Evaluation of *in vitro* Anti-Inflammatory Activity of Pirandai Uppu Using Inhibition of Protein Denaturation Method

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Abstract

Background: Pirandai uppu (salt derived from *Cissus quadrangularis*) is used in the treatment of ailments like hemorrhoids and dysmenorrhea. Inflammatory reactions are evident in both cases. Prostaglandin plays significant role in the pathomechanism of Dysmenorrhea. It is responsible for the generation of an inflammatory response. Several inflammatory markers such as prostaglandin F2 alpha, interleukin-6, and vasopressin are involved in Dysmenorrhea. Inflammatory markers such as PGF2α, IL-6, vasopressin, VEGF are found in higher concentrations in case of Dysmenorrhea. Hemorrhoidal specimens are found to have a severe inflammatory reaction involving the vascular wall and surrounding connective tissue. This inflammatory process results in the vulnerability of the arterioles causing erosion during defecation causing hemorrhage. Pirandai uppu is effective in the treatment of such conditions involving inflammatory pathways. Hence, strongly suggesting that it could have potent anti-inflammatory activity.

Objective:

To prove the *In vitro* anti-inflammatory activity of Pirandai uppu by Inhibition of albumin denaturation and Inhibition of protein denaturation method.

Method:

The *invitro* anti-inflammatory assay consists of assaying the effect of extracts of Pirandai uppu against protein denaturation of protein (egg albumin and serum albumin) and measuring the absorbance. The percentage inhibition of protein denaturation is calculated and compared with the obtained result of Standard -Diclofenac sodium.

Results and Conclusion:

Based on the results of the percentage of Inhibition of denaturation of protein obtained by calculations using the absorbance it is concluded that Pirandai uppu has good anti-inflammatory activity. The percentage inhibition of 200µg/mL of Pirandai uppu obtained by egg albumin method and bovine serum albumin method was found to be 3.8 and 1.22 times respectively greater than that of 200µg/mL aqueous stem extract of *Cissus quadrangularis*.It can be concluded that the process of deriving concentrated salts from medicinal herbs devised by Siddhars is of immense scientific significance.As denaturation of tissue protein is one of the causes of inflammatory and arthritic diseases the results of our study reveal that Pirandai uppu exerts potent anti-inflammatory activity and can be used to effectively treat inflammatory and arthritic conditions.

Keywords: Pirandai uppu, Anti-inflammatory, Cissus quadrangularis, Dysmenorrhea

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Introduction:

Inflammation is defined as the local protective response of the body due to disturbance in homeostasis, injury, invasion caused by an agent[1].Acute inflammation is of short duration which resolves quickly followed by healing process[2].Controlled inflammatory response is beneficial and plays a crucial role in our health. If it is unresolving it leads to the development of chronic inflammation and can become inimical if not regulated[3].Chronic inflammation is associated with several diseases like rheumatoid arthritis, cancer, inflammatory bowel disease, allergic asthma, chronic obstructive pulmonary disease, cardiovascular diseases etc[4]. It has been mentioned in Siddha text that Pirandai uppu (salt derived from *Cissus quadrangularis*) can be used to cure many ailments like stomach ache, hemorrhoids, dysentery, dysmenorrhea, skin diseases etc[5].These ailments are all associated with inflammatory mechanisms. The processes starting from ovulation upto menstruation involves inflammation which in this case is the inflow of immune cells into the reproductive tissue[6]. Prostaglandin plays significant role in the pathomechanism of Dysmenorrhea. It is responsible for the generation of an inflammatory response [7] several inflammatory markers such as prostaglandin F2 alpha, interleukin-6, vasopressin are involved in Dysmenorrhea. Inflammatory markers such as PGF2α, IL-6, vasopressin, VEGF are found in higher concentrations in case of Dysmenorrhea[8].Hemorrhoidal specimens are found to have a severe inflammatory reaction involving the vascular wall and surrounding connective tissue. This inflammatory process results in the vulnerability of the arterioles causing erosion during defecation causing hemorrhage [9].Based on literature evidence it can be said that Pirandai uppu could have good anti-inflammatory activity.

Aim & Objective:

To prove the Invitro anti-inflammatory activity of Pirandai uppu by Inhibition of albumin denaturation and Inhibition of protein denaturation method.

Materials and Methodology:

Anti-inflammatory Activity - Egg Albumin Denaturation Assay

The egg albumin denaturation assay according to the procedure outlined by Mizushima and Kobayashi [10] with minor modification. Sample of different concentrations is mixed with 0.5 mL of egg albumin and 1 mL of phosphate buffer (pH = 6.5) saline. Incubation is done for at 37 °C for twenty minutes. Then heating is done for fifteen minutes at 70°C. Absorbance is measured after cooling to room, temperature and the percentage of inhibition is calculated.[Fig1]

Anti-inflammatory Activity - Bovine serum albumin Denaturation Assay

The assay was performed by following the procedure reported by Anoop and Bindu [11] with minor modification. Various concentrations of the sample was mixed in 0.5 ml BSA (0.5% w/v). Then it is incubated at 37 °C for twenty minutes.

The temperature is then increased to 60°C for 3 minutes. After cooling, 1 ml of phosphate buffer is added to the above solutions. The absorbance was measured and percentage of inhibition is calculated [Fig 2].

Results and Discussion:

200µg, 100µg, 75 µg, 50µg of Pirandai uppu was found to have 53.40909%, 34.09091%, 19.31818%, 9.090909% percentage of inhibition in egg albumin denaturation assay and 46.05263%, 27.63158%, 17.10526%, 6.578947% percentage of inhibition in Bovine serum albumin denaturation assay. Based on these results it is concluded that Pirandai uppu has good anti-inflammatory activity. Inhibition of denaturation of protein is a good method to establish anti-inflammatory activity.Stem extract of *Cissus quadrangularis* has been proven to have good anti-arthritic activity [12].Denaturation of tissue proteins is one of the main causes of inflammatory and arthritic diseases. It results in the production of autoantigens in arthritic conditions [13]. The percentage inhibition of 200µg/mL of Pirandai uppu obtained by egg albumin method and bovine serum albumin method was found to be 3.8 and 1.22 times respectively greater than that of 200µg/mL aqueous stem extract of *Cissus quadrangularis*.It can be concluded that the process of deriving concentrated salts from medicinal herbs devised by Siddhars is of immense scientific significance and that Pirandai uppu can be used in the effective treatment of inflammatory and arthritic conditions.

Figure 1. Egg Albumin Denaturation Assay

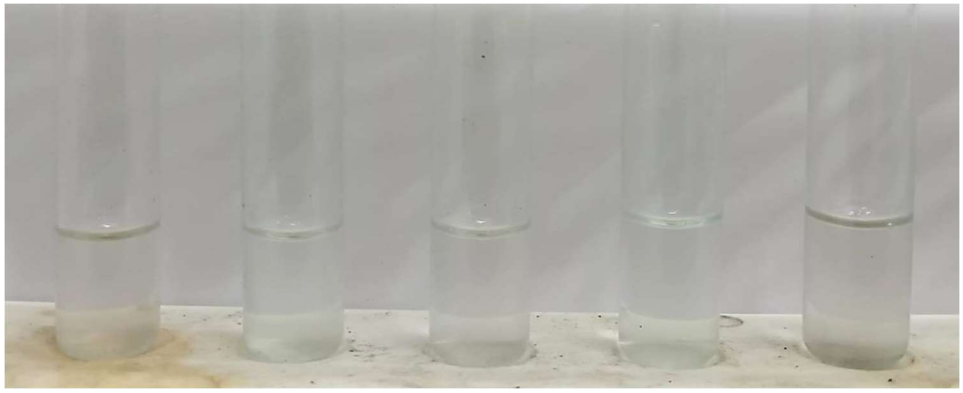
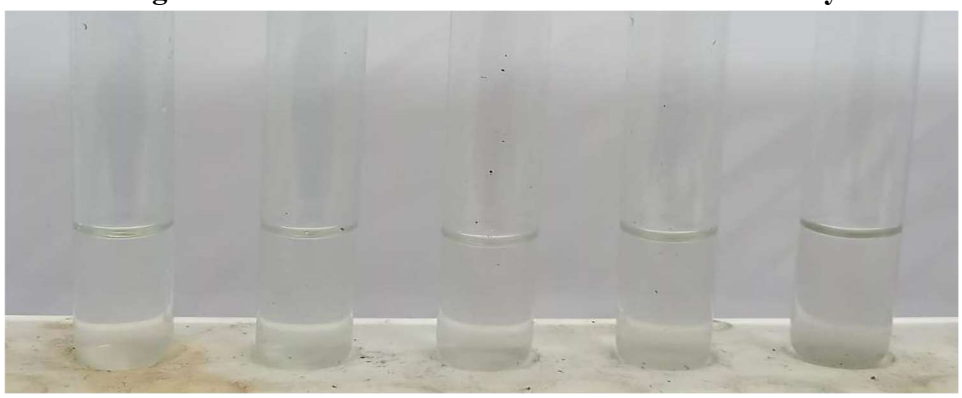


Figure 2. Bovine serum albumin Denaturation Assay



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