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FORMULATION, STANDARDIZATION, PHARMACOLOGICAL INVESTIGATION OF THE ANTI-INFLAMMATORY POTENTIAL OF *RASNA ERANDADI KWATH*: AN *IN-VITRO* EXPERIMENTAL APPROACH

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Keywords:

Anti-Inflammatory, *Rasna Erandadi Kwath*, Bovine Serum Albumin (BSA) Assay, Human Red Blood Cell (HRBC) Membrane Stabilization

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ABSTRACT: Inflammation is the body's natural response to injury, infection, or tissue damage, characterized by redness, swelling, heat, pain, and functional changes. While conventional Anti-Inflammatory drugs are effective, their prolonged use can lead to adverse effects, prompting the search for safer, natural alternatives. Ayurvedic herbal decoctions like *Rasna Erandadi Kwath*, *Sareevadi Kwatham*, and *Rasnadi Kwatham* have demonstrated notable Anti-Inflammatory properties. These Kwaths are traditional water-based preparations made by boiling medicinal herbs to extract active phytochemicals. *Rasna*, *Guduchi*, and *Devadaru* are key ingredients known for their Anti-Inflammatory benefits. This study evaluates the Anti-Inflammatory potential of *Rasna Erandadi Kwath* at three concentrations-1:4 (*Mrudu*), 1:8 (*Madhyam*), and 1:16 (*Kathin*)-using *In-vitro* methods: BSA Protein Denaturation Assay and HRBC Membrane Stabilization Test. All three concentrations showed significant Anti-Inflammatory activity, with the 1:16 (*Kathin*) dilution showing the highest effect. These findings support *Rasna Erandadi Kwath* as a natural and holistic alternative to synthetic drugs for managing inflammation through Ayurvedic medicine.

INTRODUCTION: Inflammation is a vital response required for the successful recovery from injury, trauma (surgically induced), sepsis and infections. Inflammation is body's natural protection mechanism that is essential to healthiness [1]. It is a complicated process that is often linked to pain and includes things like increased vascular permeability, increased denaturation of proteins, and changes in membranes [2].

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Damaged cells, irritants, or pathogens are examples of harmful stimuli that cause inflammation in vascular tissue. The body uses inflammation as a defense mechanism to flush out harmful stimuli and start the tissue's healing process [3]. However, disorders including vasomotor rhinorrhea, rheumatoid arthritis, and atherosclerosis develop if inflammation is not managed [4].

The cells become activated and release inflammatory mediators at the start of an inflammatory response. Histamine, prostaglandins, serotonin, the complement and other plasma enzyme systems, slow-reacting substances of anaphylaxis (SRS-A), fibrinolytic, coagulation, and kinin systems, are examples of these mediators [5]. Inflammation can be categorized into acute and chronic types [6].

Acute inflammation represents the body's primary reaction to harmful stimuli and involves an enhanced flow of white blood cells and plasma from the circulation into the injured tissues. This procedure begins with cells that are already located in the affected tissues. It is marked by significant changes in blood vessels, such as dilation and heightened capillary permeability, which are triggered by various inflammatory mediators. Conversely, Concurrent tissue injury and inflammatory process repair are hallmarks of chronic inflammation and is a persistent inflammatory reaction that gradually changes the types of cells present at the inflammation site [7].

Inflammation has been found to be caused by protein denaturation. There are indications that inflammation occurs when living tissues are damaged. In addition to loss of function in the affected area, this is characterized by redness, discomfort, heat, and swelling. The protein structure's disulfide, hydrophobic, hydrogen, and electrostatic linkages are all broken. Furthermore, the protein loses its molecular structure and functions or becomes denatured as a result of a complicated series of events that include enzyme activation, mediator release, cell migration, tissue disintegration, and repair [8, 9, 10, 11]. Often, non-steroidal anti-inflammatory drugs or NSAIDs, are used to treat infectious infections and inflammatory diseases like rheumatoid arthritis. According to reports, they attach to plasma albumin and stop or slow down albumin's thermal denaturation. However, long-term use of these drugs frequently results in toxic or secondary side effects that harm the liver, gastrointestinal system, cardiovascular system, and kidneys [12, 13, 14]. Therefore, it is necessary to investigate alternate plant-based sources of anti-inflammatory medications [15]. Ayurveda is an age-old medical science that treats patients holistically and with few negative pharmacological side effects. In Ayurvedic pharmaceutics, *Kwatha Kalpana* is the most important and frequently utilized type of dosing [16]. The ancient experts were looking for a way to extract as much water-soluble herb compounds as possible. Thus, they used water to boil plants. The greatest volume of extract was produced using this technique. *Kwath* or *Kashayam* is the name given to such a dose form [17].

***Rasna Erandadi Kwath* (REK):** *Rasna Erandadi Kwath* (REK) is aqueous base decoction which contains 14 ingredients.

TABLE 1: FORMULARY OF REK [18]

Ayurvedic Name	Parts Used	Botanical Name
Rasna	Leaf/ Root	*Pluchea lanceolata*
Eranda (Mula)	Root	*Ricinus communis*
Bala	Root	*Sida cordifolia Linn*
Sahacara	Pulp	*Baeleria prionitis Linn.*
Vari (Shatavari)	Root	*Aspragus racemosus*
Dusparsa (Yavasaka)	Pulp	*Alhagi pseudalhagi*
Vasa	Root	*Adhatoda beddomei*
Amrta (Guduchi)	Stem	*Tinospora cordifolia*
Devahva (Devadaru)	Heart wood	*Cedrus deodara*
Ativisa	Root	*Aconitum heterophyllum*
Ghans (Musta)	Rhizome	*Lithacodia musta*
Iksura	Root	*kokilaksaka mula*
Sathi	Rhizome	*Hedychium spicatum Ham.*
Visva (Sunthi)	Rhizome	*Zinziber officinalis*

This *kwath* was prepared according to the further shloka.

***Rasnerandadi kashayam* [19]:** Rāsnairaṇḍabalāsahacaravarī duḥsparśa vāsāmṛtā Devāhyātiviṣā ghane kṣura śaṭhī viśvai kaṣāya śṛtaḥ II. Sarpis taila-vimiśritaṁ praśamayet vāyuṁ ca śūlaṁ tathā, Jaṅghoru-trika-pṛṣṭha-pārśva-hanugataṁ śophaṁ ca vātāsrajam II.

The shloka is mentioned from the *Kashaya Prakarana* of the traditional Ayurvedic text, *Sahasra Yoga*, which contains numerous therapeutic formulations. *Rasnerandadi Kashayam* specifically targets *Vata* imbalance, helping relieve conditions like sciatica, arthritis, and joint pain by reducing inflammation and improving mobility.

Meaning: *Rasna, Eranda* (castor root), *Bala, Sahachara, Vari, Duhsparsha, Vasa, Amrita, Devadaru, Ativisha, Ghana, Ikshura, Shathi, Vishvabheshaja*, and other herbs are used in this formulation (*Rasnerandadi Kashayam*).

These are combined with oil (*taila*) and ghee (*sarpis*) after being boiled into a decoction (*Kashayam*). The final preparation Reduces pain (*Shula*) and alleviates swelling (*Shotha*) by pacifying aggravated *Vata*.It is especially effective in the thighs, knees, sacrum, back, sides, and jaw regions.

Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. Molecular Docking is used to positioning the computer-generated 3D structure of small ligands into a receptor structure in a variety of orientations, conformations and positions [20].

Molecular docking consists of three main connected goals: pose prediction, virtual screening and binding affinity estimation. A successful docking methodology must be able to correctly predict the native ligand pose within the receptor binding site (i.e. to find the experimental ligand geometry within a certain tolerance limit) and the associated physical chemical molecular interactions [21].

MATERIALS AND METHODS:

Plant Powders and Reagents: The following plant powders were purchased and authenticated from Sanchomee Herboveda Pvt. Ltd., Manakarnika Aushadhalaya, Pune: *Rasna, Eranda, Devdaru, Bala, Guduchi, Dusparsha, Ativisha, Sahachara, Ghana, Sunthi, Sathi, Vasa, Satavari*, and *Iksura* (Manakarnika aushadhalaya, Pune, AD/439/03/25).

Sodium phosphate buffer, monosodium phosphate, glacial acetic acid, and diclofenac sodium were kindly supplied by Research Lab Fine Chem Industries. Sodium chloride, aspirin, and disodium hydrogen phosphate were purchased from Vishal Chem. Methanol was obtained from Loba Chemie. All chemicals were of analytical grade. These reagents were used without further purification.

Formulation of Kwath: Ayurvedic kwaths are made in three different concentrations, according the SHARNGAGHARA-SAMHITA. According to classical literature, the amounts of water needed (4, 8, and 16) differ according to the hardness and quantity of the herb utilized.

Mrudu Pak, for example, uses a 4:1 ratio for softer herbs (those that use leaves and flowers), *Madhyam* Pak uses an 8:1 ratio for medium-hard herbs (which include soft barks, shrub roots, and medium tubers), and *Kathin* Pak uses a 16:1 ratio for very hard plants (such as hard tree barks and tree and climber root barks) [22].

All fourteen plant powders were metered out in equal amounts, and water was added in the following ratios: *Madhyam* Pak (1:8 ml water), *Kathin* Pak (1:16 ml water), and *Mrudu* Pak (1:4 ml water).A gas stove was used to bring the mixture to a boil. The kwath was filtered through muslin fabric and then placed into another vessel once the water volume had been cut by one-fourth. The remaining substance that was on the cloth was thrown away [23].

Physicochemical Analysis: The Following Physicochemical Parameter Were Carried Out For Standardization of Prepared Kwath.

pH: The pH of Formulations Identified Using pH Meter. Samples Were Placed in Beaker and pH of Kwath Samples Was Determined.

Specific Gravity: To determine a substance's specific gravity using a 10 mL specific gravity bottle, first clean, dry, and weigh the empty bottle. Next, fill the bottle completely with distilled water at the specified temperature, ensuring no air bubbles are present. Weigh the bottle again to obtain the mass of water. Add sample to the bottle until it reaches the calibration mark. Weigh the bottle containing the sample.

The Specific Gravity Was Calculated by,

Specific gravity = (Mass of liquid / Sample (Gm) / (Mass of water (Gm)

Total Solid Content: The term "Total Solid"refers to the residue left behind after acertain quantity of preparation has been dried to aconsistent weight under predetermined circumstances. It is calculated by using following formula,

$$\% \text{ Solid Content} = (C-A) / (B-A) \times 100$$

Refractive Index: It is determined by using Abbe's Refractometer, small sample placed in prism and refractive index of sample compared with water as a standard.

Viscosity: The Determination of Viscosity of Liquid Conducted By Using Capillary Viscometer / Oswald Viscometer. Calculated by using,

Viscosity of Liquid = p2t1/p1t1×n1 [24]

Phytochemical Screening: The Phytochemical screening of Kwath were carried out using standard qualitative methods to detect the presence of bioactive compounds. The results indicated the presence of carbohydrates, phenols, flavonoids, Glycosides, Alkaloids, steroids, and quinine. Carbohydrates were detected using the Fehling's test, while phenols were identified through the Lead Acetate test. Flavonoids were confirmed by the lead acetate and alkaline reagent tests. The Liebermann-Burchard test was used to confirm the presence of steroids. Quinine, an alkaloid, was detected using Dragendorff's reagent. The positive results suggest the potential pharmacological significance, medicinal value of the formulation [25].

***In-silico* Molecular Docking:** To perform protein-ligand docking studies using AutoDock Vina, follow these steps:

To perform a protein-ligand docking simulation, begin by downloading the PDB file of your target protein from the RCSB PDB database. Open this file in Discovery Studio Visualizer 3.5, remove heteroatoms and chain B, and save the cleaned structure as 4QFH_1.pdb. Next, obtain the 3D SDF file of your desired ligand from databases like DrugBank or PubChem. Open the SDF file in Discovery Studio Visualizer and save it as a PDB file. Then, use AutoDockTools (MGLTools) to convert both the protein and ligand PDB files to PDBQT format. For the protein, add polar hydrogens, compute Kollman charges, and define the grid box. For the ligand, detect torsions and save it as glucose6-phosphate.pdbqt. Create a configuration file specifying the grid box dimensions and center, and place the PDBQT files along with the configuration file in the AutoDock Vina installation directory. Open a command prompt as administrator, navigate to the AutoDock Vina directory, and execute the docking command with appropriate parameters. Finally, use PyMOL or Chimera to visualize the docking poses, assess binding modes, and analyze interactions such as hydrogen bonds and binding energies. AutoDock Vina is a molecular modeling simulation software that is especially effective for protein-ligand docking [26].

***In-vitro* Anti-Inflammatory Activity:**

HRBC Stabilization Method: Fresh human blood (10 ml) will be collected in heparinized centrifuge tubes and centrifuged at 3000 rpm for 10 min and washed 3× with an equal volume of normal saline solution. The volume of the blood will be measured and reconstituted as a 10% v/v suspension with normal saline. The reaction mixture consisted of formulation of 1 ml and 1 ml of 10% red blood cell suspension. For the control, saline will be added. Aspirin will be used as a standard drug (positive control). The samples will beincubated at 56 °C for 30 min, centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant measured at 560 nm. Percent membrane stabilization activity will be calculated by the following.

Percent of protection: 1 − OD of test / OD of control × 100

Here "OD of test" is optical density or the test sample's absorbance and "OD of control" is optical density or absorbance of the negative control [27].

Bovine Serum Albumin Assay (BSA): The reaction mixture (10 mL) consisted of 0.4 mL Bovine Protein Fraction, 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 100 µL of different concentration sample. Similar volume of double-distilled water served as control.

Then the mixtures were incubated at (37°C ±2) in a incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the concentration was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula,

% Inhibition = C –T/ C

Where, T = absorbance of test sample,C = absorbance of control [28, 29].

RESULT AND DISCUSSION:

Kwath Formulation: Three different concentration Kwaths *i.e. Mrudu (1:4), Madhyam (1:8), Kathin (1:16) Dravya* were prepared.

Physicochemical Parameters:

TABLE 2: PHYSICOCHEMICAL PARAMETERS

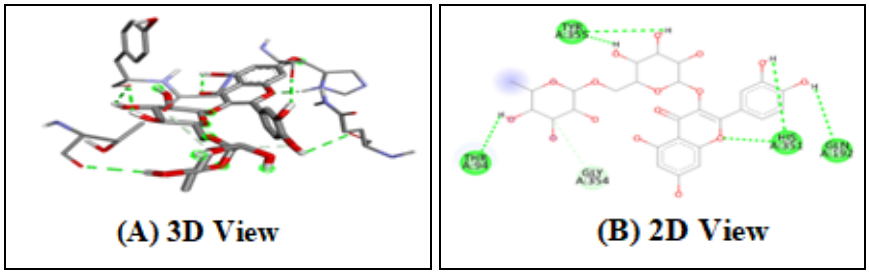
Physicochemical Tests	Kwath	Observations
		Mean ± SD
pH	1:4	5.51 ± 0.4246
	1:8	5.50 ± 0.4272
	1:16	5.14 ± 0.1616
Specific Gravity	1:4	0.9666 ± 0.0152
	1:8	0.0430 ± 0.0012
	1:16	0.3680 ± 0.5646
Total Solid Content	1:4	111.6 ± 0.0697
	1:8	115.52 ± 0.0531
	1:16	118.76 ± 0.0402
Refractive Index	1:4	0.0636 ± 0.0040
	1:8	0.064 ± 0.0006
	1:16	0.0652 ± 0.0040
Viscosity	1:4	0.1958 ± 0.0326
	1:8	0.447 ± 0.0237
	1:16	02.33 ± 0.0083

Phytochemical Parameters:

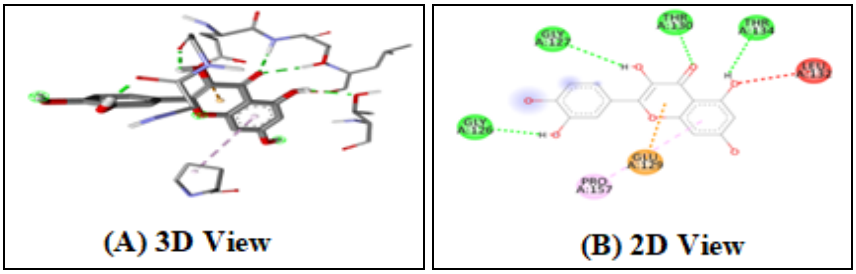
TABLE 3: PHYTOCHEMICAL PARAMETERS

Phytochemical Tests	Phytochemical Parameters	Kwath	Inference
Detection of Carbohydrates	FehlingsTest	1:4	Present
		1:8	Present
		1:16	Present
	Barfoed Test	1:4	Present
		1:8	Present
		1:16	Present
	Benedict Test	1:4	Present
		1:8	Present
		1:16	Present
Detection of Phenols	Lead Acetate	1:4	Present
		1:8	Present
		1:16	Present
Detection of Flavonoids	Detection of Flavonoids	1:4	Present
		1:8	Present
		1:16	Present
Detection of Glycosides	Detection of Glycosides	1:4	Present
		1:8	Present
		1:16	Present
Detection of Alkaloids	Wagner's Test	1:4	Present
		1:8	Present
		1:16	Present
	Dragendroffs	1:4	Present
		1:8	Present
		1:16	Present
Detection of Steroids	Steroids	1:4	Present
		1:8	Present
		1:16	Present
Detection of Quinone	Quinone	1:4	Present
		1:8	Present
		1:16	Present

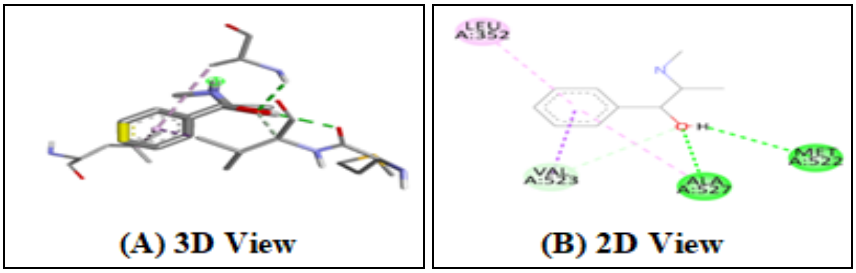
Molecular Docking: *Shatavari*: Ligand: Rutin, Protein: COX-2**,** Binding Affinity: -8.0 kcal/mol.



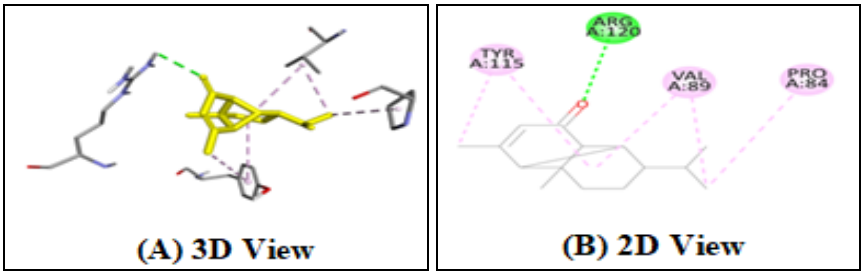
Rasna: Ligand: Quercetin, Protein: Interleukin-6 (IL-6), Binding Affinity: -6.7 kcal/mol



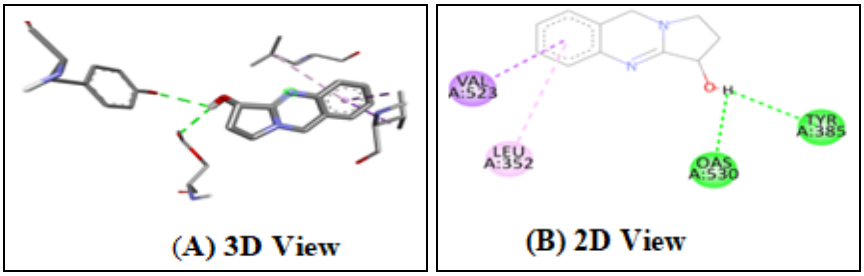
Bala: Ligand: Ephedrine, Protein: COX-2, Binding Affinity: -6.4 kcal/mol



Ghana: Ligand: Mustakone, Protein: COX-2, Binding Affinity: -6.3 kcal/mol



Vasa: Ligand: Vasicine, Protein: COX-2, Binding Affinity: -5.9



Molecular docking studies have identified several plant-derived compounds with strong binding affinities for COX-2 and IL-6, key targets in inflammation. Rutin from **Shatavari** exhibited the highest binding affinity to COX-2 (-8.0 kcal/mol), while quercetin from **Rasna** demonstrated a strong affinity for IL-6 (-6.7 kcal/mol), suggesting potential roles in regulating cytokine-induced inflammation.

Other ligands, such as ephedrine, mustakone, and vasicine, showed modest COX-2 binding, indicating varying levels of anti-inflammatory potential. The stability of these ligand-protein complexes was significantly influenced by the number and type of hydrogen bonds formed, highlighting the importance of these interactions in the design of effective anti-inflammatory agents.

In-vitro Anti-Inflammatory Activity:

Bovine Fraction Assay:

TABLE 4: BOVINE FRACTION ASSAY OF STANDARD DRUG (DICLOFENAC), MRUDU DRAVYA, MADHYAM DRAVYA, KATHIN DRAVYA

Sample Code	Concentration (µg/ml)	Absorbance ± SD	% Inhibition
Standard (Diclofenac Sodium)	10	1.52 ± 0.0124	11.62%
	50	1.31 ± 0.0244	23.83%
	100	0.84 ± 0.0205	51.16%
	500	0.74 ± 0.0169	56.97%
	750	0.32 ± 0.0169	81.39%
	1000	0.23 ± 0.0216	86.62%
1:4 REK *(Mrudu)*	10	1.71 ± 0.0124	0.33%
	50	1.62 ± 0.0205	5.81%
	100	1.51 ± 0.0286	12.20%
	500	1.41 ± 0.0141	18.02%
	750	1.38 ± 0.0205	19.76%
	1000	1.07 ± 0.0124	37.79%
1:8 REK *(Madhyam)*	10	1.63 ± 0.0141	5.23%
	50	1.57 ± 0.0169	8.72%
	100	1.27 ± 0.0169	26.16%
	500	0.92 ± 0.0124	46.51%
	750	0.82 ± 0.0188	52.32%
	1000	0.78 ± 0.0216	54.65%
1:16 REK *(Kathin)*	10	1.51 ± 0.0169	12.20%
	50	1.46 ± 0.0169	15.11%
	100	1.05 ± 0.0124	38.95%
	500	0.90 ± 0.0124	47.67%
	750	0.74 ± 0.0081	56.97%
	1000	0.64 ± 0.0188	62.79%

Based on their capacity to prevent protein denaturation, the Bovine Fraction Assay results showed that the three test formulations - *Mrudu* (1:4 REK), *Madhyam* (1:8 REK), and *Kathin* (1:16 REK) - all exhibited dose-dependent anti-inflammatory effectiveness. *Madhyam* (1:8) had 54.65% and *Mrudu* (1:4) had 37.79% of the total percentage inhibition, whereas Kathin (1:16) had the greatest percentage at 1000 µg/ml (62.79%). Its considerable anti-inflammatory action was confirmed by the significantly higher inhibition of 86.62% at 1000 µg/ml shown by the standard medicine Diclofenac Sodium. According to these findings, *Kathin* (1:16) once again stands out as the most potent test sample, and the Anti-Inflammatory action appears to grow with both concentration and dilution.

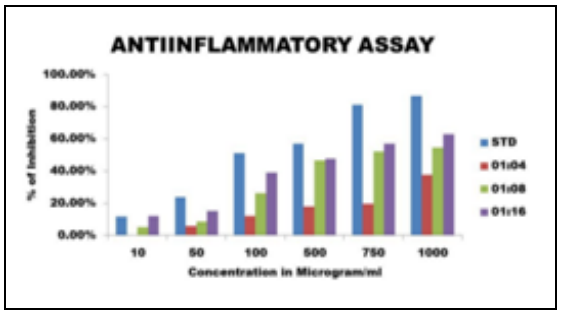


FIG. 1: GRAPHICAL REPRESENTATION OF % INHIBITION OF STANDARD, *MRUDU DRAVYA, MADHYAM DRAVYA, KATHIN DRAVYA* OF BOVINE FRACTION ASSAY

HRBC Stabilization Method:

TABLE 5: HRBC STABILIZATION METHOD FOR SATNDARD (ASPIRIN), *MRUDU DRAVYA, MADHYAM DRAVYA, KATHIN DRAVYA*

Sample Code	Concentration (µg/ml)	Absorbance ± SD	% Stabilization
Standard (Aspirin)	10	1.72 ± 0.0244	12.69%
	50	1.45 ± 0.0169	26.39%
	100	1.32 ± 0.0163	32.99%
	500	0.83 ± 0.0163	57.86%
	750	0.50 ± 0.0163	74.61%
	1000	0.38 ± 0.0216	80.71%
1:4 (Mrudu)	10	1.90 ± 0.0205	3.55%
	50	1.83 ± 0.0163	7.10%
	100	1.72 ± 0.0249	12.69%
	500	1.29 ± 0.0124	34.51%
	750	1.04 ± 0.0124	47.20%
	1000	0.92 ± 0.0169	53.29%
1:8 (Madhyam)	10	1.80 ± 0.0047	8.62%
	50	1.64 ± 0.0047	16.75%
	100	1.48 ± 0.0294	24.87%
	500	1.07 ± 0.0205	45.68%
	750	0.81 ± 0.0205	58.88%
	1000	0.74 ± 0.0216	62.43%
1:16 (Kathin)	10	1.70 ± 0.0163	13.70%
	50	1.51 ± 0.0141	23.35%
	100	1.38 ± 0.0169	29.94%
	500	0.94 ± 0.0163	52.28%
	750	0.78 ± 0.0216	60.40%
	1000	0.66 ± 0.0124	66.49%

All three test formulations - *Mrudu* (1:4), *Madhyam* (1:8), and *Kathin* (1:16) showed dose-dependent anti-inflammatory efficacy, according to the HRBC membrane stabilization assay. Among the test samples, Mrudu demonstrated 53.29% stability at the maximum concentration of 1000 µg/ml, *Madhyam* 62.43%, and *Kathin* 66.49%. Aspirin, a common medication, showed 80.71% stability at the same concentration. According to these findings, *Kathin* (1:16) is the most potent of the three formulations, and its anti-inflammatory effectiveness rises with dilution.

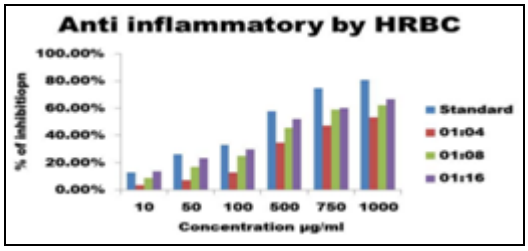


FIG. 2: GRAPHICAL REPRESENTATION OF % INHIBITION OF STANDARD, *MRUDU DRAVYA, MADHYAM DRAVYA, KATHIN DRAVYA* OF HRBC STABILIZATION METHOD

CONCLUSION: Inflammation is essential for protecting the body, but when prolonged, it can contribute to chronic diseases. While conventional Anti-Inflammatory drugs are effective, they often come with side effects, prompting the need for safer alternatives. Ayurvedic formulations like *Rasna Erandadi Kwath* (REK) are gaining popularity due to their safety and holistic benefits. This study evaluated the Anti-Inflammatory activity of REK at three dilutions (1:4, 1:8, and 1:16) using *in-vitro* methods such as BSA protein denaturation and HRBC membrane stabilization. The formulation was also standardized and subjected to molecular docking (*In-silico*) to investigate potential mechanisms. Standardization yielded consistent results, and phytochemical screening confirmed active constituents. *In-silico* studies revealed how these compounds may interact with inflammatory mediators, supporting their Anti-Inflammatory potential. Among the concentrations, the 1:16 dilution (*Kathin Kwath*) showed the most significant *in-vitro* Anti-Inflammatory activity compared to 1:4 (*Mrudu Kwath*) and 1:8 (*Madhyam Kwath*). These findings highlight REK's strong potential. Further standardization, along with advanced *in-vitro* and

in-vivo studies, is needed to validate its safety and therapeutic effectiveness.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

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