

# Evaluation of *in-vitro* anti-inflammatory activity of chebulinic acid from *Terminalia chebula* Linn. against the denaturation of protein

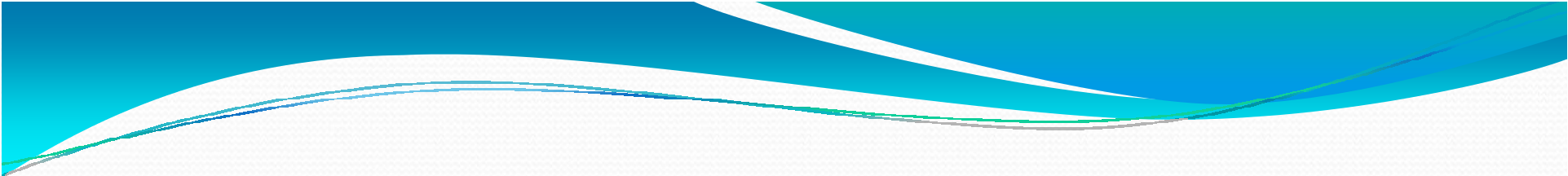
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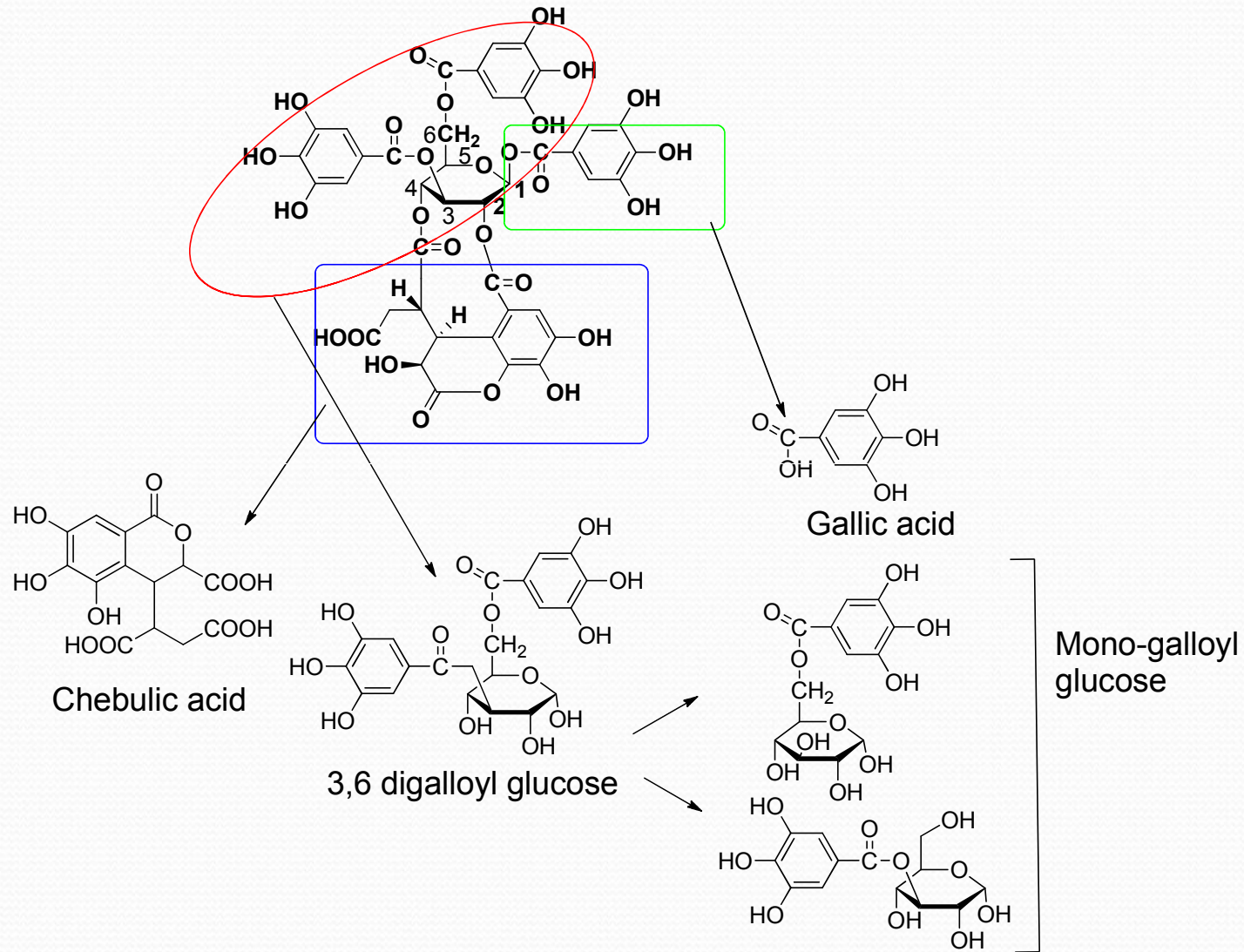
# Introduction

- *Terminalia chebula* (Combrataceae) is commonly known as *Haritaki* in India. It is found throughout India and Southeast Asia in deciduous forest and areas of light rainfall.
- It is used extensively in the preparations of many ayurvedic formulations. Chebulinic acid, chebulagic acid, chebulic acid, gallic acid, ethyl gallate and galloyl derivatives are the major phytoconstituents present in the pericarp of *Terminalia chebula* fruits .
- *Terminalia chebula* and its preparations are mainly indicated for gastrointestinal disorders

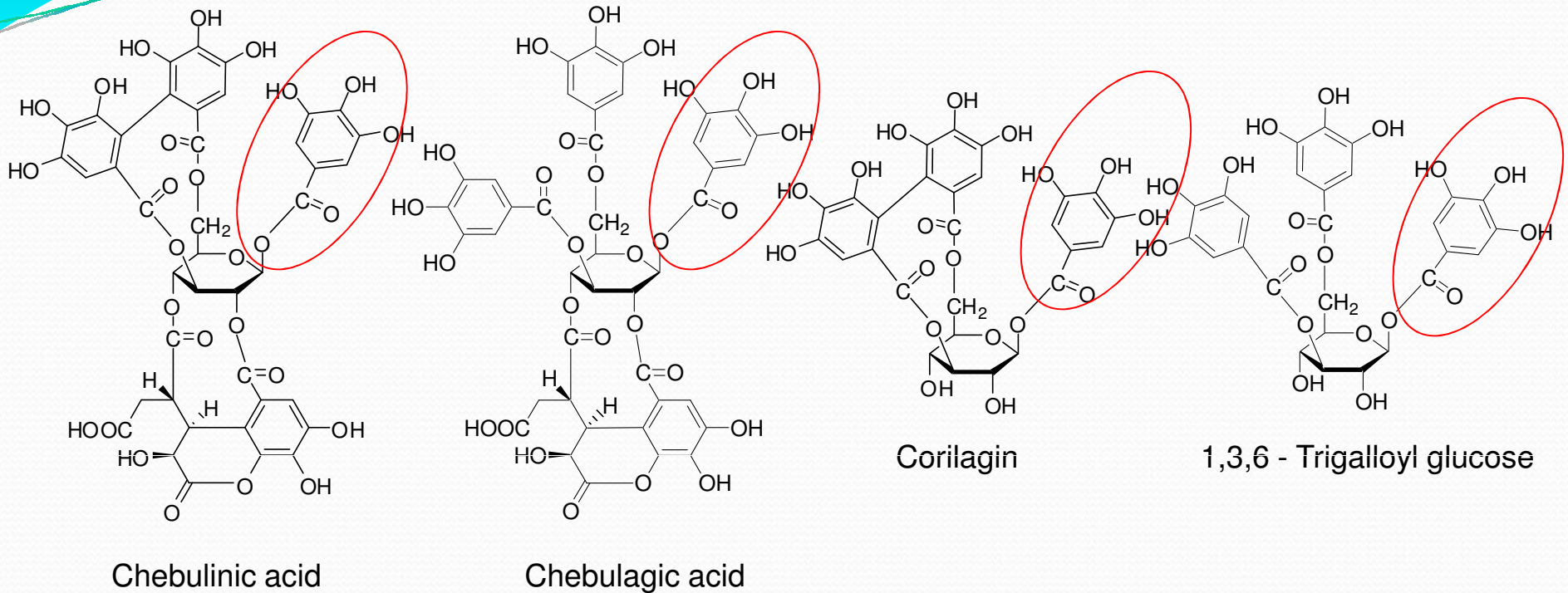
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- Its hydrolytic products are gallic acid, ellagic acid, mono and di-galloyl derivatives and chebulic acid (figure 1).
  - Mention of hydrolytic product is of importance as many of its principle preparations (ayurvedic) involve preparation of decoction of the fruit; and it is likely that during making of the formulations chebulinic acid is hydrolysed and they in turn may be biologically active.
  - Most dreaded gastro-intestinal disorders (gastric ulcers, Crohne's disease, ulcerative colitis) arise due to inflammation only.

**So, the present work is based on determination of anti-inflammatory activity of chebulinic acid *in vitro* (denaturation of protein).**

# Hydrolytic product of chebulinic acid in the formulation



# Why gallotannins are hydrolysed



- Bond formed between glucose and gallic acid is essential for hydrophobic interaction
- Contribution for hydrophobic interaction of galloyl group at anomeric carbon is larger than other galloyl groups

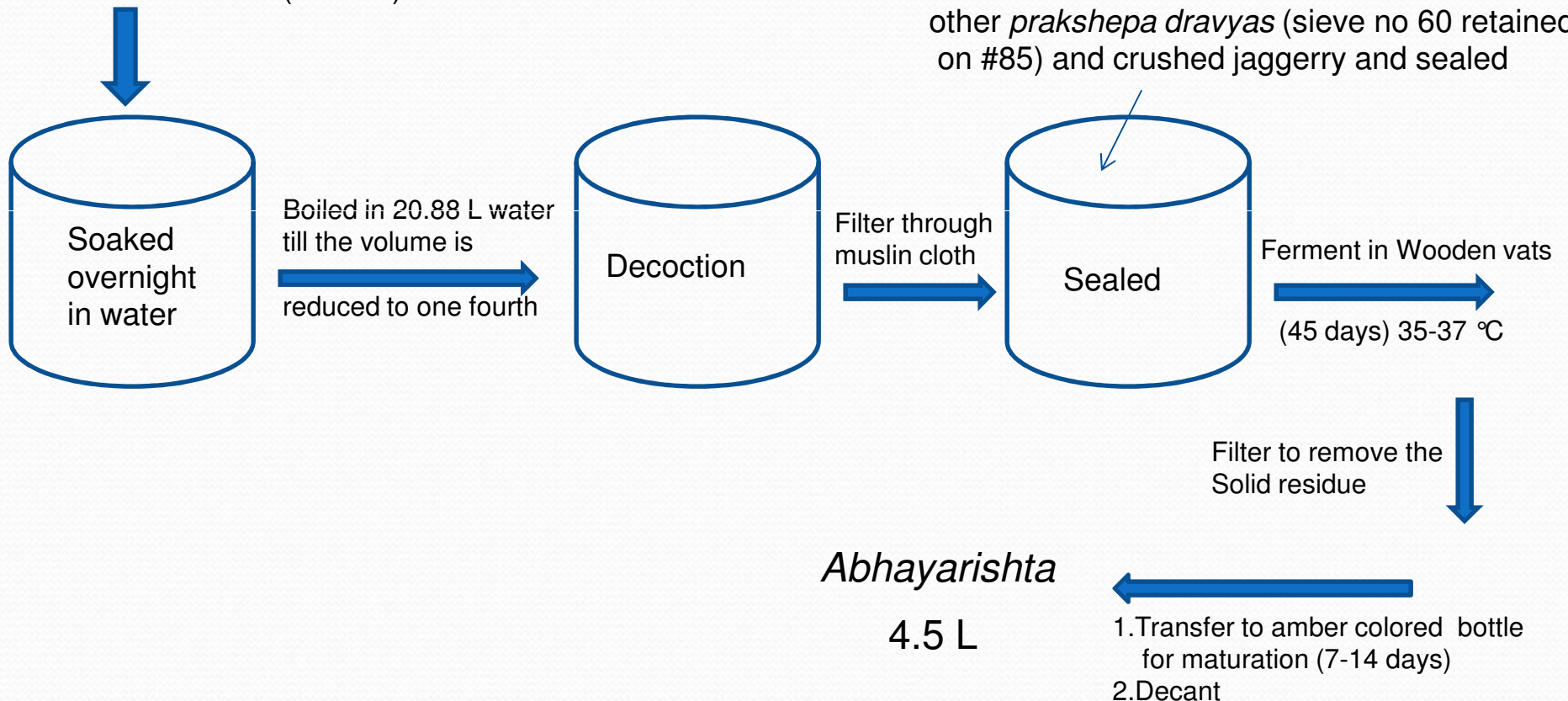
Tanaka T *et. al. Chem. Pharm. Bull.* **1997**, 45(2), 1891-1897

# Example of Preparations which involve boiling of plant material (*Abhayarishta*) along with its method of preparation

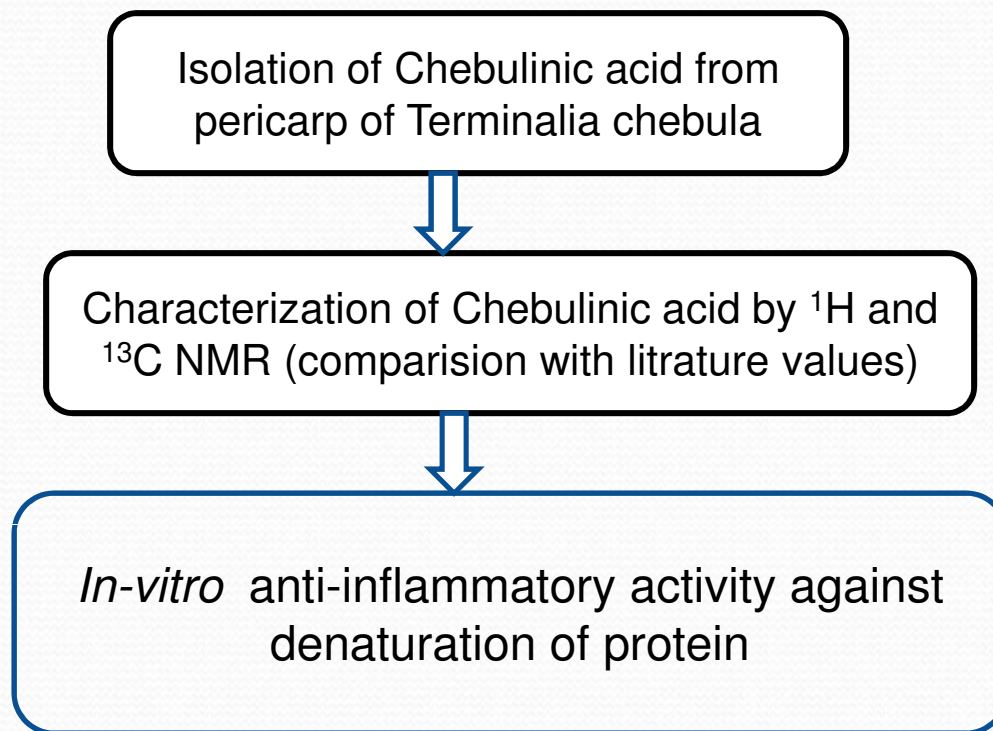
*Terminalia chebula* (pericarp)  
*Embelia ribes* } → powder passed through sieve no. 22 and retained on sieve no. 44

*Vitis vinifera* (fruits) → cut into two parts  
*Madhuca indica* (flowers) → as such

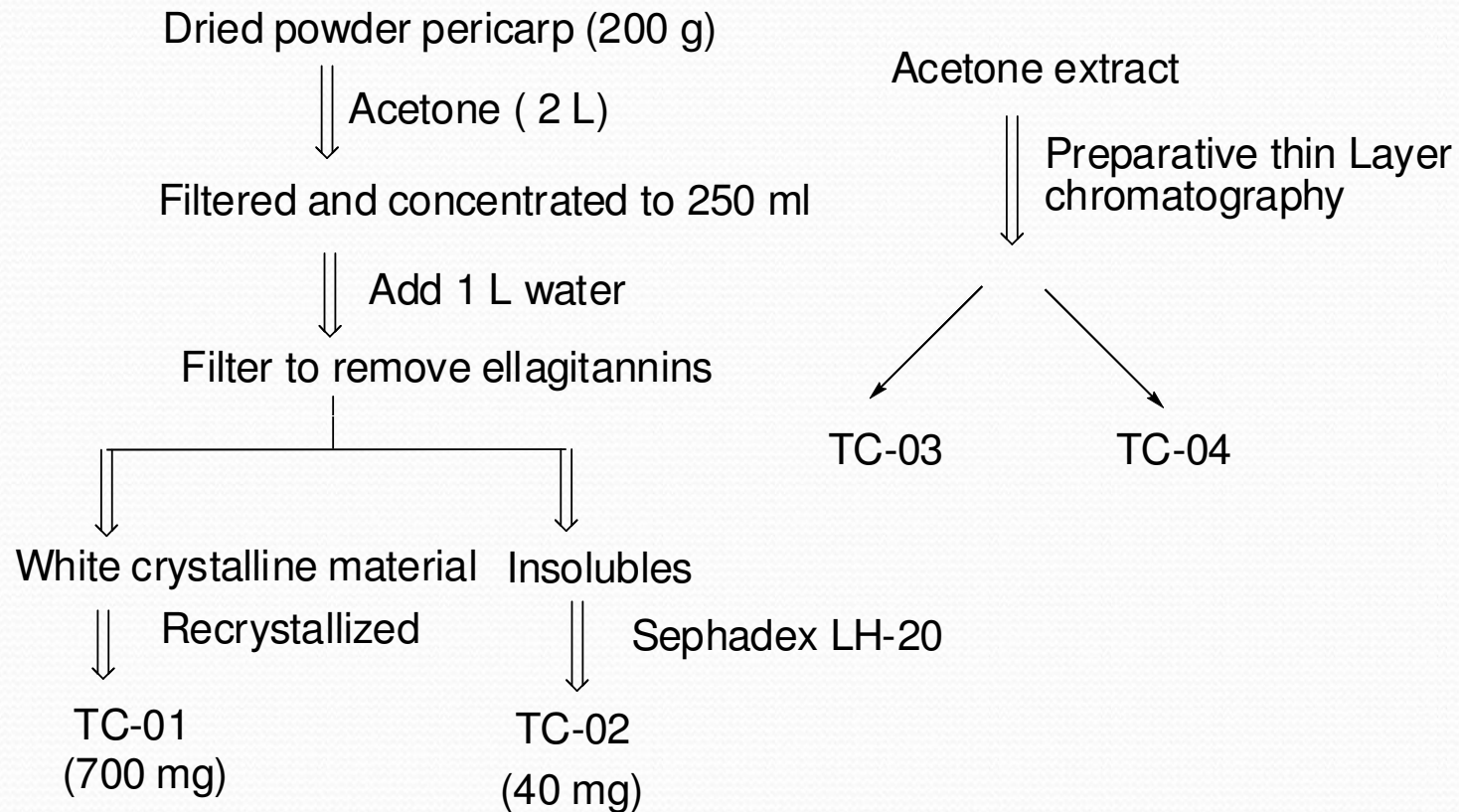
*Woodfordia fruticosa* flowers,  
other *prakshepa dravyas* (sieve no 60 retained on #85) and crushed jaggery and sealed



# Methodology



# Isolation of marker constituents from *Terminalia chebula*





## TC-01 (Chebulinic acid)

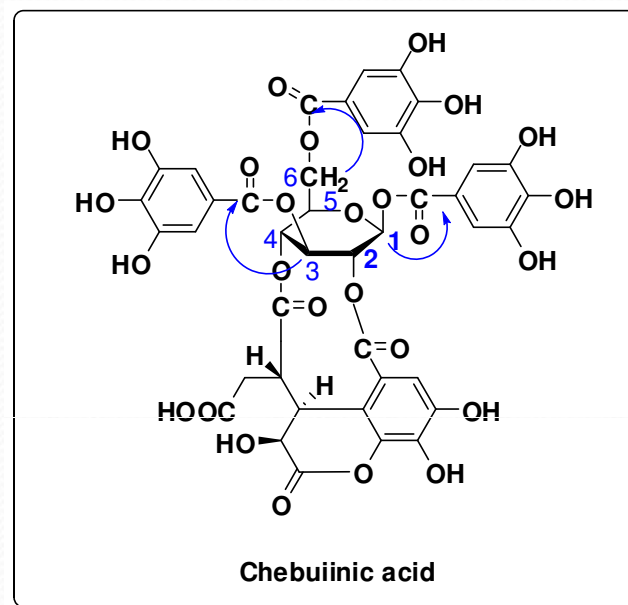
MALDI/TOF (DHB):  $m/z$   $[M+23]^+$  979

$^1\text{H}$  NMR (300 MHz, DMSO ( $d_6$ ),  $\delta$  ppm)

7.26 (s, 1H), 6.90, 6.87, 6.77 (s, 2H), 6.20 (1H, d,  $J = 2.8$ ), 5.93 (1H, d,  $J = 3.6$ ), 5.11 (1H, d,  $J = 8.0$ ), 4.82 (1H, d,  $J = 3.6$ ), 4.67 (1H, d,  $J = 8.0$ ), 4.49 (m), 4.27 (1H, m), 4.31 (1H, m), 3.6 (1H, m), 1.8 (1H, m), 1.93 (1H, m)

$^{13}\text{C}$  NMR (75 MHz, DMSO ( $d_6$ ),  $\delta$  ppm)

173.2, 172.5, 169.5, 165.6, 164.5, 164.1, 162.0, 146.1, 145.9, 145.8, 145.7, 140.5, 139.8, 139.9, 118.9, 117.7, 116.3, 115.8, 115.0, 109.2, 108.8, 91.9, 76.0, 72.3, 68.6, 65.2, 64.3, 62.2



Haslam, E. and Uddin, M. *J. Chem. Soc. (C)* **1967**, 2381-2384  
Klika, K.D. *et. al. Arkivoc* **2004**, 7, 83-105

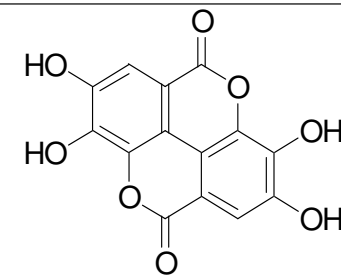
## TC-02

Light yellow powder (40 mg)

MALDI/TOF (DHB):  $m/z$  302 [M<sup>+</sup>]

<sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>,  $\delta$  ppm): 7.44 (s)

<sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>,  $\delta$  ppm) 160.0, 148.9, 140.4, 137.2, 113.2, 111.1, 108.5



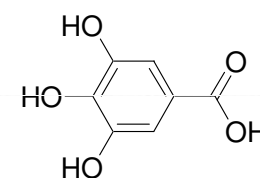
Ellagic acid

## TC-03

Off white solid (30 mg)

Positive test with NP-PEG reagent

Co-TLC with standard Gallic acid



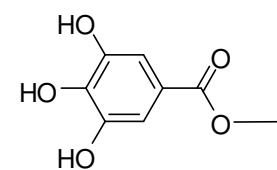
Gallic acid

## TC-04

Brown solid (20 mg)

Positive test with NP-PEG reagent

Co-TLC with standard Ethyl gallate



Ethyl gallate

# Evaluation of in vitro anti-inflammatory activity

- The 5 ml of reaction mixture consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate buffered saline (PBS, pH 7.4) and 2 ml of different concentrations of chebulinic acid so as to obtain the final concentrations.
- Equal volume of triple distilled water served as control. After that the mixtures were incubated at  $(37\pm 2)$  °C in a BOD incubator for 30 minutes and heated at 70°C for 15 minutes.
- After cooling, the absorbance was measured at 280 nm by UV spectrophotometer by using vehicle as blank and the viscosity was determined by using Ostwald Viscometer. Same procedure was followed for the standard solutions. The percentage inhibition of protein denaturation was calculated by using the following formula:

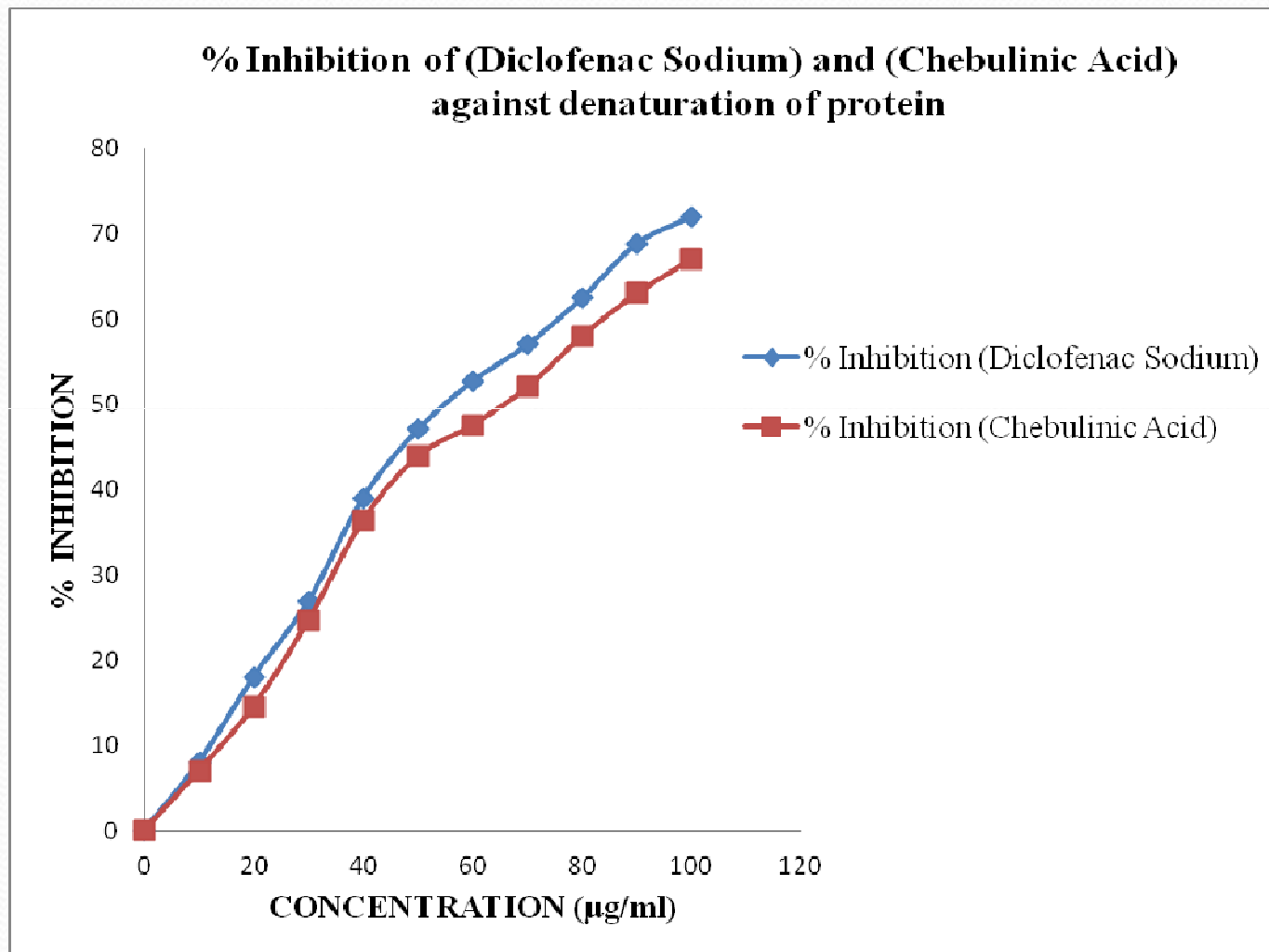
$$\% \text{ inhibition} = (Vt / Vc - 1) \times 100$$

- Where,  $Vt$  = absorbance of test sample,  $Vc$  = absorbance of control. The extract/drug concentration for 50% inhibition ( $IC_{50}$ ) was determined by plotting percentage inhibition with respect to control against treatment concentration.

**Table (1): Effect of Diclofenac Sodium and Chebulinic Acid on protein denaturation.**

S. No.	Concentration (µg/ml)	Effect of diclofenac sodium on protein denaturation		Effect of chebulinic acid on protein denaturation	
		% inhibition	Viscosity (cp)	% inhibition	Viscosity (cp)
1	0.00	0.00	0.00	0.00	0.00
2	10.0	8.00	0.54	7.00	0.49
3	20.0	18.02	0.60	14.58	0.57
4	30.0	26.90	0.66	24.65	0.62
5	40.0	39.04	0.72	36.42	0.68
6	50.0	47.04	0.76	43.92	0.72
7	60.0	52.71	0.79	47.61	0.76
8	70.0	57.04	0.84	52.04	0.82
9	80.0	62.52	0.88	58.04	0.85
10	90.0	68.86	0.92	63.04	0.89
11	100.0	72.04	0.98	67.04	0.94

**Figure 2:** Percentage inhibition of Diclofenac Sodium and Chebulinic acid against denaturation of protein



## Conclusions

- From the results it is evident that chebulinic acid efficiently reduces the denaturation of protein in terms of percentage inhibition ( $IC_{50}$  - 43.92  $\mu\text{g/ml}$ ).
- The percentage inhibition was comparable with that of standard (diclofenac sodium) having  $IC_{50}$  value 47.04  $\mu\text{g/ml}$ . Our study is the first report which focuses on anti-inflammatory response of chebulinic acid against denaturation of proteins.
- Since, the results are comparable with diclofenac sodium. The plants as well as compound can be taken for further *in-vivo* studies.