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Antitussive Activity and *in silico* Drug Profiling of *Chandramruta Rasa*

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Abstract

Chandramruta Rasa, a classical herbo-mineral formulation traditionally prescribed in *Bhaishajya Ratnavali* for respiratory disorder including *Kasa* (cough), lacks preclinical validation of its antitussive efficacy. This study evaluated its antitussive efficacy in a citric acid-induced cough model in guinea pigs. 24 Dunkin Hartley male guinea pigs were randomized into four groups (n=6): Group I served as vehicle control (0.5% carboxymethyl cellulose, 2 mL/kg), Group II (reference standard treatment) received diphenhydramine hydrochloride (10 mg/kg), and two test groups Group III and Group IV received Low-dose *Chandramruta Rasa* (LCR: 87.19 mg/kg) and High-dose *Chandramruta Rasa* (HCR: 261.56 mg/kg), respectively. One-hour post-administration, animals were exposed to 7.5% citric acid aerosol delivered through a nebuliser for 10 min and cough latency and percentage inhibition of cough frequency were recorded. Both test groups showed an increased cough latency and decrease in cough frequency ($P<0.0001$ vs vehicle control group). Both test group showed a dose-dependent antitussive efficacy relative to the control group. *In silico* analysis discovered that 36 components of *Chandramruta Rasa* satisfied Lipinski's rule of five, indicating physicochemical properties that could support oral bioavailability. Among these 36 compounds, 33 molecules revealed promising ADMET drug properties through Deep-PK webserver. Similarity Ensemble Approach (SEA) webserver predicted interaction of 3 key molecules (PubChem CID: 2998, 3084336, and 4744) with TRPV1 (maxTC=1) and histamine H1 receptors (MaxTC=0.8), supporting modulation of cough hypersensitivity pathways. These results suggested *Chandramruta Rasa's* traditional use and suggest multi-target mechanisms, potential interactions with predicted biological targets, including TRPV1 and H1 receptors.

Major Finding: *Chandramruta Rasa* displays dose-dependent antitussive activity with significant improvement in cough latency and cough frequency and *in silico* profiling reveals favourable pharmacokinetic and target prediction with TRPV1 and H1 receptors.

Keywords: ADMET, Antitussive Activity, *Chandramruta Rasa*, Guinea Pig Model, In-silico Drug Profiling

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1. Introduction

Chronic cough persists for eight weeks in nearly one out of ten adults, and it is associated with high rate of smoking, obesity, COPD/asthma, and is one of the most prevalent respiratory symptoms encountered in clinical practice, representing a major cause of healthcare visits worldwide[1]. Cough serves as the body's immune defense against foreign particles and pathogens[2]. The cough reflex can be triggered by noxious and innocuous stimuli, which often leads to chronic coughing, damaging the upper respiratory system, and impairing the quality of life and is associated with substantial physical, psychological, and social burdens[3-5].

Several Transient Receptor Potential (TRP) ion channels have been linked to sensory perception, inflammation modulation, and various diseases, such as COPD, asthma, cancer, and fibrosis[6,7]. TRPV1 sense acid, capsaicin, and pro-inflammatory mediators. Histamine H1 receptor antagonists are effective in treating cough

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in patients with atopic cough[8-10]. Crosstalk between these channels underpins refractory and unexplained chronic cough[11]. Several naturally occurring compounds, including alkaloids and glycosides have been shown to influence these pathways, thereby reducing cough reflex sensitivity in experimental models. However, the possible antitussive efficiency with respect to these molecular targets has not yet been discovered.

Despite the availability of numerous conventional antitussive medicines, such as Proton Pump Inhibitors (PPIs), Inhaled Corticosteroids (ICS), and neuromodulators, these offer partial relief in many patients, who often suffer frequent side effects (*e.g.*, dysgeusia, drowsiness). These limitations urged to increased attention in alternative and other multi-component medicines. Traditional medicines have been shown to diminish cough severity, expand excellence of life, and diminish adverse drug reactions. Preclinical studies propose natural compounds can desensitise/down regulate TRPV1/TRPA1, attenuating inflammation, hyper responsiveness, and cough impulse in relevant models[12-14].

The traditional system of medicine, particularly *Ayurveda*, describes cough (*Kasa*) as a pathological condition arising from an imbalance of *Dosha* affecting the respiratory and systemic pathology. *Ayurvedic* therapeutics employ a wide range of herbal and herbo-mineral medicines that are traditionally claimed to show sustained therapeutic effects at relatively low doses. Among these, herbo-mineral formulations are considered pharmacologically distinctive due to their enhanced stability, synergistic interactions, and improved bioavailability compared to purely herbal preparations[13].

Chandramruta Rasa, a classical herbo-mineral formulation is described in *Bhaishajya Ratnavali*, is traditionally prescribed for respiratory and systemic disorders, including *Kasa* (cough), *Shwasa* (asthma), *Rajayakshma*, *Urahkshata*, and *Jwara* (pyrexia)[15]. Despite its use in various ailments, there is a lack of systemic experimental evidence validating its antitussive activity using established preclinical models. Additionally, the molecular mechanism underlying its claimed effect have not yet been explored. *In silico* approaches are a useful method to prioritise drug targets and predict plausible molecular targets, thereby supporting mechanistic hypotheses for complex formulations, such as *Chandramruta Rasa,* and accelerating early-stage drug discovery[16]. Therefore, the present study was designed to evaluate antitussive efficacy of *Chandramruta Rasa* using the citric acid-induced cough model in Guinea pigs, and to explore its potential molecular mechanism through *in silico* pharmacokinetic and drug profiling.

2. Materials and Methods

2.1 Preparation of *Chandramruta Rasa*

Chandramruta Rasa was prepared according to the classical procedure described in *Bhaishajya Ratnavali*[15]. The components in addition to proportions are listed in the Table 1. Raw-materials requiring *Shodhana* (purification) such as *Lauha* (iron), and *Hingula* (cinnabar), underwent *Shodhana* as per traditional methods before preparation of *Chandramruta Rasa*. Subsequently, herbal components were finely powdered, precisely weighed, and thoroughly mixed as per the exact ratios shown in Table 1. The mixture then received one *Bhavna* of *Aja-Dugha* (goat milk) and *Vati* (tablets) of 1125 mg were prepared and kept in air-tight containers.

2.2 *In-silico* Study

Lipinski's rule of 5 was applied to evaluate the drug-likeness properties of phytochemicals identified from *Chandramruta Rasa*[17], although it is acknowledged that many successful drugs do not adhere strictly to these guidelines. Physicochemical properties of the compounds were retrieved from PubChem database, while toxicity profile, especially cancer toxicity was assessed and filtered using the Deep-PK webserver[18]. Potential protein targets relevant to antitussive activity were predicted using the Similarity Ensemble Approach (SEA) web server. The SEA tool estimates target-ligand interactions based on chemical similarity between query compounds and known ligands. SMILES strings of candidate compounds were submitted to the SEA server, and predicted targets were

Table 1. Grouping for antitussive activity

Groups	Dose
Vehicle control	0.5% CMC (2 mL/kg, p.o.)
Reference standard (Diphenhydramine hydrochloride)	10 mg/kg, p.o.
Low-dose *Chandramrita Rasa* (LCR)	87.19 mg/kg, p.o.
High-dose *Chandramrita Rasa* (HCR)	261.56 mg/kg, p.o.

ranked according to E-values and Maximum Tanimoto similarity (MaxTC) scores. Targets with established roles in modulating the cough impulse were ranked for further consideration[19].

2.3 Animal

Dunkin Hartley male guinea-pigs, weighing 300-400 g (Crystal Biological Solutions), were used in this study. All experimental procedures adhered to CPCSEA guidelines and were approved by the Institutional Animal Ethics Committee (IAEC) (IAEC Protocol Number: CRY/2223/101). The guinea-pigs were maintained in standard animal rooms, relative humidity of 50-60 % and temperature of 23±2 °C; lighting was adjusted to provide 12 hours of light and 12 hours of darkness. Animals were allowed to acclimate to adapt to the conditions for at least one week before use. Ad libitum quantities of standard rodent pelleted diet and purified water were provided during the study.

2.4 Animal Experimental Study

The antitussive effects of *Chandramruta Rasa* were evaluated using the citric acid-induced cough model in conscious guinea pigs, a well-established paradigm for screening antitussive agents[20,21]. *Chandramruta Rasa Vati* was suspended in 0.5% Carboxymethyl Cellulose (CMC) solution by thorough trituration in a mortar and pestle to obtain a homogenous suspension. Doses were calculated by converting the human therapeutic dose to the guinea pig equivalent based on body surface area normalisation by means of the tables of Paget and Barnes (1964)[22]. The animals were randomly allocated to four groups (n=6 animals per group):

Test and standard drugs were administered orally one hour prior to cough induction. Sixty minutes later, each animal was placed individually in a transparent plexiglass chamber (dimensions: 10 x 10 x 21cm) and exposed to 7.5% (w/v) citric acid aerosol generated by an ultrasonic nebuliser (output: 0.5mL/min) for 10 min. Cough bouts were identified by characteristic sound, abdominal contraction, and open-mouth posture, and were recorded continuously by trained observers blinded to treatment allocation. Cough latency and percentage inhibition of cough frequency (total number of coughs during the 10-min exposure period) are the two chief parameters assessed during antitussive activity.

2.5 Cough Latency

Cough latency was defined as the time interval (in seconds) from the initiation of the occurrence of the first cough. A quicker cough latency imitates enhanced sensitivity of the cough reflex, which can be modulated by various pharmacological treatments[23].

% Increase in cough Latency = Latency with stimulus – Baseline Latency/ Baseline Frequency x 100

2.6 Cough Frequency (Percentage Inhibition of Cough Count)

Cough frequency (Percentage inhibition of cough count) is the number of coughs occurring within a set time and is used to assess the severity of cough-related conditions[24,25]. A decline in cough frequency (decrease in percentage inhibition of cough count) can increase air sensitivity and contribute to other respiratory problems[26].

% Inhibition of Cough count = Baseline Frequency – Frequency with stimulus/ Baseline Frequency x 100

2.7 Statistical Analysis

Values are expressed as mean ± SEM. Number of animals of all experimental groups (n) = 6. Significant differences were determined using one-way ANOVA followed by Dunnet's multiple comparison test, with significance level indicated as *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. The vehicle control group was compared against standard and treatment groups using GraphPad prism 9 software.

3. Results and Observation

3.1 *In silico* Study

Table 2 presents the chemical constituents of *Chandramruta Rasa*, including their IUPAC names, drug classes, and PubChem IDs. A total of 35 ligands were identified. Table 3 displays the analysis of molecules for primary drug screening using Lipinski's rule of 5, including details such as molecular weight, logP, hydrogen bond donors, and hydrogen bond acceptors. 36 molecules complied with Lipinski's Rule of 5, exhibiting two or fewer violations. The molecules that passed the norms for Lipinski's Rule of 5 were selected for ADMET investigation. Table 4 presents the ADMET data for

Table 2. Molecules of *Chandramruta Rasa*

Sr. No.	Name of Compounds	Formula	PubChem CID	Drug Class
1	Ureidoglycine	$C_3H_7N_3O_3$	46173475	Amino acids and peptides
2	(S)-Edulinine	$C_{16}H_{21}NO_4$	2309	Shikimates, phenylpropanoids
3	L-beta-aspartyl-L-aspartic acid	$C_8H_{12}N_2O_7$	471583	Amino acids and peptides
4	N-Acetyl-ala-ala-ala-methylester	$C_{12}H_{21}N_3O_5$	44316829	Amino acids and peptides
5	Ruspolinone	$C_{14}H_{19}NO_3$	57359308	Alkaloids
6	Glutamyl-lysine	$C_{11}H_{21}N_3O_5$	7015704	Amino acids and peptides
7	Furmecyclox	$C_{14}H_{21}NO_3$	69765	Shikimates and phenylpropanoids
9	3-(4-Hydroxy-2-methoxyphenyl)-2-propenal	$C_{10}H_{10}O_3$	699414	Shikimates and phenylpropanoids
10	Methyl 2-furoate	$C_6H_6O_3$	11902	Ester
11	Leonurine	$C_{14}H_{21}N_3O_5$	161464	Shikimates and phenylpropanoids
12	Nitrotyrosine	$C_9H_{10}N_2O_5$	65124	Aminoacids
13	Nordihydrocapsaicin	$C_{17}H_{27}NO_3$	2998	Alkaloid
14	Perazine	$C_{20}H_{25}N_3S$	4744	Alkaloid
15	Marimastat	$C_{15}H_{29}N_3O_5$	119031	Dipeptides
16	Homocapsaicin	$C_{19}H_{29}NO_3$	123836	Alkaloids
17	Homodihydrocapsaicin	$C_{19}H_{31}NO_3$	3084336	Alkaloids
18	Ajaconine	$C_{22}H_{33}NO_3$	10839	Alkaloid
19	N-Hexadecanoylpyrrolidine	$C_{20}H_{39}NO$	247220	Fatty acids
20	N-(14-Methylhexadecanoyl) pyrrolidine	$C_{21}H_{41}NO$	6436377	Fatty acids
21	Docosanamide	$C_{22}H_{45}NO$	76468	Fatty acids
22	Citric acid	$C_6H_8O_7$	311	Carboxylic acids and derivatives
23	(+)-Chebulic acid	$C_{14}H_{12}O_{11}$	71308174	Carboxylic acids and derivatives
24	omega-hydroxy behenic	$C_{22}H_{44}O_3$	5282922	Fatty acids
25	7,8,3',4'-Tetrahydroxyisoflavone	$C_{15}H_{10}O_6$	42604622	Isoflavonoids
26	β-Erythroidine	$C_{16}H_{19}NO_3$	363463	Alkaloids
27	Mevinphos	$C_7H_{13}O_6P$	4511751	Fatty acids
28	4,4'-Diaminostilbene	$C_{14}H_{14}N_2$	53405192	Shikimates and phenylpropanoids
29	3-keto stearic acid	$C_{18}H_{34}O_3$	5283005	Fatty acids
30	(Z)-5-Hexadecenoic acid	$C_{16}H_{30}O_2$	13105359	Fatty acids
31	Linalyl caprylate	$C_{18}H_{32}O_2$	61435	Terpenoids
32	Octyl octanoate	$C_{16}H_{32}O_2$	61294	Fatty acids
33	Petroselinic acid	$C_{18}H_{34}O_2$	5281125	Unsaturated fatty acids
34	(-)-Catechin	$C_{15}H_{14}O_6$	73160	Flavonoids
35	Aloe-emodin	$C_{15}H_{10}O_5$	10207	Anthraquinones and anthrones

33 molecules covering parameters, such as Human Intestinal Absorption (HIA), Blood-Brain Barrier (BBB) permeability, excretion, and carcinogenesis are listed. Table 5 shows the outcomes of the Similarity Ensemble Approach (SEA) for TRPV1 target, identifying molecules modulating the cough reflex and targeting relevant receptors. Among the 33 analysed molecules, three showed probable interaction with the TRPV1 receptor.

3.2 Antitussive Activity

Table 7 represented cough latency and % inhibition of cough frequency data for the different groups. The

Table 3. Lipinski's rule of five screening of molecules of *Chandramruta Rasa*

Sr. No	PubChem CID	Molecular Weight (MW)	LogP (Partition Coefficient)	Hydrogen Bond Donors (HBD)	Hydrogen Bond Acceptors (HBA)	No of Violations
1	2309	291.15	1.057	2	5	0
2	2998	293.2	3.995	2	4	0
3	6368932	568.91	-	0	1	1
4	4744	339.18	2.092	0	3	0
5	10839	359.25	4.273	0	4	0
6	39468	291.18	1.082	2	4	0
7	69765	251.15	4.074	1	1	0
8	119031	331.21	0.355	5	6	0
9	123836	319.21	2.683	1	4	0
10	161464	311.15	0.731	3	8	0
11	247220	309.3	8.008	0	2	1
12	131752845	570.68	4.74	0	6	1
13	471583	248.06	-4.892	5	9	0
14	242326	500.75	6.55	0	4	2
15	699414	178.06	1.072	1	3	0
16	3084336	321.23	5.072	2	4	1
17	7015704	275.15	-3.776	5	8	0
18	12550	548.63	3.76	3	9	1
19	44316829	287.15	-3.444	4	8	0
20	46173475	133.05	-2.269	3	5	0
21	57359308	249.14	2.18	1	4	0
22	6436377	323.32	9.318	1	2	1
23	4511751	210.94	0.043	0	6	0
24	5283005	263.98	7.616	0	3	1
25	5282922	311.98	9.219	0	3	1
26	311	192.03	-2.247	4	7	0
27	10207	270.05	-0.65	3	5	0
28	11902	126.03	0.658	0	3	0
29	10328022	806.08	4.90	2	11	2
30	61294	256.24	6.965	0	2	1
31	61435	280.24	6.306	0	2	1
32	73160	290.08	0.852	5	6	0
33	13105359	254.22	7.054	1	2	1
34	42604622	286.05	1.82	4	6	0
35	53405192	212.13	2.146	2	2	0
36	71308174	356.04	-0.609	6	11	2
37	5281125	247.99	8.192	0	2	1
38	363463	273.14	-0.171	0	4	0
39	76468	339.35	10.256	1	2	1

Table 4. ADMET screening of molecules of *Chandramruta Rasa*

Sr. No.	PubChem CID	Human Intestinal Absorption (HIA)	Blood-Brain Barrier (BBB) Permeability	CYP3A4 Inhibition	[Excretion/Half-Life of Drug] Probability	Carcinogenesis
1	2309	Absorbed	0.22	Non-Inhibitor	0.355	Safe
2	2998	Absorbed	0.931	Inhibitor	0.359	Safe
3	4744	Absorbed	1	Non-Inhibitor	0.085	Safe
4	10839	Absorbed	1	Non-Inhibitor	0.212	Safe
5	39468	Absorbed	0.393	Non-Inhibitor	0.162	Safe
6	69765	Absorbed	0.948	Non-Inhibitor	0.496	Safe
7	119031	Non-Absorbed	0.366	Inhibitor	0.705	Toxic
8	123836	Absorbed	0.789	Non-Inhibitor	0.127	Safe
9	161464	Absorbed	0.903	Non-Inhibitor	0.409	Safe
10	247220	Absorbed	0.997	Non-Inhibitor	0.478	Safe
11	471583	Non-Absorbed	0.004	Non-Inhibitor	0.841	Safe
12	699414	Absorbed	0.887	Non-Inhibitor	0.391	Safe
13	3084336	Absorbed	0.919	Inhibitor	0.355	Safe
14	7015704	Absorbed	0.288	Non-Inhibitor	0.786	Safe
15	44316829	Non-Absorbed	0	Non-Inhibitor	0.831	Safe
16	46173475	Absorbed	0.999	Non-Inhibitor	0.402	Safe
17	57359308	Absorbed	0.887	Inhibitor	0.275	Safe
18	6436377	Absorbed	0.999	Non-Inhibitor	0.586	Safe
19	4511751	Absorbed	0.994	Non-Inhibitor	0.873	Toxic
20	5283005	Absorbed	0.937	Non-Inhibitor	0.486	Safe
21	5282922	Absorbed	0.239	Non-Inhibitor	0.652	Safe
22	311	Non-Absorbed	0.873	Non-Inhibitor	0.745	Safe
23	10207	Absorbed	0.688	Non-Inhibitor	0.189	Safe
24	11902	Absorbed	1	Non-Inhibitor	0.424	Safe
25	61294	Absorbed	1	Non-Inhibitor	0.561	Safe
26	61435	Absorbed	1	Non-Inhibitor	0.515	Safe
27	73160	Absorbed	0.003	Non-Inhibitor	0.362	Safe
28	13105359	Absorbed	0.972	Non-Inhibitor	0.575	Safe
29	42604622	Absorbed	0	Non-Inhibitor	0.286	Safe
30	53405192	Absorbed	0.998	Non-Inhibitor	0.334	Toxic
31	5281125	Absorbed	0	Non-Inhibitor	0.586	Safe
32	363463	Absorbed	0.976	Non-Inhibitor	0.164	Safe
33	76468	Absorbed	1	Non-Inhibitor	0.509	Safe

Table 5. Similarity Ensemble Approach (SEA) of *Chandramruta Rasa*

Sr. No	PubChem CID	Target Name	Description	P-Value	MaxTC
1	2998	TRPV1	Transient receptor potential cation channel subfamily V member 1[27]	9.00E-53	1
2	3084336	TRPV1	Transient receptor potential cation channel subfamily V member 1[27]	1.63E-51	0.76
4	4744	HRH1	Histamine H1 receptor	2.04E-26	0.8

Table 6. Body weight of *Chandramruta Rasa* for antitussive activity

Sr. No	Groups	Body Weight (g)
1	Vehicle control	331.26±5.26
2	Ref. Std.	335.89±6.48
3	LCR	341.13±5.12
4	HCR	347.47±7.85*

Values are represented as Mean±SD. Number of animals (n)=6. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

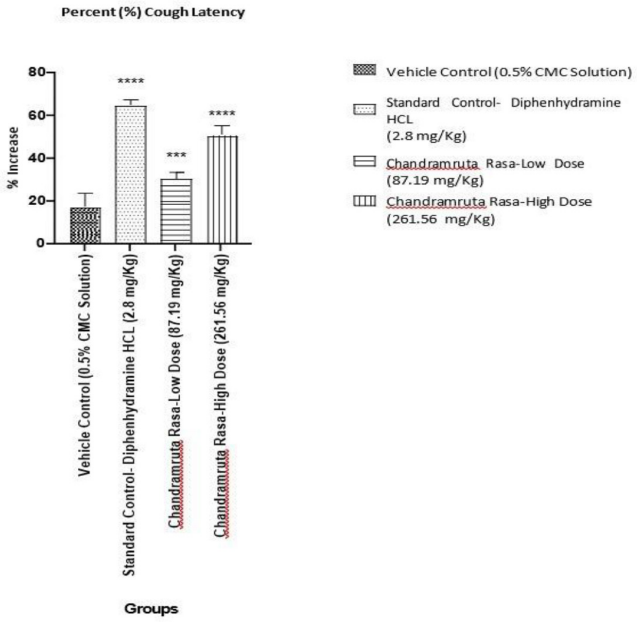


Figure 1. Effect on cough latency

present Figure 1 and 2 are the graphical representation of the cough latency and % inhibition of cough frequency.

Chandramrita Rasa significantly increased cough latency in a dose-dependent manner compared with the vehicle control group. The vehicle control group showed a cough latency of 17.18±6.63s, whereas the ref. std. group showed a markedly higher latency of 64.72±2.95s (****p<0.0001). The test drug group increased cough latency at High dose of *Chandramruta Rasa* (HCR) and at Low dose of *Chandramruta Rasa* (LCR), respectively.

Chandramruta Rasa produce a statistically significant change in % inhibition of cough frequency compared with the vehicle control group. The vehicle control group showed a cough frequency of 7.68± 3.29, while ref. std. group showed 53.77±3.76. The HCR and LCR groups increased % inhibition of cough frequency in a dose-dependent manner, respectively.

Table 7. Cough latency and inhibition of cough frequency of *Chandramruta Rasa* for antitussive activity

Groups	Cough Latency	% Inhibition of Cough counts
Vehicle Control	17.18±6.63	7.68±3.29
Ref. Std.	64.72±2.95****	53.77±3.76****
LCR	30.51±3.22***	24.43±8.29***
HCR	51.08±4.25****	39.35±6.55****

Values are represented as Mean±SD. Number of animals (n)=6. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

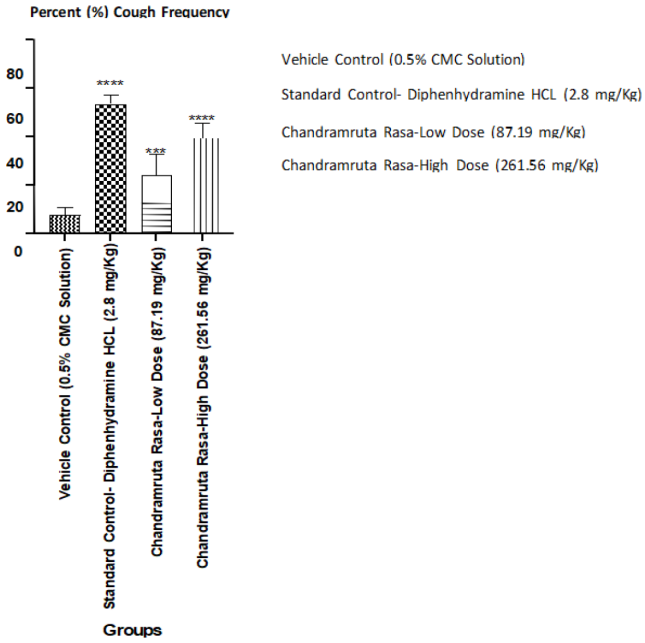


Figure 2. Effect on cough frequency (percentage inhibition of cough counts)

4. Discussion

Exposure to air pollutants and irritants, including ambient particulate matter, cigarette smoke, biomass fuel, or occupational dusts and gases, has been associated with an increased risk of chronic cough in diverse populations[28-30]. Chronic cough is increasingly linked to cough hypersensitivity and heightened sensory signalling, contributing to persistent symptoms and impaired quality of life[31]. In the present study, *Chandramruta Rasa* demonstrated significant antitussive activity in a citric acid-induced cough in guinea pigs. In parallel, *in silico* profiling and target prediction were performed to generate mechanism-based hypotheses and prioritise biologically plausible pathways for further validation.

The citric acid-induced cough model in guinea pigs is widely used to screen the antitussive effectiveness of medicines and to investigate the airway sensory reflex mechanisms. Guinea pigs are frequently used for cough research due to their reproducible tussive responses and serve as both an antitussive screen and a fundamental tool for studying the cough reflex[32].

Table 6 showed the body weight of the guinea pig across the different groups showing sign of healthy. Table 7 represented cough latency and % inhibition of cough frequency data for the different groups. Their extensive use in respiratory studies has yielded valuable information for clinical therapeutics[33,34]. The dose-dependent increases in cough latency as well as an increase in percentage inhibition of cough frequency were observed in this work indicate a reduction in cough reflex sensitivity compared to the vehicle control group. Figure 1 and 2 are the graphical representation of the Table 7, originally.

Evidence suggests that chronic cough frequently involves cough hypersensitivity, characterised by enhanced excitability of airway sensory afferents, and amplified cough signals. Effective antitussive interventions may act not only to suppress cough output but also to modulate neurogenic inflammation of the respiratory system[3]. *Chandramruta Rasa* attenuated tussive responsiveness and supports sensory-targeted pathways. In addition, diphenhydramine HCL has been reported to be used first-generation antihistamine and is used as an antitussive (cough suppressant) agent[35]. The reference standard diphenhydramine hydrochloride exhibited strong cough suppression, which further validates the responsiveness of the antitussive model.

Complex herbal and herbo-mineral formulations commonly contain multiple primary and secondary constituents that may contribute to therapeutic outcomes through additive or synergistic pharmacology. In such systems, *in silico* analysis can support mechanism-based hypothesis generation by prioritising complex constituents that may contribute to producing an effect through an additive or a synergistic effect. Computational drug-target prediction has gained importance in herbal and herbo-mineral medicines research and can guides mechanism-oriented investigations[16].

In the present study, computational drug profiling was used to evaluate drug-likeness and pharmacokinetic trends of the identified small molecules. Parameters such as the Lipinski rule of 5 are widely used in early-stage drug screening to reduce the likelihood of prioritizing compounds with unfavourable absorption/permeability profiles. Lipinski rule of five suggests that poor absorption is likely when a molecule has more than 5 hydrogen-bond donors, 10 hydrogen-bond acceptors, molecular weight over 500, or a cLogP above 5. Higher logP values indicate greater hydrophobicity[17]. For orally administered drugs from naturally derived products, 2 or 3 violations are common[36]. Overall, 36 constituents (Table 3) of *Chandramruta Rasa* met with Lipinski's rule of 5 criteria, indicating physicochemical properties consistent with potential oral bioavailability. A screening of ADMET profiling included Human intestinal absorption and the Blood-Brain Barrier (BBB) are two important pharmacokinetic tools for predicting drug bioavailability and serve as in early-stage indicators relevant to systemic exposure. Multiple approaches have been explored to enhance gastrointestinal penetration of poorly absorbed ligands[37]. A total of 24 molecules out of 33 molecules of *Chandramruta Rasa* passes the criteria for ADMET profiling. Although this does not confirm CNS activity, it suggests that some constituents may have physicochemical compatibility with CNS exposure. This may be relevant, as cough reflex regulation involves peripheral sensory afferent activation as well as brainstem integration and output patterning, and centrally acting antitussives showed efficacy in selected cough phenotypes.

Similarity Ensemble Approach (SEA) is a ligand-based target prediction has been used to discover poly-pharmacological interactions and off-target relationships, especially for complex chemical datasets and natural productss[34]. TRP ion-channels play a vital part in modulating the cough reflex and serve as molecular sensors for irritants and inflammatory stimuli[38].

Out of 24 molecules which passed the criteria for ADMET profiling, Table 7 represented top 3 molecules has been described for SEA analysis with special reference to TRPV1 and H1 receptors. Several constituents identified by the SEA webserver (PubChem CIDs: 2998, 3084336, and 4744) were associated with TRPV1-and H1-receptors related cough pathways and involvement in the modulation of cough reflex or airway sensory neuron activity. E-values provides ligand-set similarity score by random chance which was derived from Tanimoto similarity[19]. The lower the E-value, the significant the value would be for target linkage for pharmacological activity. PubChem CID 2998 represent significant lower E-value like capsaicin, implying antitussive effect through reduction/lowering of cough reflexes. The lower the

E-value of PubChem CID 4744 represent lower E-value, suggesting antitussive action through H1-mediated cough suppression and this cough suppression action is independent of their sedative and/or ventilation effects[39].

Maximum Tanimoto similarity (MaxTC) is derived as the pinnacle of pairwise ligand similarity within compared sets, utilising topological overlap on a 0-1 scale, where 1 denotes identical structure and values above 0.5 suggests pharmacologically relevant resemblances[19]. The Pubchem CID number 2998's maxTC=1 for TRPV1 indicates an exact match to known vanilloids like capsaicin, implying potent modulation; CID number 3084336's maxTC 0.76 suggest robust yet variant similarity, possibly synergistic in cough reflex desensitization. And, maxTC of Pubchem CID 4744 = 0.8, indicating high structural resemblance, confirm contribution to the formulation's dose-dependent antitussive activity[19]. Thus, these finding suggest that *in vivo* antitussive efficacy and SEA-based target prediction may modulate cough reflex sensitivity through numerous converging pathways, as well as TRPV1-mediated sensory signaling pathways. One study suggested that a TRPV1 inhibitor reduced airway inflammation, Immunoglobulin E (IgE) levels, and airway hyper-reactivity in a mouse model of allergic asthma[38]. And, it might be possible that *Chandramruta Rasa* may act in the same mode of action through TRPV1; however, these computational findings are hypotheses and must be experimentally confirmed by receptor-level functional assays and pathway-specific validation.

Chandramruta Rasa demonstrated significant antitussive effects in a citric acid-induced cough reflex in guinea pigs, with dose-dependent improvements in cough latency compared to the vehicle control group. In addition, *in silico* profiling of the formulation identified chemical constituents with predicted relevance to TRPV1 pathways, supporting a plausible mechanistic hypothesis for observed antitussive response.

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6. Conclusion

This study provides robust preclinical evidence for the antitussive efficacy of *Chandramruta Rasa*, demonstrating dose-dependent prolongation of cough latency and percentage inhibition of cough frequency in a citric acid induced cough in guinea pig model. The antitussive potential of *Chandramruta Rasa* in a citric acid induced cough in guinea pigs showed a dose-dependent increase in cough latency and percentage inhibition of cough frequency, which supports conventional use of *Chandramruta Rasa* in cough-related illnesses. The *in silico* pharmacokinetic profiling discovered compounds that may be linked to biologically significant pathways regulating the cough reflexes, especially in context to TRPV1 and H1-receptors. However, to validate its anticipated actions, pathway-specific assays and receptor-level validation are required.

7. References

1. Yang X, Chung KF, Huang K. Worldwide prevalence, risk factors and burden of chronic cough in the general population: a narrative review. J Thorac Dis. 2023; 15(4):2300-2313. https://doi.org/10.21037/jtd-22-1435 PMid: 37197554. PMCid: PMC10183506.
2. Widdicombe JG. Neurophysiology of the cough reflex. Eur Respir J. 1995; 8(7):1193-1202. https://doi.org/10.1183/09031936.95.08071193 PMid: 7589405
3. Niimi A, Chung KF. Airway inflammation and remodelling changes in patients with chronic cough: Do they tell us about the cause of cough? Pulm Pharmacol Ther. 2004; 17(6):441-446. https://doi.org/10.1016/j.pupt.2004.09.004 PMid: 15564089.
4. Jawla S, Gupta AK, Singla R, Gupta V. General awareness and relative popularity of allopathic, *ayurvedic* and homeopathic systems. J Chem Pharm Res. 2009; 1(1):105-112. https://www.jocpr.com/articles/general-awareness-and-relative-popularity-of-allopathic-ayurvedic-and-homeopathic-systems.pdf
5. Singh VC. Nicholas Piramal India Ltd, Mumbai, Herbal (*Ayurvedic*) drug industry for compliance to quality parameters, regional training course at India international centre, New Delhi.
6. Zhu G, Gulsvik A, Bakke P, Ghatta S, Anderson W, Lomas DA, *et al*. Association of TRPV4 gene polymorphisms with chronic obstructive pulmonary disease. Hum Mol Genet. 2009; 18(11):2053-2062. https://doi.org/10.1093/hmg/ddp111 PMid: 19279160.
7. Bessac BF, Jordt SE. Breathtaking TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control. Physiology 2008; 23:60e70. https://doi.org/10.1152/physiol.00026.2008 PMid: 19074743. PMCid: PMC2735846.
8. Fujimura M, Kamio Y, Hashimoto T, Matsuda T. Cough receptor sensitivity and bronchial responsiveness in

patients with only chronic nonproductive cough: In view of effect of bronchodilator therapy. J Asthma. 1994; 31(6):463-472. https://doi.org/10.3109/02770909409089488 PMid: 7961323.
9. Fujimura M, Songur N, Kamio Y, Matsuda T. Detection of eosinophils in hypertonic saline-induced sputum in patients with chronic nonproductive cough. J Asthma. 1997; 34(2):119-126. https://doi.org/10.3109/02770909709075656 PMid: 9088298.
10. Fujimura M, Ogawa H, Yasui M, Matsuda T. *Eosinophilic tracheobronchitis* and airway cough hypersensitivity in chronic non-productive cough. Clin Exp Allergy. 2000; 30(1):41-47. https://doi.org/10.1046/j.1365-2222.2000.00698.x PMid: 10606929.
11. Drake MG, McGarvey LP, Morice AH. From bench to bedside: The role of cough hypersensitivity in chronic cough. Clin Transl Med. 2023; 13(8):e1343. https://doi.org/10.1002/ctm2.1343 PMid: 37501282. PMCid: PMC10374883.
12. Abozid H, Patel J, Burney P, Hartl S, Breyer-Kohansal R, Mortimer K, Nafees AA, Al Ghobain M, Welte T, Harrabi I, Denguezli M. Prevalence of chronic cough, its risk factors and population attributable risk in the Burden of Obstructive Lung Disease (BOLD) study: A multinational cross-sectional study. EClinicalMedicine. 2024; 68. https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(24)00002-6/fulltext?uuid=uuid%3Acf496d95-4ed4-4b60-aab9-d6faab93b890
13. Lee B, Kwon CY, Suh HW, Kim YJ, Kim KI, Lee BJ, Lee JH. Herbal medicine for the treatment of chronic cough: A systematic review and meta-analysis. Front Pharmacol. 2023; 14:1230604. https://doi.org/10.3389/fphar.2023.1230604 PMid: 37920213. PMCid: PMC10619915.
14. Hu X, Zhang K, Liu T, Zhu XJ. Chronic cough: A review and prospects. Medicine (Baltimore). 2025; 104(41):e45162. https://doi.org/10.1097/MD.0000000000045162 PMid: 41088639. PMCid: PMC12517813.
15. Khemraj K. *Bhaishajya Ratnavali, Kasarogachikitsa*. Ver. Mumbai: Steem Press. 2009; 83:514-515.
16. Koutsoukas A, Simms B, Kirchmair J, *et al*. From *in silico* target prediction to multi-target drug design: Current databases, methods and applications. J Proteomics. 2011; 74(12):2554-2574. https://doi.org/10.1016/j.jprot.2011.05.011 PMid: 21621023.
17. Lipinski CA, Lombardo F, Dominy BW, *et al*. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 1997; 23(1-3):3-25. https://doi.org/10.1016/S0169-409X(96)00423-1
18. Myung Y, Alex GC de Sa, Ascher DB. Deep-PK: Deep learning for small molecule pharmacokinetic and toxicity prediction. Nucleic Acid Res. 2024; 52(W1):W469-W475. https://doi.org/10.1093/nar/gkae254 PMid: 38634808. PMCid: PMC11223837.
19. Keiser MJ, Roth BL, Armbruster BN, Ernsberger P, Irwin JJ, Shoichet BK. Relating protein pharmacology by ligand chemistry. Nat Biotech. 2007; 25(2):197-206. https://doi.org/10.1038/nbt1284 PMid:17287757
20. Forsberg K, Karlsson JA, Theodorsson E, Lundberg JM, Persson CGA. Cough and bronchoconstriction mediated by capsaicin-sensitive sensory neurons in the guinea pig. Pulm Pharmacol. 1988; 1(1):33-39. https://doi.org/10.1016/0952-0600(88)90008-7 PMid: 2980286.
21. Karlsson JA, Lanner AS, Persson CG. Airway opioid receptors mediate inhibition of cough and reflex bronchoconstriction in guinea pigs. J Pharmacol Exp Ther. 1990; 252(2):863-868. https://doi.org/10.1016/S0022-3565(25)20030-1 PMid: 2156065.
22. Paget, GE, Barnes JM. Toxicity Tests. Evaluation of Drug Activities: Pharmacokinetics. Academic Press, London and New York. 1964; 1:135-166. https://doi.org/10.1016/B978-1-4832-2845-7.50012-8
23. Martinez MN. Factors influencing the use and interpretation of animal models in the development of parenteral drug delivery systems. AAPS J. 2011; 13(4):632-649. https://doi.org/10.1208/s12248-011-9303-8 PMid: 21971647. PMCid: PMC3231859.
24. Chung KF, McGarvey L, Song WJ, *et al*., Cough hypersensitivity and chronic cough. Nat Rev Dis Primers. 2022; 8(1):45. https://doi.org/10.1038/s41572-022-00370-w PMid: 35773287. PMCid: PMC9244241.
25. Irwin RS, *et al*. Diagnosis and management of cough: ACCP evidence-based clinical practice guidelines. Chest. 2006; 129(1):1S-23S. https://doi.org/10.1378/chest.129.1_suppl.24S
26. Hirons B, Rhatigan K, Kesavan H, Turner RD, Birring SS, Cho PS. Cough in chronic lung disease: a state of the art review. J Thorac Dis. 2023; 15(10):5823. https://doi.org/10.21037/jtd-22-1776 PMid: 37969279. PMCid: PMC10636467.
27. Belvisi MG, Dubuis E, Birrell MA. Transient receptor potential A1 channels: Insights into cough and airway inflammatory disease. Chest. 2011; 140(4):1040-1047. https://doi.org/10.1378/chest.10-3327 PMid: 21972382. PMCid: PMC3186687.
28. Song WJ, Faruqi S, Klaewsongkram J, Lee SE, Chang YS. Chronic cough: An Asian perspective. Part 1: Epidemiology. Asia Pac Allergy. 2015; 5(3):136-144. https://doi.org/10.5415/apallergy.2015.5.3.136 PMid: 26240790. PMCid: PMC4521162.
29. Tarlo SM, Altman KW, Oppenheimer, *et al*. Occupational and environmental contributions to chronic cough in adults: Chest expert panel report. Chest. 2016; 150(4):894-

907. https://doi.org/10.1016/j.chest.2016.07.029 PMID: 27521735.
30. Szram J, Schofield SJ, Cosgrove MP, Cullinan P. Welding, longitudinal lung function decline and chronic respiratory symptoms: A systematic review of cohort studies. Eur Respir J. 2013; 42(5):1186-1193. https://doi.org/10.1183/09031936.00206011 PMid:23258779
31. Irwin RS, Curley FJ, French CL. Chronic cough: The spectrum and frequency of causes, key components of the diagnostic evaluation, and outcome of specific therapy. Am Rev Respir Dis. 1990; 141(3):640-647. https://doi.org/10.1164/ajrccm/141.3.640 PMid:2178528
32. Mackenzie AJ, Spina D, Page CP, *et al*. Models used in the development of antitussive drugs. Drug Discov Today Dis Models. 2004; 1(3):297-302. https://doi.org/10.1016/j.ddmod.2004.10.009
33. Forsberg K, Karlsson JA. Cough induced by stimulation of capsaicin-sensitive sensory neurons in conscious guinea pigs. Acta Physiol Scand. 1986; 128(2):319-320. https://doi.org/10.1111/j.1748-1716.1986.tb07981.x PMid:3776651
34. Lalloo UG, Fox Aj, Belvisi MG, Chung KF, Barnes PJ. Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea pigs. J Appl Physiol. 1995; 79(4):1082-1087. https://doi.org/10.1152/jappl.1995.79.4.1082 PMid: 8567546.
35. Dicpinigaitis PV, Dhar S, Johnson A, *et al*. Inhibition of cough reflex sensitivity by diphenhydramine during acute viral respiratory tract infection. Int J Clin Pharm. 2015; 37:471-474. https://doi.org/10.1007/s11096-015-0081-8 PMid: 25673148. PMCid: PMC4409637.
36. Benet LZ, Hosey CM, Ursu O, Oprea TI. BDDCS, the rule of 5 and drugability. Adv Drug Deliv Rev. 2016; 176:139-148. https://pubmed.ncbi.nlm.nih.gov/27182629/
37. Azman M, Sabri AH, Anjani QK, Mustaffa MF, Hamid KA. Intestinal absorption study: Challenges and absorption enhancement strategies in improving oral drug delivery. Pharmaceuticals. 2022; 15(8):975. https://doi.org/10.3390/ph15080975 PMid: 36015123. PMCid: PMC9412385.
38. Baker K, Raemdonck K, Dekkak B, Snelgrove RJ, Ford J, Shala F, Belvisi MG, Birrell MA. Role of the ion channel, transient receptor potential cation channel subfamily v member 1 (TRPV1), in allergic asthma. Respir Res. 2016; 17(1):67. https://doi.org/10.1186/s12931-016-0384-x PMid: 27255083. PMCid: PMC4890475.
39. McLeod RL, Mingo G, O'Reilly S, Ruck LA, Bolser DC, Hey JA. Antitussive action of antihistamines is independent of sedative and ventilation activity in the guinea pig. Pharmacology. 1998; 57(2):57-64. https://doi.org/10.1159/000028226 PMid:9691225

Author Contributions

Barad Sandhya prepared *Chandramruta Rasa*, performed in vivo experiment, and wrote full original draft. Paragunde Sheela conceptualized the study and critically review the preparation of the Chandramruta Rasa and data collection.

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