Phytochemical characterization, antioxidant and immunomodulatory evaluation of C-Health Forte: An ayurvedic proprietary medicine

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Abstract

Full Text

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ABSTRACT

Objective: C-Health Forte (CHF) is an Ayurvedic proprietary formulation designed to promote overall health, well-being and immune regulation. It comprises eleven medicinal plants widely used in Ayurveda. This study aimed at phytochemical profiling and pharmacological evaluation of CHF using standardized methods.
Methods: In this study, pharmacological evaluation, including antioxidant assays, immunomodulatory testing by mast cell degranulation assay and metabolite profiling using liquid chromatography mass spectrometry were conducted to assess its efficacy and bioactive contents.
Results: Metabolite profiling using LC-MS revealed the presence of numerous bioactive secondary metabolites, including curcumin derivatives, phenolic compounds, cinnamic acid derivatives and a diverse range of flavonoids, chalcones, ellagitannins, and diterpenoids. A major phenolic compound, gallic acid was quantified using High-Performance Thin-Layer Chromatography (HPTLC). The formulation exhibited promising results in phytochemical analyses, showing a high content of total phenolics and flavonoids. *In vitro* antioxidant assays—such

as DPPH radical scavenging, reducing power assay, hydroxyl radical scavenging, and nitric oxide inhibition—demonstrated significant activity, comparable to the standard antioxidant, quercetin. Immunomodulatory potential was evaluated through the mast cell degranulation assay. The formulation showed dose-dependent inhibition of histamine release induced by compound 48/80.
Conclusion: The results support the therapeutic potential of C-Health Forte as an effective Ayurvedic formulation for promoting immune health and overall well-being.

Introduction

The search for natural remedies to treat human ailments dates back to prehistoric times. Knowledge accumulated through centuries of observation, trial, and error laid the foundation for traditional medical systems such as Ayurveda, Traditional Chinese Medicine (TCM), and Indigenous healing practices across the world. In addition to classical Ayurvedic formulations, contemporary research on medicinal plants has contributed to the development of Ayurvedic proprietary medicines—novel, polyherbal combinations formulated with scientifically validated ingredients aimed at specific therapeutic benefits. Unlike classical formulations detailed in ancient Ayurvedic texts, proprietary medicines are unique, modern innovations that synergize traditional wisdom with current scientific insights. CHF is one such Ayurvedic proprietary formulation, developed to support immune function, promote overall health, and address conditions such as immune deficiency, general debility, and emaciation. Its formulation draws inspiration from classical Rasayana principles—Ayurvedic rejuvenation therapies known for their immunomodulatory and adaptogenic properties.

CHF is a propriety herbal formulation of Arya Vaidya Sala Kottakkal and it contains a blend of eleven well-known medicinal plants: *Hordeum vulgare* (fruit), *Vitis vinifera* (fruit), *Phyllanthus emblica* (fruit), *Withania somnifera* (root), *Musa paradisiaca* (fruit), *Clitoria ternatea* (root), *Terminalia arjuna* (stem bark), *Tribulus terrestris* (fruit), *Piper longum*

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(fruit), *Curcuma longa* (rhizome), and *Saccharum officinarum* (stem). These ingredients are widely recognized in traditional medicine for their broad spectrum of pharmacological properties. Several of the constituents are classified in Ayurveda as Rasayana drugs. For instance, *P. emblica* and *W. somnifera* are known for their antioxidant, anti-aging, and immunomodulatory activities. *W. somnifera* contains withanolides, a group of active compounds shown to modulate immune responses.1 *V. vinifera* and *P. emblica* fruits are rich in antioxidant phytochemicals, making them effective in managing oxidative stress and lifestyle-related disorders.2,3 *C. ternatea* is traditionally used as a nootropic agent to enhance memory and intelligence, while *T. arjuna* bark is valued in cardiovascular health management.4,5 *P. longum* is effective in treating digestive disturbances, enhancing appetite, and aiding detoxification.6 *C. longa* is one of the most extensively studied plants, with applications in inflammation, infection, diabetes, and parasitic diseases.7 *T. terrestris* has also demonstrated diverse pharmacological properties, including hormone regulation and anti-inflammatory activity.8 From an immunological standpoint, allergens or environmental antigens often trigger mast cells to release histamine, leading to allergic responses. Drugs that inhibit this degranulation process—thereby suppressing histamine release—are considered effective anti-allergic or immunomodulatory agents. Ayurveda offers prophylactic approaches that help modulate immunity and maintain health during seasonal, dietary, and lifestyle transitions. CHF represents a contemporary Ayurvedic solution that integrates traditional plant-based wisdom with modern pharmaceutical standards. To gain global acceptance, polyherbal formulations like this require scientific validation and regulatory harmonization using rigorous phytochemical and pharmacological evaluation methods. Establishing the chemical composition, standardizing the preparation, and confirming efficacy through validated *in vitro* and *in vivo* models are essential steps toward its integration into evidence-based medicine.

The present study aims to validate CHF through a multi-faceted approach that includes in vitro antioxidant assays, immunomodulatory testing (mast cell degranulation model), and comprehensive phytochemical profiling using techniques such as LC-MS and HPTLC. These findings will contribute to establishing its safety, efficacy, and quality, thereby supporting its therapeutic application and broader scientific acceptance.

Materials and methods

Extraction

One gram of CHF was extracted separately with 10 mL each of ethanol, hydroalcohol (ethanol: water, 1:1 v/v) and distilled water using the sonication method. The samples were sonicated for 30 min at room temperature, followed by filtration through Whatman No. 1 filter paper. The filtrates were collected and stored at 4 °C until further use for phytochemical and analytical studies. For antioxidant assays, the water extract was used at a concentration of 1 mg/mL.

Total phenolics and flavonoid assay

The total phenolic content (TPC) of the samples was determined using the Folin-Ciocalteu assay as described by Singleton et al.9 The reaction mixture consisted of Folin–Ciocalteu phenol reagent, sodium carbonate (Na_2CO_3) solution, and the sample or standard. Gallic acid was used as the standard, and the results were expressed as mg gallic acid equivalents (GAE) per gram of extract. Total flavonoid content (TFC) was measured using the aluminum chloride colorimetric assay described by Zhishen et al.10 The assay involved sequential addition of sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), and sodium hydroxide (NaOH) to the sample or standard. Quercetin was used as the standard, and results were expressed as mg quercetin equivalents (QE) per gram of extract.

High performance thin layer chromatography

High Performance Thin Layer Chromatography (HPTLC) profiling was performed on aluminium backed pre-coated silica gel 60 F_{254} TLC plates (20 × 10 cm; Merck, Darmstadt, Germany). Samples were applied to the plates by CAMAG Automatic sampler IV. TLC images were documented using CAMAG TLC visualizer on irradiating under 254 nm and 366 nm. Densitometric scanning of the developed plate was performed with a CAMAG TLC Scanner 3 at 254 nm, and 366 nm. Integrated Software Wincats was used for the detection as well as for the evaluation of data. The Rf values of each separated bands were noted.

Quantification of gallic acid

Preparation of standard solution Gallic acid (≥ 98% purity, Sigma-Aldrich) was used as the reference standard. A stock solution was prepared by dissolving 1 mg of gallic acid in 10 mL of methanol. Working standards were prepared by applying varying concentrations of 0.1, 0.3, 0.5, 0.7, and 1.0 μg per spot on silica gel 60 F_{254} TLC plates.

Chromatographic conditions The plates were developed in a pre-saturated twin-trough chamber using the mobile phase: Ethyl acetate: Toluene: Methanol: Formic acid (7:3:1:0.1 v/v/v/v). After development, the plates were air-dried and scanned densitometrically using a CAMAG TLC Scanner at a wavelength of 254 nm. Peak areas corresponding to each concentration of gallic acid were recorded and a calibration curve was constructed by plotting peak area versus concentration (μg/spot).

Sample analysis Test extracts were applied under identical conditions, and the peak area for the gallic acid band in each sample track was recorded. The amount of gallic acid present in the test samples was calculated based on the calibration curve. All measurements were performed in triplicate, and results were expressed as mean ± standard deviation.

LC/MS analysis

LC/MS analysis was done using Agilent 6530 C Q-TOF LC/MS system with HPLC 1260 Infinity II Prime LC series. The separation was done with C−18 reverse phased column (Agilent poroshell 120 EC-C8 μm, 3.0 × 150 mm) with a mobile phase that consists of Acetonitrile (A) and 0.1% formic acid in water (D) in a gradient elution as follows % 0-10-90,2-10-90,6-20-80,10-30-70,14-40-60,20-50-50. ESI ionization technique was employed in negative mode and the general MS parameters were optimized as Nebulizer: 40 psi; source temperature: 325 °C; Nitrogen flow: 10 L/min; jet stream sheath temperature: 345 °C. Total ion chromatogram was extracted using Agilent Mass Hunter software (version 10). The data sets were subjected to spectral peak extraction with a minimum peak height of 1000 counts, and the charge state for each metabolite was restricted to two. Metabolite identification was carried out through the embedded METLIN metabolite database. Metabolites were tentatively identified based on accurate mass (< 2 ppm mass error) and then confirmed through MS/MS fragmentation and spectral library searches. The mass fragmentation was performed with varying collision energy 4 V/100 DA with an offset of 8 V.

Anti-oxidant assays

Various antioxidant activities like diphenyl picryl hydrazyl (DPPH) radical scavenging activity, determination of reducing power activity, hydroxyl radical scavenging activity (OH) and nitric oxide (NO) radical scavenging activity were carried out using reported methods. Quercetin

was used as a standard.11-13

Evaluation of immunomodulatory activity

Experimental animals

Four healthy adult male Wistar rats were used for the study. The animals were maintained under standard laboratory conditions with free access to food and water. All procedures involving animals were carried out in accordance with institutional ethical guidelines and approved by the relevant animal ethics committee.

Mast cell isolation

Mast cells were isolated from the peritoneal cavity of the rats. After humane euthanasia by decapitation and exsanguination, 50 mL of Hank' s Balanced Salt Solution (HBSS) was injected into the peritoneal cavity. The abdomen was gently massaged for uniform distribution, and then the abdominal wall was opened to aspirate the peritoneal fluid containing peritoneal cells. The fluid was centrifuged at 2000 rpm for 10 min, and the cell pellet was resuspended in fresh HBSS. The final concentration of mast cells was adjusted to 1×10^5 cells per 100 μL.

Treatment with test compound and histamine release induction

To evaluate the inhibitory effect of CHF on mast cell degranulation, 1 mL of the test extract at concentrations of 1 mg/mL and 2 mg/mL was added to the mast cell suspension (1×10^5 cells/100 μL). The mixture was incubated at 37 °C for 15 min. Subsequently, the volume was made up to 3 mL with HBSS. An equal volume of calcium ionophore (10^{-6} g/mL) and compound 48/80 (5 μg/mL)—used to induce histamine release—was added to each tube. The suspension was further incubated at 37 °C for 30 min and centrifuged at 2500 rpm for 10 min.

Histamine extraction and o-phthalaldehyde complexing reaction

To quantify released histamine, 1 mL of the supernatant was transferred into a tube containing 300 mg NaCl and 1.25 mL n-butanol. The solution was alkalized by adding 1 mL of 3 N NaOH, vortexed, and centrifuged. From the top organic layer, 1 mL was transferred to a fresh tube containing 2 mL n-heptane and 0.4 mL of 0.12 N HCl. After phase separation, 0.5 mL of the aqueous (bottom) phase was collected for derivatization. To this, 100 μL of 1 N NaOH was added under constant stirring, followed immediately by 100 μL of 0.2% o-phthalaldehyde solution. After exactly 2 min, the reaction was stopped with 50 μL of 3 N HCl. The fluorescence of the resulting complex was measured using a fluorescence spectrophotometer at an excitation wavelength of 350 nm and an emission wavelength of 450 nm, following the method described by Kim et al.14

Results

Total phenolic content

The total phenolic content (TPC) of the extracts was expressed as milligrams of gallic acid equivalents per gram (mg GAE/g) of extract (Table 1). The calibration curve for gallic acid was linear in the concentration range of 100–500 μg/mL, with a correlation coefficient (R^2) of 0.9995, indicating high analytical precision. Among the three extracts tested, the water extract exhibited the highest phenolic content, followed by the hydroalcoholic extract. Similarly, the total flavonoid content (TFC) was quantified and expressed as milligrams of quercetin equivalents per gram (mg QE/g). The standard calibration curve for quercetin was linear in the range of 50–250 μg/mL, with an R^2 value of 0.9993. Consistent with TPC results, the water extract again demonstrated the highest flavonoid content, supporting the presence of a substantial proportion of water-soluble phenolics and flavonoids in the formulation. These results highlight the polarity-driven extractability of bioactive constituents and suggest that the aqueous extract is a rich source of antioxidant phytochemicals.

Table 1
Total phenolics and flavonoid in CHF.

Extract	Phenolic content (mgGAE/g)	Flavonoid content (mgQE/g)
Ethanol extract	2.0405 ± 0.31	1.9032 ± 0.01
Hydroalcohol extract	2.584 ± 0.3	2.5689 ± 0.05

Extract	Phenolic content (mgGAE/g)	Flavonoid content (mgQE/g)
Water extract	2.9630 ± 0.13	2.7687 ± 0.03

HPTLC analysis and Gallic acid quantification

Chromatographic profiling of the samples using High-Performance Thin Layer Chromatography (HPTLC) provided detailed identification and comparative information on the chemical compounds present in the extracts, along with their relative quantities (Fig. 1). This profiling led to the identification of a major phenolic acid, gallic acid, which is reported as the principal compound in *P. emblica* fruit.15 A suitable solvent system was developed for the quantification of gallic acid, providing good resolution. Both the samples and standard gallic acid showed a distinct band at an R_f value of 0.51. The calibration curve was linear over the range of 100–500 ng, with an R^2 value of 0.9997. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 25.75 ng and 78.02 ng, respectively. Among the different extracts evaluated, the percentage of gallic acid increased in the following order: Ethanol extract < Hydroalcohol extract < Water extract, indicating that the water extract is the most effective solvent medium for phenolic acids due to its higher polarity (Table 2). Gallic acid is a well-known phenolic acid with versatile pharmacological efficacy, particularly noted for its potent antioxidant activity. The higher content of gallic acid in the water extract contributes significantly to the strong antioxidant properties exhibited by the sample. Additionally, gallic acid offers numerous health benefits, including antioxidant, anti-allergy, and immunomodulatory effects through mechanisms such as inhibition of lipid peroxidation and enhancement of antioxidant enzyme activity.16 The inhibitory effect of gallic acid on histamine release is mediated by modulation of intracellular cyclic AMP (cAMP) levels and calcium influx. Gallic acid suppresses mast cell-derived inflammatory allergic reactions by blocking histamine release and downregulating pro-inflammatory cytokine expression, which is suggested as the mechanism underlying its immunomodulatory action.17

LC MS/MS analyses

The Q-TOF LC/MS analysis in negative ionization mode led to the identification of numerous active metabolites present in the sample extract. The Base Peak Chromatogram showed many molecular ions which further led to the compound identification (Fig. 2) Most detected compounds were phenolic in nature, encompassing diverse classes such as cinnamic acid derivatives, flavonols, flavonol glycosides, bio-flavonoids, flavones, flavone glycosides, aromatic acids, flavanones, flavanone glycosides, isoflavone glycosides, isoflavanes, chalcone glycosides, phenolic compound glycosides, a labdane diterpenoid, benzoic acid, and ellagitannins (Table 3). Notably, compounds such as bisdemethoxycurcumin and demethoxycurcumin were identified as major constituents derived from *C. longa*.18 The sample also contained cis-resveratrol, a key phenolic compound abundant in *V. vinifera* fruit, along with polydatin, a stilbenoid polyphenol

and bioavailable resveratrol derivative. Several flavonoids and their derivatives commonly reported from *V. vinifera* were also detected, including quercetin, syringetin-3-O-glucoside, rhamnetin, kaempferitrin, and myricetin. Other compounds such as vitexin, phlorizin, naringenin, acacetin, diosmin, quercitrin, and daidzin were also consistent with previous reports from *V. vinifera*.19 From *P. emblica*, major compounds like gallic acid and its derivatives, including methyl gallate and corilagin, were

Fig. 1. Quantification of gallic acid in different extracts of CHF. Track 1 Ethanol extract; track 2 Hydroalcohol extract; track 3 Water extract; track 4-6 Gallic acid.

Table 2
Percentage of gallic acid in CHF.

Extract	Percentage of gallic acid
Ethanol extract	0.071 ± 0.003
Hydroalcohol extract	0.073 ± 0.01
Water extract	0.0917 ± 0.005

identified. Flavonoids such as quercetin and apigenin, as well as cinnamic acid derivatives like caffeic acid, were also prominent.3 Compounds like vitexin, quercitrin, quercetin, andrographolide, myricetin, and methyl gallate were previously reported from the bark of *T. arjuna*.5 Additionally, *C. ternatea* contributed flavonoids such as quercetin, kaempferitrin, and myricetin.20 From *T. terrestris*, important metabolites including dihydroferulic acid, quercetin-3-O-glucoside, quercetin-7-β-O-diglucoside, and rutin were identified.21

Overall, the LC/MS analysis identified 37 flavonoids, representing the largest group of secondary metabolites in the sample extract. These flavonoids included various subclasses such as flavonols, flavones, biflavonoids, flavanones, isoflavones, and their glycosides. Four cinnamic acid derivatives were also documented. Many of the bioactive secondary metabolites identified correspond to those previously reported in the ingredient plants, suggesting that the therapeutic efficacy of CHF arises from the synergistic interactions of these compounds. The combined presence of diverse phenolic compounds and flavonoids may contribute to a broad spectrum of pharmacological activities, thereby supporting the formulation' s beneficial effects on health.

Anti-oxidant activity

The antioxidant potential of the water extract of CHF was evaluated using four standard *in vitro* assays: DPPH radical scavenging, reducing power assay, hydroxyl radical (OH) scavenging, and nitric oxide (NO) scavenging assays. The results were expressed as IC_{50} values ($\mu g/\mu L$), representing the concentration of the extract required to inhibit or achieve 50% activity in each respective assay

(Table 4). The extract demonstrated notable antioxidant activity in all four assays, with performance comparable to the standard antioxidant compound quercetin. In the DPPH assay, the extract showed a strong ability to donate hydrogen atoms or electrons (IC_{50} 1.8433 ± 0.09), efficiently neutralizing free radicals—thus potentially preventing oxidative stress associated with aging and degenerative diseases. The hydroxyl radical scavenging activity indicates the extract's ability to mitigate highly reactive OH radicals (IC_{50} 0.1 ± 0.06) which are known to cause DNA damage, lipid peroxidation, and contribute to inflammatory and neurodegenerative conditions. The NO scavenging assay highlighted the extract's potential in reducing nitric oxide-mediated oxidative damage (IC_{50} 0.85 ± 1.05) which can otherwise form harmful peroxynitrite species when combined with superoxide radicals. Additionally, the extract' s reducing power reflected its electron-donating ability, further supporting its role in neutralizing reactive oxygen species (ROS) and preventing cellular damage.22 Overall, the results suggest that CHF possesses significant antioxidant potential, which may contribute to its therapeutic efficacy in preventing oxidative stress-related disorders.

Fig. 2. LC/MS base peak chromatogram of CHF.

Table 3
Compounds identified in CHF by LC/MS analysis.

Sl. No	Retention time	Molecular ion	Compound name	Fragment ions	Nature of compound
1.	0.359	341.111	Caffeic acid hexoside	179.0577, 101.0251	Cinnamic acid derivative
2.	0.409	377.0880	Oleuropein aglycone	341.1103, 217.0287, 179.0569	Phenolic compound
3.	0.459	463.1487	Quercetin -3-O-glucoside	341.1106, 179.0571, 121.0304	Flavonol glycoside
4.	0.526	431.1434	Vitexin	341.1087	Flavone glycoside
5.	0.559	195.0527	Dihydroferulic acid	159.0311, 129.0202	Hydroxycinnamic acid derivative
6.	0.576	537.1705	Amentoflavone	195.0515	biflavonoid
7.	0.660	447.1384	Quercitrin	105.0202	Flavonol glycoside

Sl. No	Retention time	Molecular ion	Compound name	Fragment ions	Nature of compound
8.	0.676	507.1605	Syringetin -3-O-glucoside	165.0419, 129.0214	Flavonol glycoside
9.	0.710	315.2566	Rhamnetin	141.1303	Flavonol
10.	0.861	295.2302	Coutaric acid	171.1005	Cinnamic acid derivative
11.	0.977	297.2459	3-Hydroxy -3′,4′-dimethoxyflavone	185.1216	Flavone
12.	1.078	165.0421	3-(2-Hydroxyphenyl)propionic acid	125.0242	Aromatic acid
13.	1.212	301.1680	Quercetin	217.0726, 145.0456	Flavonol
14.	1.278	577.3619	Kaempferitrin	281.2471, 154.9970	Flavonol
15.	1.412	299.2615	5-Hydroxy -3′,4′-dimethoxyflavanone	217.0045	Flavanone
16.	1.513	607.3726	Diosmin	546.7273, 393.8734,283.2679	Flavone glycoside
17.	1.529	531.2816	Malonylglycitin	470.7839, 348.8117	Isoflavone glycoside
18.	1.630	307.1912	Bisdemethoxycurcumin	274.8778, 227.1056, 165.4786	Phenolic compound
19.	1.964	269.2142	Apigenin	252.9176 225.2236, 192.8963	Flavone
20.	2.416	435.2107	Phlorizin	378.8624 318.8470, 274.8676	Chalcone glycoside
21.	2.483	271.2304	Naringenin	252.9137 192.8994 118.9413	Flavanone
22.	2.516	239.2037	4′-Hydroxyflavanone	162.8919	Flavanone

Sl. No	Retention time	Molecular ion	Compound name	Fragment ions	Nature of compound
23.	2.567	227.2036	cis-Resveratrol	114.9721	Phenolic compound
24.	2.683	283.2666	Acacetin	224.8945 121.0289	Flavone
25.	2.784	389.2937	Polydatin	309.2039 227.2036 141.0202	Phenolic compound glycoside
26.	2.817	323.1919	Glabridin	281.1101 227.2028	Isoflavane
27.	2.884	477.3957	Isorhamnetin-3-O-glucoside	399.8056 358.8870 290.9165,	Flavonol glycoside
28.	3.068	253.2194	2′,6-Dihydroxyflavone		Flavone
29.	3.085	595.2927	Neoeriocitrin	475.9211,315.1489,270.2357,	Flavanone glycoside
30.	3.185	415.3090	Daidzin	336.2180 253.2198 174.9412,	Isoflavone glycoside
31.	3.420	303.2355	Dihydroquercetin	208.9235 141.0186	Flavanonol
32.	4.490	254.9597	Pinocembrin	216.9232 154.9478	Flavanone
33.	4.691	349.2386	Andrographolide	267.2342 192.8987 112.9830	Labdane diterpenoid
34.	5.243	236.9938	3′-Hydroxyflavone	223.0384 158.9604 114.9714	Flavonol
35.	5.577	255.2349	Liquiritigenin	154.9467	Flavanone
36.	5.694	337.2387	Demethoxycurcumin	255.2343	Phenolic compound
37.	5.744	419.2420	4-Deoxyphloridzin	337.2352 255.2339 164.9933	Chalcone glycoside
38.	5.845	609.4449	Rutin	349.1694 255.2342	Flavonol glycoside
39.	5.995	317.2532	Myricetin	255.2336	flavonol

Sl. No	Retention time	Molecular ion	Compound name	Fragment ions	Nature of compound
40.	6.196	501.2446	Malonyldaidzin	337.2393 255.2345 141.0153	Isoflavone glycoside
41.	6.447	445.2576	Glycitin	363.2487 281.2511 141.0161	Isoflavone glycoside
42.	6.481	585.4902	Quercetin -7-O-galloyl-glucoside	281.2503	Flavonol glycoside
43.	6.681	417.2263	Liquiritin	323.2380 281.2498 112.986	Flavanone glycoside
44.	6.698	625.4338	Quercetin -7-β-O-diglucoside	281.2501	Flavonol glycoside
45.	6.748	521.2441	Iridin	439.2412 377.2068 281.2496	Isoflavone glycoside
46.	6.782	639.5602	Isorhamnetin - 3,7-di-O-glucoside	281.2503	Flavonol glycoside
47.	6.798	487.2299	Acetylglycitin	323.2394 281.2506 182.9844,	Isoflavone glycoside
48.	6.882	182.9905	Methyl gallate	112.9867	Benzoic acid
49.	7.099	311.2980	Caftaric acid	276.8820 196.9070 126.0307	Cinnamic acid derivative
50.	7.501	475.3044	Cirsimarin	358.8935, 311.2941,274.9413,	Flavone glycoside
51.	9.140	633.4409	corilagin	534.7547,438.8930,353.8740	Ellagitannin
52.	9.692	433.2580	Reynoutrin	336.8634 269.2478 208.9292	Flavonol

Immunomodulatory activity

Immunomodulatory activity refers to the ability to regulate or modify the im-

mune response—either enhancing or suppressing it—to prevent or treat diseases. The immunomodulatory potential of CHF was evaluated using a mast cell degranulation assay induced by compound 48/80.23 Compound 48/80 is a basic secretagogue and a potent mast cell activator that triggers degranulation through a receptor-independent mechanism by activating G-proteins involved in mast cell exocytosis, leading to the release of inflammatory mediators such as histamine.24 In the assay, CHF demonstrated a dose-dependent inhibition of histamine release induced by compound 48/80 (Fig. 3, Table 5). Unstimulated mast cells (control) released very low levels of histamine, whereas cells stimulated with compound 48/80 (5 µg/mL) showed a significant increase in histamine release. Treatment with the formulation at low (1 mg/mL) and high (2 mg/mL) concentrations resulted in

Table 4
anti-oxidant activity of CHF.

Sample	DPPH (µg/µl)	Reducing power (µg/µl)	Hydroxyl radical (µg/µl)	Nitric oxide (µg/µl)
CHF	1.8433 ± 0.09	0.68 ± 0.07	0.1 ± 0.06	0.85 ± 1.05
Quercetin	0.04 ± 0.0	0.18 ± 0.08	0.03 ± 0.05	0.11 ± 0.05

Values are mean ± SD, n = 3

Fig. 3. Percent Histamine release.

Table 5
Percent Histamine release.

Sample name	Percent Histamine release	% inhibition of Histamine release
Control	18.16 ± 9.04	-
Compound 48/80	66.73 ± 12.94	− 2.6
Sample low dose (1 mg/mL)	36.49 ± 19.39	45.2
Sample high dose (2 mg/mL)	21.02 ± 11.07	68.4

Values are mean ± SD, n = 3

histamine release inhibition of 45.2% and 68.4%, respectively. These results indicate the immunomodulatory potential of CHF, suggesting its ability to modulate allergic and inflammatory responses by stabilizing mast cells and inhibiting histamine release.

Discussion

Quantitative analyses demonstrated a significant presence of natural phenolic compounds and flavonoids in the sample extracts. Flavonoids emerged as the predominant group, as confirmed by both spectrometric estimations and mass spectrometry profiling. These polyphenolic compounds are well-known for their dual roles in antioxidant defense and immunomodulation. Flavonoids neutralize free radicals and reactive oxygen species, thereby mitigating oxidative stress linked to chronic inflammation and immune dysregulation. Moreover, flavonoids act as natural antihistamines by stabilizing mast cells and basophils, preventing their degranulation and excessive histamine release. They inhibit key enzymes such as protein kinase C and suppress calcium influx, crucial steps in mast cell activation, making them valuable in treating allergies, inflammation, and histamine intolerance.25 Previous studies have shown flavonoids like quercetin and naringenin modulate macrophage functions by downregulating glycolytic activity, enhancing antioxidant defenses, exerting anti-inflammatory effects, and modulating osmoregulation and membrane properties.26 Further research indicated that the biological properties of flavonoids may result from synergistic interactions, potentially generating novel bioactivities beyond those of individual components.27 Flavonoids such as apigenin, luteolin, 3,6-dihydroxy flavone, fisetin, kaempferol, quercetin, and myricetin have been shown to inhibit hexosaminidase release, a key marker in allergic responses, with their structure-activity relationships elucidated.28 Additionally, luteolin, apigenin, and fisetin inhibited histamine and cytokines (IL-4, IL-13) and CD40 ligand expression in basophils and mast cells, highlighting the structural basis for their immunosuppressive activity.29

The immunomodulatory potential of CHF was evaluated using a mast cell degranulation assay, where the sample dose-dependently inhibited histamine release induced by compound 48/80. Alongside this, the sample exhibited notable antioxidant activity across four different assays, showing comparable efficacy to the standard compound quercetin. A positive correlation between the immunomodulatory and antioxidant activities was observed, suggesting intertwined mechanisms. It has been reported that populations consuming diets high in flavonoids demonstrate lower rates of asthma and reduced allergic symptoms.30 Besides flavonoids, cinnamic acid derivatives such as caffeic, ferulic, and p-coumaric acids also exhibited immunomodulatory effects, mainly attributed to their antioxidant capacity and modulation of immune cell functions.31 Overall, plant-derived polyphenols hold promise as anti-allergic agents and key sources for novel drug discovery.32 The component plants of CHF contribute various protective effects. *V. vinifera* fruit offers antioxidant, cardioprotective, hepatoprotective, and neuroprotective benefits.33,34 *P. emblica* fruit enhances immune function by elevating CD4, CD8, CD16, CD19, IgM, IgG, albumin, and globulin levels and exhibits chemoprotective and cardioprotective properties.3 *W. somnifera*, a well-known immunomodulator in traditional medicine, stimulates immune activity and has a role in type 2 allergic diseases,1,35 with extensive

studies confirming its efficacy in immune inflammation models.36 Immune regulation remains a vital focus amid lifestyle-driven health changes. Natural products from medicinal plants, such as those in CHF, continue to gain prominence as prophylactic agents due to their multifaceted bioactivities and lower side-effect profiles compared to synthetic drugs. Ayurvedic formulations, traditionally used to promote overall well-being, require further large-scale clinical trials and detailed pharmacological investigations to fully optimize their integration into modern healthcare.

Conclusion

The scientific validation of CHF, an Ayurvedic proprietary formulation, was conducted by integrating modern scientific methodologies. Pharmacological evaluations revealed that CHF possesses notable antioxidant activity and significant immunomodulatory effects. Active metabolites in the sample were profiled using LC-MS which identified a substantial quantity of flavonoids, known immunomodulators likely contributing to the formulation' s beneficial effects. Quantification of gallic acid, the major phenolic compound detected, provides a reliable marker for routine quality control. This study underscores the importance of systematic research on newly developed Ayurvedic formulations like CHF, bridging traditional knowledge and modern science to advance holistic and effective healthcare aimed at maintaining health.

CRediT authorship contribution statement

C T Sulaiman: Conceptualization, Project administration, Supervision, Writing –original draft. **Deepak M:** Investigation, Methodology. **Syamily Sivadas:** Formal analysis, Investigation. **Anandan E M:** Resources. **Indira Balachandran:** Writing –review & editing. **P M Varier:** Project administration, Supervision.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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