CE	ME	WE	CE	ME	WE
Alkaloid	-	-	-	-	-	-
Glycoside	-	+	+	+	+	+
Saponin	-	-	-	-	+	+
Flavonoid	-	+	+	-	+	+
Steroid	-	-	-	-	-	-
Sugar	-	-	-	-	+	+
Tannin	-	+	+	-	+	+
Phenolic	-	+	+	-	+	+

Table.8 Phytochemical screening of different extract of stem of plant

Plant	*E. nivulia*		
Extracts	CE	ME	WE
Alkaloid	-	-	-
Glycoside	-	+	+
Saponin	-	+	+
Flavonoid	+	+	+
Steroid	+	+	+
Sugar	-	-	+
Tannin	-	-	+
Phenolic	-	-	+

Table.9 *In vitro* anti-inflammatory activity of different extracts of selected medicinal plants by inhibition of albumin denaturation method

Name of plant/Std.	Type of extract	Percent inhibition at Concentration (µg/mL)				
		100	**200**	**300**	**400**	**500**
Aspirin	Std.	35.49±1.91^{a}	43.15±0.38^{a}	67.71±3.53^{b}	85.13±0.99^{c}	90.17±1.28^{c}
***Adansonia digitata* leaves**	CE	40.78±0.18^{a}	41.09±0.13^{a}	44.81±0.34^{a}	45.06±0.58^{a}	49.46±0.21^{a}
	ME	58.31±0.17^{c}	61.53±0.17^{b}	66.7±0.17^{a}	87.26±0.21^{d}	87.54±0.17^{c}
	WE	46.12±0.17^{a}	61.97±0.08^{b}	88.52±0.26^{e}	88.75±0.38^{d}	89.71±0.17^{c}
***Flueggea leucopyrus* leaves**	CE	45.19±0.13^{a}	45.47±0.19^{a}	58.22±0.18^{a}	61.1±0.23^{a}	65.55±0.29^{a}
	ME	24.28±0.36^{a}	30.39±0.30^{a}	48.49±0.25^{a}	52.16±0.25^{a}	54.49±0.17^{a}
	WE	59.87±0.13^{c}	62.13±1.83^{b}	72.9±0.18^{c}	75.78±0.23^{b}	80.23±0.30^{b}
***Peltophorum pterocarpum* bark**	ME	52.29±0.30^{b}	53.37±0.29^{a}	55.86±0.21^{a}	56.99±0.39^{a}	58.65±0.29^{a}
	WE	62.52±0.34^{d}	64.52±0.34^{b}	66.77±0.29^{a}	69.47±0.25^{a}	71.42±0.25^{a}
***Solanum xanthocarpum* aerial part**	CE	29.87±0.21^{a}	42.17±0.17^{a}	59.19±0.30^{a}	68.35±0.29^{a}	71.24±0.17^{b}
	ME	31.19±0.29^{a}	31.49±0.21^{a}	52.73±0.25^{a}	76.45±0.38^{b}	81.79±0.25^{b}
	WE	73.04±0.40^{e}	74.21±0.26^{d}	76.07±0.39^{d}	77.05±0.21^{b}	80.38±0.26^{b}
***Vitex negundo* leaves**	ME	60.96±0.25^{c}	67.61±0.21^{c}	69.25±0.29^{b}	70.88±0.21^{b}	71.47±0.21^{a}
	WE	42.21±0.40^{a}	47.01±0.30^{a}	52.42±0.23^{a}	54.36±0.42^{a}	55.44±0.34^{a}

Values with same superscript in a column were not significantly different (p>0.05) different from each other.
CE- Chloroform extract; ME-Methanol extract; WE-Water extract

Table.10 *In-vitro* anti-inflammatory activity of different extracts of selected medicinal plants by protease inhibition method

Name of plant/Std.	Type of extract	Percent inhibition at Concentration (µg/mL)				
		100	200	300	400	500
Aspirin	**Std.**	63.93±1.24^{b}	70.99±0.76^{b}	78.44±0.47^{b}	82.28±0.51^{c}	84.79±0.44^{c}
***Adansonia digitata* leaves**	CE	47.72±0.25^{b}	48.62±0.37^{b}	53.39±0.21^{a}	54.54±0.15^{a}	55.58±0.36^{a}
	ME	44.17±0.30^{b}	64.79±0.20^{b}	74.94±0.10^{b}	80.86±0.05^{b}	84.1±0.02^{c}
	WE	56.59±0.21^{b}	70.4±0.10^{b}	77.29±0.07^{b}	83.53±0.28^{c}	85.47±0.01^{c}
***Flueggea leucopyrus* leaves**	CE	51.84±0.32^{b}	53.40±0.39^{b}	53.90±0.51^{a}	54.49±0.39^{a}	54.89±0.28^{a}
	ME	71.24±0.15^{c}	81.64±0.07^{d}	86.78±0.03^{c}	89.35±0.01^{d}	91.94±0.01^{d}
	WE	59.99±0.33^{b}	72.23±0.10^{b}	78.28±0.07^{b}	82.43±0.05^{c}	85.65±0.02^{c}
***Peltophorum pterocarpum* bark**	ME	49.36±0.41^{b}	61.43±0.23^{b}	67.17±0.14^{b}	73.16±0.11^{b}	77.69±0.09^{b}
	WE	61.99±0.29^{b}	75.53±0.09^{c}	82.21±0.04^{c}	87.16±0.03^{d}	89.06±0.04^{d}
***Punica granatum* fruit epicarp**	ME	41.21±0.55^{b}	62.18±0.19^{b}	71.48±0.13^{b}	75.34±0.11^{b}	81.07±0.04^{b}
	WE	57.23±0.25^{b}	68.79±0.16^{b}	79.04±0.07^{b}	82.48±0.05^{c}	82.76±0.04^{c}
***Solanum xanthocarpum* aerial part**	CE	48.02±0.31^{b}	52.53±0.43^{b}	54.22±0.19^{a}	56.6±0.17^{a}	58.6±0.28^{a}
	ME	43.05±0.37^{b}	49.32±0.20^{b}	53.73±0.19^{a}	57.98±0.20^{a}	60.65±0.18^{a}
	WE	62.29±0.20^{b}	65.59±0.93^{b}	64.62±0.14^{b}	65.29±0.11^{a}	67.41±0.09^{a}
***Vitex negundo* leaves**	ME	49.68±0.35^{b}	64.96±0.17^{b}	76.2±0.12^{b}	76.72±0.08^{b}	78.55±0.05^{b}
	WE	12.92±0.87^{a}	24±0.67^{a}	32.38±0.52^{a}	41.3±0.32^{a}	45.97±0.33^{a}

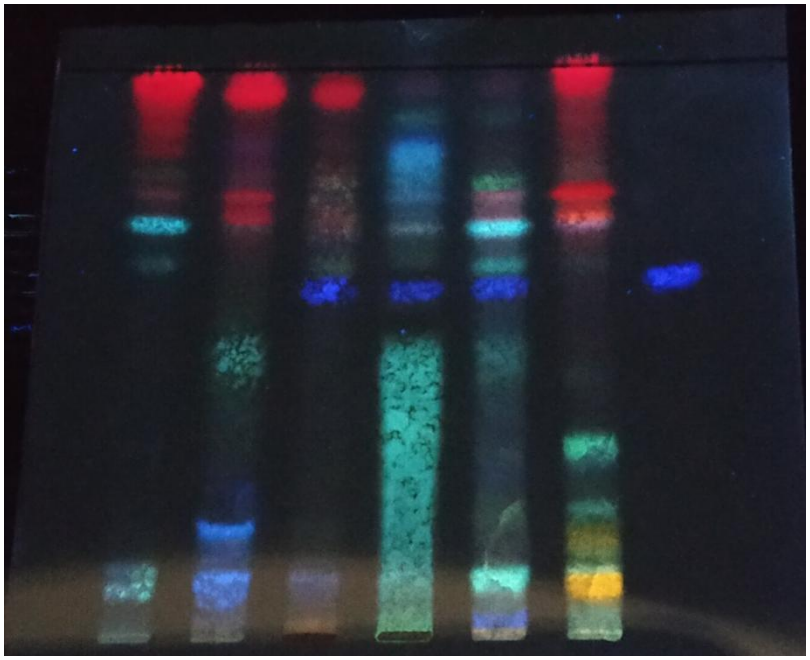
Values with same superscript in a column were not significantly different (p>0.05) different from each other.
CE- Chloroform extract; ME-Methanol extract; WE-Water extract

Table.11 Rf value of each plant extracts and gallic acid

Name of plant and standard	Rf values*
Adansonia digitata leaves	0.075, 0.1, 0.71
Flueggea leucopyrus leaves	0.11, 0.18, 0.72, 0.96
Peltophorum pterocarpum bark	**0.6**, 0.73, 0.96
***Punica granatum* aerial part**	**0.6**, 0.7, 0.82, 0.9
***Solanam xanthocarpum* fruit pericarp**	0.03, 0.1, **0.6**, 0.62, 0.68, 0.78,0.91
Vitex negundo leaves	0.05, 0.08, 0.175, 0.22, 0.26, 0.71,0.76,0.97
Gallic acid	**0.6**

* The bold values in plant extracts are matching with standard gallic acid

Plate.1 Thin-Layer Chromatography of selected medicinal plants showed *in vitro* anti-inflammatory activity (1-*Adansonia digitata* leaves; 2-*Flueggea leucopyrus* leaves; 3-*Peltophorum pterocarpum* bark; 4-*Punica* granatum fruit epicarp; 5-*Solanam xanthocarpum* aerial part; 6-*Vitex negundo* leaves; 7-Gallic acid)



1 2 3 4 5 6 7

It is chemically a trihydroxybenzoic acid. Gallic acid is responsible for various pharmacological actions like antioxidant, anticancer, astringent *etc*. The gallic acid was identified at Rf value 0.6 in the plant extracts which was matched with reference standard compound (Table 11). The presence of phenolic compounds like gallic acid might be the reason of *in-vitro* anti-inflammatory action of few plants among tested varieties of plants.

In conclusion, the results of present study indicated that the extract of *Adansonia digitata* leaves, *Flueggea leucopyrus* leaves, *Solanum xanthocarum* aerial part, *Adansonia digitata* leaves, *Flueggea leucopyrus* leaves and *Punica granatum* epicarp possess promising anti-inflammatory effect against protein denaturation and proteinase inhibitors. The presence of flavonoids and related polyphenols may be responsible for the activity. Base on this study was investigated the anti-inflammatory activity from natural sources with more potent and less side effects. Therefore, it can be used as a natural source of inflammatory agents. Further research is required to invention active component of the plant extract and their mechanism of action.

References

Alexandrina, L. D. 2010. Antibiotics and Antiseptics in Periodontal Therapy. Berlin/Heidelberg, Springer Verlag.

Alhakmani, F., Khan, S. A. and Ahmad, A. 2014. Determination of total phenol, *in vitro* antioxidant and anti-inflammatory activity of seeds and fruits of *Zizyphus spina-christi* grown in Oman. *Asia Pacific J. Trop. Biomed.* 4(2): S656-S660.

Boursions, L.A., Karachalios, T., Poultsides, L. and Malizos, K.N. 2009. Do steroids, conventional non-steroidal anti-inflammatory drugs and selective Cox-2 inhibitors adversely affect fracture healing? *J. Musculoskelet Neuronal Interact*. 9(1): 44-52.

Chick, H, Martin, C.J. 1910. On the heat coagulation of protein. *J Physiol.* 4: 404-430.

Coughlin, S.R. 2000. Thrombin signaling and protease-activated receptors. *Nature*. 407: 258.

Das, S. N. and Chatterjee, S. 1995. Long term toxicity study of ART-400. *Indian Indg Med.* 16 (2): 117-123.

Dharmalingam, S. R., Chidambaram, K., Ramamurthy, S. and Nadaraju, S. 2014. Effects of nanosuspension and inclusion complex techniques on the *in vitro* protease inhibitory activity of naproxen. *Brazilian J Pharm Sci*. 50(1): 165-171.

Gerard, J. T. and Sandra R. 1993. Principles of Anatomy and Physiology. Harper Collins College Publishers, 7th edition: pp 695.

Gerard, J. T. and Sandra, R.G. 2000. Principles of anatomy and physiology (9th ed). San Francisco Benjamin Cummings.

Grant, N. H., Alburn, H. E. and Kryzanauska, C. 1970. Stabilization of serum albumin by anti-inflammatory drugs. *Biochem Pharmacol*. 19: 715–22.

Harborne, A. J. 1998. Phytochemical methods-A guide to modern techniques of plant analysis. Springer science, 3rd edn.

Ingle, P. V. and Patel, D. M. 2011. C-reactive protein in various disease condition – an overview. *Asian J Pharm Clin Res.* 4(1): 9–13.

Khare, C. P. (2007). Indian Medicinal Plants: An Illustrated Dictionary. 1st Edn. Springer-Verlag New York.

Leelaprakash, G. and Mohan, D. S. 2010.*In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*. *International Journal of Drug Development and Research*. 3:189- 196.

Mann, G. 1906. Chemistry of the proteins, London and New York. Pp. 336-344.

Mizushima, Y. 1966. Screening test for anti-rheumatic drugs. *Lancet*. 2: 443.

Roach, J.T. and Sufka, K. J. 2003. Characterization of the chick carrageenan response. *Brain Res.* 994: 216–25

Robertson, T. B. 1918. The physical chemistry of the proteins, New York and London.

Sakat, S., Juvekar, A. R. and Gambhire, M. N. 2010. In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. I. *J Pharm Sci.* 2(1): 146- 155.

Sengupta, R., Sheorey, S. D. and Hinge, M. A. 2012. Analgesic and anti-inflammatory plants: An updated review. *Int J Pharm Sci Rev Res*. 12(2): 114-119.

Shah, M., Patel, H. and Raj, H. 2017. Methods for the estimation of ellagic acid and curcumin in antidiabetic herbal formulations – A Review. *Eurasian journal of analytical chemistry*, 12 (4), 295-311.

Vane, J. R. and Botting, R. M. 1995. New insights into the mode of action of anti-inflammatory drugs. *Inflammation Research.* 44(1): 1-10.

Williams, L., O'Connar, A., Latore, L., Dennis, O., Ringer, S., Whittaker, J. A., Conrad, J., Vogler, B., Rosner, H. and Kraus, W. 2008. The *in vitro* Anti-denaturation Effects Induced by Natural Products and Non-steroidal Compounds in Heat Treated (Immunogenic) Bovine Serum Albumin is proposed as a screening

assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *West Indian Med J*. 57 (4): 327-331.

How to cite this article:

Chirag M. Modi, Punit R. Bhatt, Kajal B. Pandya, Harshad B. Patel and Urvesh D. Patel. 2019. Comparative Evaluation of *in vitro* Anti-Inflammatory Activity of Different Extracts of Selected Medicinal Plants from Saurashtra Region, Gujarat, India. *Int.J.Curr.Microbiol.App.Sci.* 8(05): 1686-1698. doi: https://doi.org/10.20546/ijcmas.2019.805.195