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Original Article

In vitro Anti-Arthritic and Anti-Inflammatory Activity of Siddha Formulation Pirandai Uppu

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ABSTRACT

Background: Siddha medicine uses unique herbal salts like Pirandai Uppu, derived from *Cissus quadrangularis*, for inflammatory conditions. As a concentrated form, it is expected to be richer in bioactive constituents than the raw plant. **Objectives:** To establish the potential of Pirandai Uppu as an anti-arthritic and anti-inflammatory drug through *in vitro* studies. **Materials and Methods:** Anti-inflammatory activity was assessed using the HRBC membrane stabilization method. Anti-arthritic activity was evaluated via the inhibition of egg albumin and bovine serum albumin denaturation methods. **Results:** Pirandai Uppu showed concentration-dependent activity in all assays. In the HRBC method, hemolysis decreased as concentration increased. In protein denaturation assays (Egg and Bovine serum albumin), the percentage of inhibition increased with higher concentrations of the formulation. **Conclusion:** Pirandai Uppu possesses significant anti-inflammatory and anti-arthritic activity comparable to the standard drug Diclofenac sodium, making it a promising candidate for clinical trials**.**

Keywords: Anti-arthritic, Anti-inflammatory, *Cissus quadrangularis*, *Pirandai Uppu*, Protein denaturation, Siddha.

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INTRODUCTION

Siddha system of medicine is one of the most ancient systems of medicine and possesses several polyherbal, herbomineral and metallomineral preparations. Herbal formulations are generally safe and highly effective. Medicinal herbs are rich in bioactive constituents which attributes to its pharmacological properties. Herbal salts is a unique concept of Siddha system of medicine which involves the process of acquiring concentrated herbal salts rich in active principles from the medicinal herb. Thus, the medicine in the form of herbal salt delivers the desired pharmacological action in the best manner. *Pirandai Uppu* is one such herbal salt derived from *Cissus quadrangularis* which can be used for the treatment of conditions involving inflammatory mechanisms. *Cissus quadrangularis* is a perennial climber belonging to the family Vitaceae (Hamid & Patil, 2023). It is rich in various chemical constituents including triterpenoids like friedelin, flavonoids like quercetin, phytosterols like β- sitosterol, Vitamin C and Calcium. Further, the stem of *Cissus quadrangularis* is found to contain tannins, phenols, carotene, calcium and phosphorus (Sundaran *et al*., 2020). Pharmacological studies have revealed that it has anti-inflammatory, anticonvulsant, antimicrobial, anticancer, analgesic and anti-osteoporotic activity (Bafna *et al*., 2021). Animal studies also indicate that it reduces osteoclastic activity and facilitates bone formation. This property could be due to the phytogenic steroids present in it (Potu *et al*., 2009). Results of *in vitro* studies have proved that *Cissus quadrangularis* exhibits potent anti-inflammatory and anti-arthritic activity (Gopinathan *et al*., 2015).

Pirandai Uppu which is the concentrated form of the plant will be rich in bioactive constituents and pharmacological properties than the plant itself. It could serve as a promising anti-arthritic and anti-inflammatory drug and also it has been mentioned in Siddha Literature that *Pirandai Uppu* can be used to treat hemorrhoids, dysmenorrhea, stomach ache, dysentery and skin diseases (Innasimuthu, 2016). These ailments are all associated with inflammatory mechanisms. Generally Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are used to treat inflammatory and arthritic conditions. However, they cause several adverse effects such as gastric ulcer, renal dysfunction, myocardial infarction, thromboembolism and thrombocytopenia (Ghlichloo & Gerriets, 2023). *Pirandai Uppu* is safe when taken in the prescribed manner.

Inflammation is defined as the biological response of the body to harmful stimuli, which include pathogens, radiation or toxic

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compounds. It is a defence mechanism mediated by the immune system (Chen *et al.*, 2017). Lysosomal enzymes released during inflammation play a key role in the initiation and maintenance of inflammatory reactions (Brade,1980). This results in tissue injury and damage. Lysosomal enzymes can be inhibited by stabilizing the lysosomal membrane. Generally, the ability of a substance to act as an anti-inflammatory drug is tested and determined based on its capacity to stabilize the lysosomal membrane, thus inhibiting the lysosomal enzymes (Smith *et al.*, 1976). As the Human Red Blood Cell (HRBC) membrane is similar to the lysosomal membrane, the stabilization of the erythrocyte membrane is taken as a method for estimating the anti-inflammatory activity of a drug (Saleem *et al.*, 2011). Denaturation of protein is the process that involves the loss of the secondary and tertiary structure of the protein as a result of stress due to heat or any chemical compound (Leelaprakash & Mohan, 2011). It is often related to inflammatory response and results in inflammatory conditions like arthritis (Osman *et al.*, 2016). Inhibition of protein denaturation by a potential drug indicates that it has promising anti-arthritic activity. This can be proved by the *in vitro* egg albumin denaturation assay and bovine serum albumin denaturation assay.

MATERIALS AND METHODS

Anti-inflammatory study by HRBC membrane stabilization method

Blood sample of 1 mL is freshly collected after obtaining informed consent and mixed with an equal amount of Alsever solution. Alsever solution is added to prevent coagulation of blood and is composed of 2% dextrose, 0.8% Sodium citrate, 0.5% Citric acid and 0.42% Sodium chloride. Sample is then centrifuged at 3,000 rpm for 5 min. Supernatant was discarded and the lower layer containing RBC is collected. 0.85% NaCl (isosaline) is used to wash the RBC's and centrifuged at 3000 rpm for 5 min. 1 mL of RBC's is collected, and 9 mL of isosaline solution is added. The solution obtained is HRBC (Human Red Blood Cell). Varying concentrations of the sample is taken in test tubes and by using distilled water it is made up to 1 mL. 1 mL of phosphate buffer, 2 mL of hyposaline (0.36% NaCl) and 0.5 mL of HRBC suspension was added to each tube. The test tubes were then incubated at 37 °C for 30 min and are centrifuged at 3,000 rpm for 20 min. Supernatant was collected, and its absorbance was read at 560 nm. Diclofenac (1 mg/mL) was used as standard and a control was prepared without sample. The percentage (%) of HRBC stabilization was calculated using the following formula:

$$\text{Percentage of Hemolysis (\%)} = \frac{\text{OD of sample}}{\text{OD of Control}} \times 100$$

$$\text{Percentage of Stabilization (\%)} = 100 - \frac{\text{OD of sample}}{\text{OD of Control}} \times 100$$

Anti-arthritic study by Egg albumin denaturation method

Different concentration of samples was taken in all the test tubes and made up to 1 mL using phosphate buffer saline (pH 6.4). 4 mL of egg albumin was added to all the tubes including control tube. Then the test tubes were incubated at 37°C for 15 min and further heated at 72°C for 5 min. After incubation, samples and control were allowed to cool for 10 min. Following this, centrifugation at 3,000 rpm for 20 min was performed, and the clear supernatant is collected. The absorbance of the supernatant was measured at 660 nm. Diclofenac (1 mg/mL) was used as a standard, and a control was prepared without sample. The percentage inhibition of protein denaturation (%) was calculated using the following formula:

$$\text{\% of Inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Anti-arthritic study by Bovine serum albumin denaturation method

Different concentration of samples was taken in all the test tubes and made upto1 mL using phosphate buffer saline (pH 6.4). 0.5 mL of 5% aqueous solution of Bovine albumin fraction is added. The pH (6.3) of the solution was adjusted using 0.1N HCl and mixed well. The test tubes are then incubated at 37°C for 20 min and then heated at 57°C for 3 min. After incubation samples and control were allowed to cool for 10 min, followed by centrifugation at 3,000 rpm for 20 min. The clear supernatant was collected. The absorbance was measured at 660 nm. Diclofenac (1 mg/mL) was used as standard and control was prepared without sample. The percentage inhibition of albumin denaturation (%) was calculated by using the following formula (Dragan *et al.*, 2015):

$$\text{\% of Inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Statistical analysis

The statistical significance of the results obtained was analysed by 1-way ANOVA. Pearson product-moment correlation was used to determine the relationship in the activities between various concentrations of *Pirandai Uppu* and pharmacological responses.

RESULTS

Anti-inflammatory study by HRBC membrane stabilization method

The results of the anti-inflammatory activity determined by human red blood cell membrane stabilization method is shown in Figures 1 and 2. The percentage of stabilization of *Pirandai Uppu* is found to increase with the increasing concentrations of the sample. The percentage of hemolysis is decreasing with the increasing concentrations of the sample, thus indicating that *Pirandai Uppu* has concentration-dependent potent

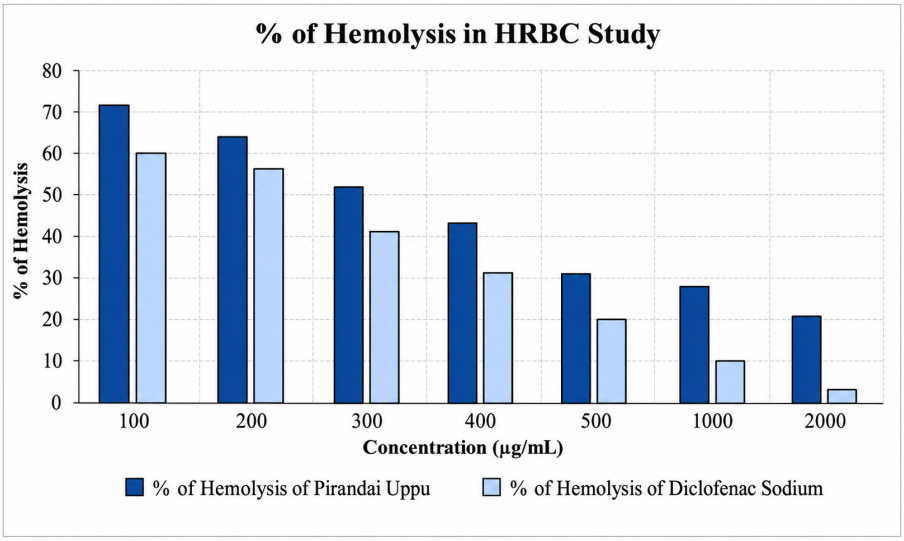


Figure 1: Hemolysis values of *Pirandai Uppu* and Diclofenac Sodium in percentage.

Table 1: Hemolysis and Stabilization values of *Pirandai Uppu* and Diclofenac sodium in percentage obtained by HRBC Method.

Concentration (µg/mL)	Hemolysis of Pirandai Uppu (%)	Hemolysis of Diclofenac sodium (%)	Stabilization of Pirandai Uppu (%)	Stabilization of Diclofenac sodium (%)
100	71.4	60.2	28.6	39.8
200	64.8	55.9	35.2	44.1
300	52.49	41.3	47.51	58.7
400	42.68	31.5	57.32	68.5
500	31.86	19.7	68.14	80.3
1000	28.44	9.82	71.56	90.18
2000	21.2	2.46	78.8	97.54

Table 2: One-way ANOVA for Anti-inflammatory study.

Source	D_f	Sum of square	Mean Square	F Statistic	*p*-value
Groups (between groups)	1	1208264.259	1208264.259	5.4522	0.03773
Error (within groups)	12	2659306.028	221608.8356		
Total	13	3867570.287	297505.4067		

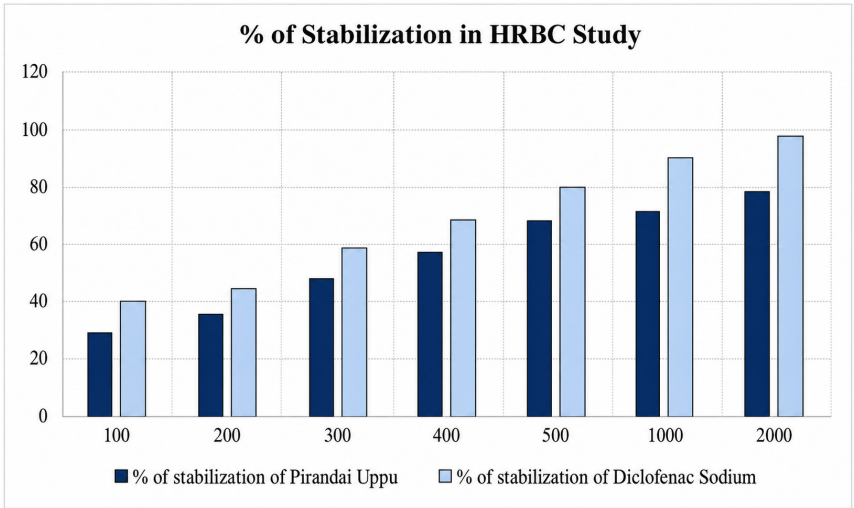


Figure 2: Stabilization values of *Pirandai Uppu* and Diclofenac Sodium in percentage.

anti-inflammatory activity. The highest and lowest percentage values of stabilization were observed at concentrations of 2000 µg/mL and 100 µg/mL, respectively. The results are tabulated in Table 1.

There is a statistically significant difference in the percentage of protection among the various concentrations of *Pirandai Uppu* in the anti-inflammatory assay, as determined by 1-way ANOVA with p=0.03773, as shown in Table 2. Since the p-value <0.05, the null hypothesis has been rejected. Results of the Pearson correlation analysis indicated a significant large positive relationship between the various concentrations of *Pirandai Uppu* and HRBC membrane stabilization activity, ($r(5)$=0.81,p=0.027), as shown in Table 3.

Table 3: Pearson correlation between *Pirandai Uppu* and HRBC membrane stabilization activity.

Parameter	Value
Pearson correlation coefficient (r)	0.8104
r^2	0.6568
p-value	0.02706
Covariance	10240.2857
Sample size (n)	7
Statistic	3.0932

Anti-arthritic study by Egg albumin denaturation method

Anti-arthritic activity of *Pirandai Uppu* is evaluated against denaturation of egg albumin method. The percentage of inhibition increased with the increasing concentrations of the sample indicating the increase in anti-arthritic activity as shown in Figure 3. The lowest value (9.09%) was observed at a concentration of 50 µg/mL, while the highest value (74.2%) was observed at a concentration of 500 µg/mL. The results are tabulated in Table 4.

There is a statistically significant difference in the percentage of inhibition among the various concentrations of *Pirandai Uppu* in the anti-arthritic assay, as determined by 1-way ANOVA with

Table 4: Inhibition percentage values of *Pirandai Uppu* and Diclofenac sodium in Egg albumin denaturation assay.

Sample Concentration (µg)	Inhibition by Pirandai Uppu (%)	Inhibition by Diclofenac sodium (%)
50	9.09	22.5
75	19.31	30.2
100	34.09	38.4
200	53.4	55.8
300	57.2	61.5
400	63.54	69.2
500	74.2	84.6

Table 5: One-way ANOVA for Anti-arthritic study by Egg albumin denaturation method.

Source	D_f	Sum of Square	Mean Square	F Statistic	*p*-value
Groups (between groups)	1	123360.1986	123360.1986	8.0289	0.01508
Error (within groups)	12	184374.6759	15364.5563		
Total	13	307734.8746	23671.9134		

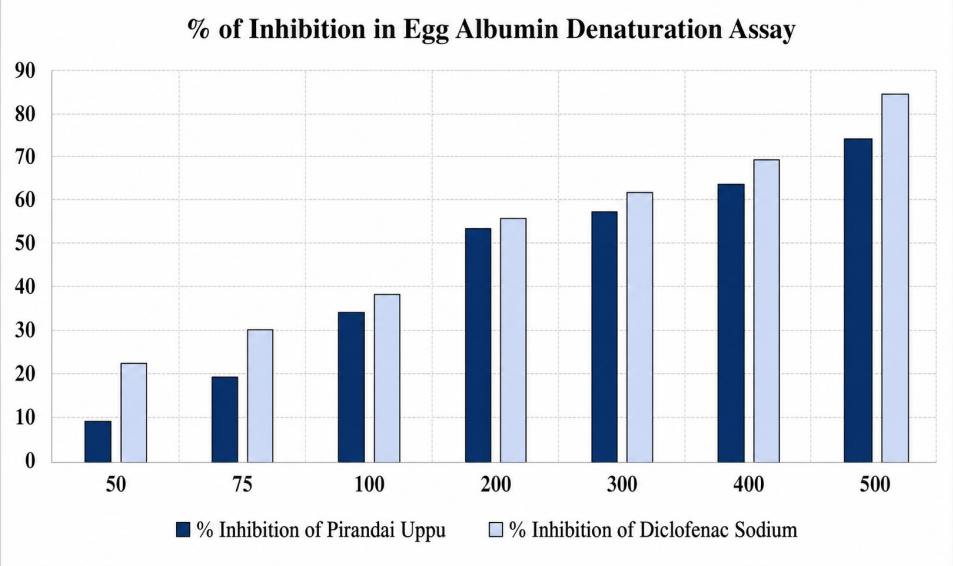


Figure 3: Percentage of Inhibition values in Egg albumin denaturation assay.

p=0.01508, as shown in Table 5. Since p-value <0.05, the null hypothesis has been rejected. Results of the Pearson correlation analysis indicated a significant large positive relationship between various concentrations of *Pirandai Uppu* and inhibitory effect on egg albumin denaturation, (r(5) =0.937,p=0.002), as shown in Table 6.

Anti-arthritic study by Bovine serum albumin denaturation method

Anti-arthritic activity of *Pirandai Uppu* is evaluated against denaturation of bovine serum albumin method. The percentage of inhibition increased with the increasing concentrations of the sample indicating the increase in anti-arthritic activity as shown in Figure 4. The lowest value (6.57%) was observed at a concentration of 50 µg/mL, while the highest value (82.1%) was observed at the concentration of 500 µg/mL. The results are tabulated in Table 7.

There is a statistically significant difference in the percentage of inhibition among the various concentrations of *Pirandai Uppu* in the anti-arthritic assay, as determined by 1-way ANOVA with p=0.0151, as shown in Table 8. Since p-value <0.05, the null hypothesis has been rejected. Results of the Pearson correlation analysis indicated a significant large positive relationship between various concentrations of *Pirandai Uppu* and inhibitory effect on bovine serum albumin denaturation, (r(5)=0.979,p<0.001), as shown in Table 9.

Table 6: **Pearson correlation between *Pirandai Uppu* and inhibition of Egg albumin denaturation.**

Parameter	Value
Pearson correlation coefficient (r)	0.9368
r^2	0.8776
p-value	0.001863
Covariance	3918.4643
Sample size (n)	7
Statistic	5.988

Table 7: **Inhibition percentage values of *Pirandai Uppu* and Diclofenac sodium in Bovine serum albumin denaturation assay.**

Sample Concentration (µg)	Inhibition by Pirandai Uppu (%)	Inhibition by Diclofenac sodium (%)
50	6.57	12.14
75	17.10	19.23
100	27.63	30.5
200	46.05	49.5
300	51.23	62.5
400	75.47	79.5
500	82.1	89.5

Table 8: **One-way ANOVA for Anti-arthritic study by Bovine serum albumin denaturation method.**

Source	D_f	Sum of Square	Mean Square	F Statistic	*p*-value
Groups (between groups)	1	124240.3804	124240.3804	8.0249	0.0151
Error (within groups)	12	185783.4054	15481.9505		
Total	13	310023.7858	23847.9835		

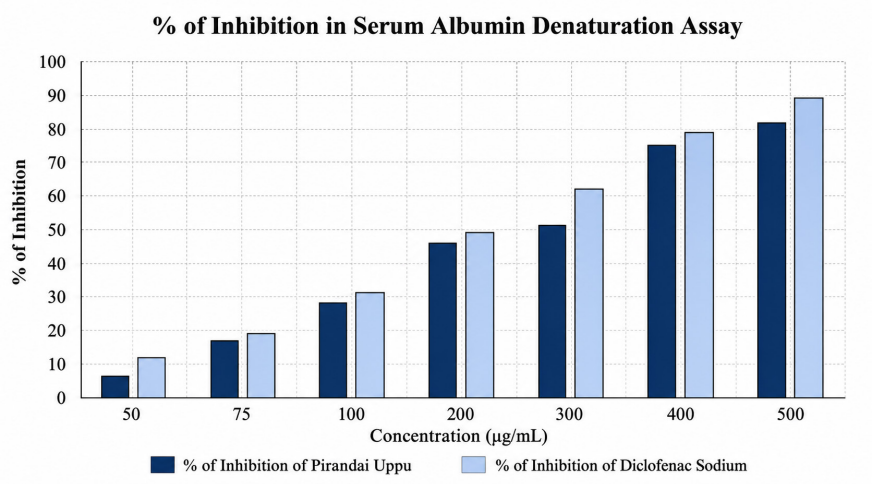


Figure 4: Percentage of Inhibition values in Bovine serum albumin denaturation assay.

Table 9: Pearson correlation between *Pirandai Uppu* and inhibition of Bovine serum albumin denaturation.

Parameter	Value
Pearson correlation coefficient (r)	0.9791
r^2	0.9586
p-value	0.0001205
Covariance	4853.4107
Sample size (n)	7
Statistic	10.7538

DISCUSSION

Hence, it is proven that *Pirandai Uppu* has potent anti-inflammatory and anti-arthritic activities. It possesses significant activity comparable with that of the standard Diclofenac sodium. Inflammation is one of the defense mechanisms of the body. Extracellular release of the lysosomal constituents is responsible for the inflammation and damage caused in tissues. Stabilizing lysosomal membrane is crucial in mediating anti-inflammatory response (Azeem *et al*., 2010). Hence HRBC method was selected as erythrocyte membrane is analogous to lysosomal membrane. The results indicate that *Pirandai Uppu* stabilized the erythrocyte membrane thereby proving its significant anti-inflammatory activity. Protein denaturation mechanism involves modification of electrostatic, hydrophobic, hydrogen and disulphide bonds. It is the reason for production of autoantigens in some arthritic conditions (Elisha *et al*., 2016). From the results of the study, it is evident that *Pirandai Uppu* is capable of controlling the production of autoantigens and inhibits the process of protein denaturation. It is observed that *Pirandai Uppu* dose-dependently prevents heat-treated egg albumin and bovine serum albumin denaturation indicating that it has potent anti-arthritic activity. Statistical analysis using one-way ANOVA revealed significant differences among the different concentrations of *Pirandai Uppu* in all assay models. Further, Pearson correlation analysis demonstrated a strong positive correlation between various concentrations of *Pirandai Uppu* and pharmacological activity.

This potent anti-inflammatory and anti-arthritic activity is attributed to the presence of rich phytoconstituents in *Cissus quadrangularis* which is further enhanced in *Pirandai Uppu* due to the medicine preparation process. Extracts of *Cissus quadrangularis* is found to contain alkaloids, saponins, coumarins and phenolic compounds (Garg *et al*., 2024). It is also rich in phytoestrogens (Aswar *et al*., 2012). It has been found that phenols and flavonoids exert protective effect in degenerative conditions including arthritis. Inhibition of articular swelling in arthritic rats has been attributed to the activity of alkaloids and flavonoids (Niazi *et al*., 2017). Some coumarins are known to possess Anti-inflammatory activity capable of inhibiting cyclooxygenase and lipoxygenase pathways (Anjali *et al*., 2020). Phenolic compounds are capable of inhibition of pro-inflammatory mediators (Fawole *et al*., 2009). Phytoestrogens are found to have strong anti-inflammatory activity (Desmawati *et al*., 2019). The potential of *Pirandai Uppu* as anti-arthritic and anti-inflammatory agent can be further established by Carrageenan-induced paw edema and adjuvant arthritis model.

CONCLUSION

The findings of the present study demonstrate the strong anti-inflammatory and anti-arthritic activity of *Pirandai Uppu*. It also validates the indications mentioned in Siddha literature and the usage of this drug by traditional physicians to treat inflammatory and arthritic conditions. Thus, *Pirandai Uppu* is a promising candidate for clinical trials on inflammatory and arthritic conditions.

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ABBREVIATIONS

HRBC: Human Red Blood Cell; **NSAIDs:** Non-steroidal Anti-Inflammatory Drugs; **NaCl:** Sodium Chloride; **OD:** Optical Density; **pH:** Potential of Hydrogen; **RBC:** Red Blood Cell.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Pirandai Uppu, a Siddha herbal salt prepared from *Cissus quadrangularis*, demonstrated significant concentration-dependent *in vitro* anti-inflammatory and anti-arthritic activities through HRBC membrane stabilization and protein denaturation inhibition assays. The findings support the pharmacological potential of *Pirandai Uppu* as a promising candidate for the management of inflammatory and arthritic conditions. Further preclinical and clinical studies are required to substantiate and establish its efficacy and safety.

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