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Immunomodulatory and AntiOxidant Potential of Polyherbal *Dhatryadi Rasayana* in the Form of Churna and Granules

Sheenam Rani,* Usha Sharma, Manish Purushottam Deshmukh, Vipin Kumar, Khem Chand Sharma, Mayank Kumar Malik, and Vetriselvan Subramaniyan

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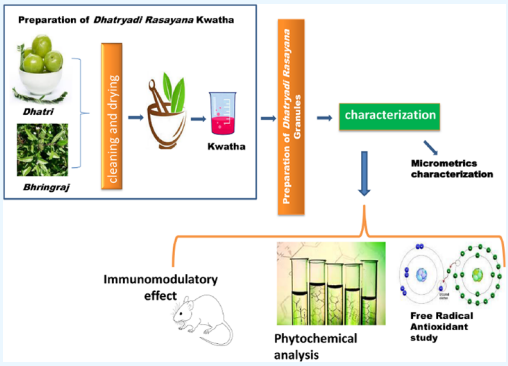
ABSTRACT: *Dhatryadi Rasayana* revitalizes the human body and helps in maintaining health with the elimination of ill effects of various diseases. The effective delivery systems for *Rasayana* may affect the profound effect of active principles in the body. The present study deals with investigation and evaluation of phytochemical constituents, physicochemical characteristics, along with antioxidant and immunomodulatory effects of *Dhatryadi Rasayana* in churna (powder) and granule formulations. *Dhatryadi Rasayana* churna and its granules were studied for various physicochemical parameters, e.g., moisture content, ash-value, acid-insoluble ash content, water-soluble extractive, alcohol-soluble extractive, bulk density, tapped density, angle of repose, Carr's index, Hausner's ratio, total sugar, reducing sugar, non-reducing sugar, heavy metals, total microbial load, etc. *In vitro* antioxidant potential of *Dhatryadi Rasayana* churna and its granules was determined by scavenging the DPPH and FRAP assays. The immunomodulatory activities of *Dhatryadi Rasayana* churna and its granules were studied in Wistar albino rats and the complete blood count (CBC), delayed-type hypersensitivity reaction (DTH), and hemagglutination antibody titer were assessed. *Dhatryadi Rasayana* churna contained alkaloids (0.50 ± 0.298% w/w), tannins (9.84 ± 1.527% w/w), saponins (4.18 ± 2.126% w/w), and flavonoids (9.34 ± 1.026% w/w), while its granules contained 11.08 ± 2.468% w/w total tannins, 2.40 ± 1.132% w/w alkaloids, and 12.46 ± 2.645% w/w total flavonoids. The DPPH scavenging effect was determined by IC_{50} (churna - 23.89 μg/mL; granules - 9.33 μg/mL), and the antioxidant capacity assessed by FRAP was 77.0 mmol/100 g equivalent of ascorbic acid for churna and 50 mmol/100 g equivalent of ascorbic acid for granules. *Dhatryadi Rasayana* churna and its granules reflected a significant immunostimulatory effect on both the cell-mediated and humoral immune systems in Wistar albino rats. Moreover, churna and granules of *Dhatryadi Rasayana* revealed significant antioxidant and immunomodulatory activities and these may be applied for treating different diseases as well as improving the immunity of the body.



1. INTRODUCTION

Rasashastra and *Bhaishajya Kalpana* are the foremost branch of "Ayurveda" that chiefly deal with the formulation of medicines from various natural resources. The mechanism of trans-formation where the drug is altered or its characteristics are augmented, is known as "Samskara."[1] Conversion of the formulation into a more suitable form is a pressing priority along with additional benefits of palatability and dosage forms. *Khanda Kalpana* is an amended form of *Avaleha* preparations. The root meaning of the term "*khanda*" or "*khandaka*" is "a fragment or a piece." The "*Khanda Kalpana*" is the granular form of the formulation.[2] Granules are commonly applied in the production of the pharmaceutical oral dosage form and "granulation" is defined as the size enlargement process in which fine and smaller particles are aggregated to form strong and stable particles called granules.[3,4] Granules produce particle-size uniformity and hence are responsible for content uniformity. Granules are usually more stable toward the impacts of atmospheric humidity and are less likely to harden upon standing.

Dhatryadi Rasayana is one of the valuable formulations mentioned in Chakradutta,[5] Bhaishajya Ratnavali,[6] Rasa Kamadhenu,[7] Vrindamadhava, or Siddha Yoga[8] and Yoga Ratnakara.[9] It consists of three ingredients: Dhatri (*Emblica officinalis* Gaertn.), Bhringraj (*Eclipta alba* Hassk.), and Tila (*Sesamum indicum* Linn.). This *Rasayana* revitalizes the human body, helps in prolonging health, eliminates the ill effects of various diseases, and provides long, strong, as well as black hair. Dhatri (*Emblica officinalis* Gaertn.) has been known for its

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antioxidant, antiaging, and hepatoprotective properties.[10] In the ethnic system of medicine, Bhringraj is also known as the "King of hair", reported as a hepatoprotective, anti-inflammatory, analgesic, and promoter of blackening and growth of hair.[11] Tila (*Sesamum indicum* Linn.) seeds are depicted as "seeds of immortality" for their resistance to oxidation and rancidity even when stored at ambient air temperature and are also known as "the king of oil" due to the rich oil content (50−60%) of its seed.[12] In the current investigation, an attempt has been made to prepare *Dhatryadi Rasayana* churna and its granules, along with a comparison of their analytical parameters. Pharmaceutical standardization of both of these formulations has not been established yet, which is the first step toward research on any formulation.

The current scenario is witnessing a number of lifestyle disorders due to mankind's hectic, sedentary, and busy schedule. Poor dietary choices and negligence toward one's health have deteriorated the situation further. There are several infectious diseases that are the leading causes of morbidity and mortality.[13] The immune system has two distinct but overlapping defense mechanisms with which it fights invading organisms: the cell-mediated defense system and the antibody-mediated defense system. A strong well-functioning immune system is the cornerstone of good health.[14,15]

There are several Ayurvedic formulations that enhance the overall natural resistance of the body to infectious agents. Such formulations possessing immunomodulatory effects are referred to as *Rasayana* in Ayurvedic classics. An immunomodulator is a substance of biological and/or synthetic origin, which can suppress (immunosuppressors) or modulate or stimulate (immunostimulants) any of the components of the immune system including both adaptive and innate arms of the immune response. *Rasayanas* are claimed to possess rejuvenating and immunomodulatory properties.[6] *Rasayana* therapy is defined as a rejuvenating agent for the prevention and promotion of health and cure from diseases in all ages and stages of life. One such *Rasayana* formulation is "*Dhatryadi Rasayana*" that is a polyherbal formulation consisting of Amalaki (*Emblica officinalis* Gaertn.), *Bhringraj* (*Eclipta alba* Hassk.), and Tila (*Sesamum indicum* Linn.).[7] *Dhatryadi Rasayana* keeps hair long, strong, and black, maintains proper functioning of the body, and provides longevity devoid of illness.[8]

The objective of the current investigation was to develop the granules of the *Dhatryadi Rasayana* and to compare the immunomodulatory benefits of the *Dhatryadi Rasayana* granules over *Dhatryadi Rasayana* churna. In the current study, the classical *Dhatryadi Rasayana* churna and its granules were analytically investigated for their organoleptic and other physicochemical parameters. Heavy metal analysis as well as the total microbial overload were also determined. Phytochemical components, such as total tannins, total alkaloids, total saponins, total flavonoids, and *in vitro* antioxidant potential, of *Dhatryadi Rasayana* churna and its granules were determined by scavenging DPPH and FRAP assays.

Further, the immunomodulatory activity of *Dhatryadi Rasayana* churna and its granules was also studied in Wistar albino rats. In this study, cyclophosphamide administered orally in animals was used as control, and the delayed-type hypersensitivity reaction (DTH), complete blood count (CBC), and hemagglutination antibody titer were explored using standard protocols.

2. MATERIALS AND METHODS

The raw materials required for the preparation of *Dhatryadi Rasayana* churna and its granules like *Dhatri* and *Bhringraj* were collected from Rishikul Campus, Haridwar, and *Tila* was collected from the local market of Haridwar and certified by the P.G. Department of Dravyaguna. In this study, fruits of *Dhatri*, whole plant of *Bhringraj*, and seeds of *Tila* were applied. All of the reagents and chemicals applied in this investigation were of analytical grade.

2.1. Preparation of *Dhatryadi Rasayana* Churna. *Dhatri* (*Emblica officinalis* Gaertn.) *and Bhringraj* (*Eclipta alba* Hassk.) were dried in sunlight and shady area, respectively. They were powdered individually in a mixer grinder and sieved through mesh no. 80 to obtain a fine powder. *Tila* was dried in sunlight to make it free of moisture and grinding was done in a mixer grinder. All the powders were taken in equal amounts and mixed in mortar and pestle. After mixing, sieving was done through the sieve (#80) and filled in an airtight container.[7]

2.2. Preparation of *Dhatryadi Rasayana Kwatha* (Decoction). After proper identification, cleaning, and drying, all the ingredients were taken in a specific amount. Each and every drug was crushed into *Yavkut* form separately with the help of an iron mortar and pestle. The raw drugs were mixed with portable drinking water in a stainless-steel vessel and kept for overnight soaking (12 h). Constant mild heat was applied to enable the evaporation, and stirring was performed internmentally until the volume reduced to one-fourth of the initial volume to obtain maximum water solvent extractives. After that, *Kwatha* was filtered through four-folded muslin cloth and stored in a separate vessel for further processing. The residue was discarded. The components of *Kwatha* are listed in Table 1.

Table 1. Preparation of *Kwatha* (decoction) from the Ingredients of *Dhatryadi Rasayana*

components	quantity
initial qty of *Kwatha Churna*	200 g (each 67 g)
total quantity of water	1600 mL
total time for soaking	12 h
total time taken for *Kwatha*	1:45 h
total quantity of *Kwatha* obtained	400 mL
weight of residue after filtration	428 g

2.3. Preparation of *Dhatryadi Rasayana* Granules. After the *Kwatha* preparation, *Sharkara* (sugar) was added to the prepared *Kwatha* (*Sharkara* was taken in equal quantitites as *Kwatha*) and heat was applied (95−100 °C). It was then boiled for evaporation of the water content up to the desired level. After 20 min, sodium benzoate as a preservative was added and stirred properly. During this process, there was a reduction in the volume of the liquid as well as a change in consistency. After attaining two threads consistency of *Drava Dravyas,* a homogenous blend was formed in lumps during continuous stirring. The lump was passed through the sieve (#10) to get granules. Later on, *Dhatryadi Rasayana* granules were kept for drying at 55 °C in a hot air oven. The dried granules were weighed and stored in air-tight containers. The composition of granules is shown in Table 2.

2.4. Estimation of Physiochemical Parameters of *Dhatryadi Rasayana* Churna and Its Granules. The powder and granule form of *Dhatryadi Rasayana* were

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Table 2. Formulation Composition of *Dhatryadi Rasayana* Granules

basic ingredients	ingredients	quantity
Drava Dravyas	*Dhatryadi Rasayana Kwatha*	400 mL
sweetening agent	*Sharkara* (sugar)	400 g
preservative	sodium benzoate	80 mg

investigated for various properties including total ash content, acid-insoluble ash content, loss on drying, pH, alcohol, and water-soluble extractive. For the estimation of total ash content, pH, and loss on drying, the methods used in our earlier reported literature were applied.[16] Briefly, the total ash content was determined by ignition (500 °C) of 2 g of the air dried powder and granules in a muffle furnace. Further, for the estimation of the acid-insoluble ash content, hydrochloric acid (25 ml) was added to the ash (100 mg) stored in a silica crucible. The pH of the powder and granules were measured from the aqueous dispersion (1% w/v) of each of the formulation after filtration through Whatman filter paper (No. 42) at room temperature using a digital pH meter. The measurement of each sample was performed in triplicate and the results were expressed as the mean of measured observations. The content of total sugar was calculated by considering contents of reducing and non-reducing sugars. The protocols followed by Hernández-López et al. were used without modification for the estimation of the reducing and non-reducing sugars.[17]

2.4.1. Water-Soluble and Alcohol-Soluble Extractive. A 2 g sample of each air-dried formulation was macerated in a closed flask with ethanol (100 mL, 95% v/v) for 24 h with intermittent shaking for the initial 6 h and then allowed to stand for 18 h. Thereafter, the filtrate was evaporated to dryness (dried at 105 °C) and weighed. Finally, the percentage of ethanol-soluble extractive with reference to air-dried formulation was calculated. The similar method was followed for the estimation of water-soluble extractive. However, ethanol was replaced with water.

2.4.2. Heavy Metal Detection. Detection of heavy metals was done by atomic absorption spectrophotometry by the procedure reported elsewhere.[18,19]

2.4.3. Total Microbial Load Estimation. The microbial load of each of the formulations was carried out by the pour plate method. Casein soy bean digest agar medium was used for this purpose. The soyabean agar medium containing 1 g/mL of the formulation was kept at 10 °C for 1 h and incubated at 37 °C for 24 h in the BOD incubator (NSW-152, New Delhi). The number of colonies formed after the incubation period were counted. Further, for the determination of the total fungal count, the potato dextrose agar medium was used. The plates were incubated for 72 h at 25 °C. The existence of designated microbial species in the samples was assessed by applying selected media like cetrimide agar (*Pseudomonas sps.*), mannitol salt agar medium (*Staphylococcus aureus*), deoxycholate citrate agar medium (*Salmonella*sps.), and MacConkey agar medium (*Escherichia coli*).[19]

2.4.4. Micrometrics Characterization. The formulations were characterized for various micrometrics properties like bulk and tapped density, porosity, Hausner's ratio, Carr's index, and angle of repose.

Density of Powder. The liquid displacement method was applied for the determination of the density of the powder. For that, pycnometer and the following expression were used:

$$\text{Density} = \frac{w}{[(a + w) - b] \times \text{SG}} \quad (1)$$

where "*w*" is the weight of the preparation, "SG" is the specific gravity of the solvent (*n*-hexane), "*a*" is the weight of the pycnometer with solvent, and "*b*" is the weight of the pycnometer with powder and solvent.

Bulk Density and Tapped Density. Each formulation was poured freely in a measuring cylinder (10 mL) upto the mark. The initial volume occupied by the powder was noted down and represented as bulk volume and the weight as bulk mass.[16,20] The bulk density was determined by using the following expression:

$$\text{Bulk density} = \frac{\text{Mass of formulation}}{\text{Bulk volume occupied by formulation}} \quad (2)$$

While, for the determination of tapped density, the measuring cylinder filled with formulation was tapped up and down until the filled formulation acquired a consistent volume. Aluminum foil was placed over the measurement cylinder's open end to stop fines from being lost from dusting during the tapping process. The final constant volume was measured, recorded, and shown as the tapped volume. The tapped density was calculated by using the following formula:

$$\text{Tapped density} = \frac{\text{Tapped mass of the formulation}}{\text{Tapped volume of the formulation}} \quad (3)$$

Porosity. Porosity is the empty space that is occupied by the mass of the powder along with the entrapped air.[16,20] It was determined by the following expression:

$$\text{Porosity (\%)} = \frac{\text{Bulk volume} - \text{Tapped volume}}{\text{Tapped volume}} \times 100 \quad (4)$$

Carr's Index and Hausner's Ratio. Carr's index has been implemented as an indirect technique to evaluate the powder flow ability from bulk density.

The lower the number, the more the free-flowing powder. The % compressibility of a powder is a direct indicator of the possible powder arch or bridge strength and stability. Both the Hausner's ratio and Carr's index were calculated by using the following equations:[19,21,22]

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{bulk density}}{\text{Tapped density}} \times 100 \quad (5)$$

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad (6)$$

Angle of Repose. It was determined by heap method reported elsewhere[13,22] using the following expression:

$$\text{Angle of repose} = \frac{h}{r} \quad (7)$$

where "*r*" is the radius of heap made by the powder and "*h*" is the height of heap.

2.5. Phytochemical Screening of Powder and Granules of *Dhatryadi Rasayana*. *2.5.1. Estimation of Total Alkaloids and Total Tannins.* In this study, the sample of the formulation was macerated with aqueous acetic acid (2% v/v), filtered, and the filtrate was concentrated to one-third of the

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original volume under reduced pressure at 45 °C. The pH was adjusted to 2 by 4 M HCl. The yellow precipitate obtained was separated from solution A and dissolved it in 0.1 M HCl to give solution B. Mayer's reagent was added to solutions A and B to give a precipitate of alkaloid−Mayers reagent complex. It was dissolved again in acetone−methanol−water (6:2:10 v/v/v) to give the resultant mixture and then, it was passed finally through Amberlite IRA 400 anion exchange resin (500 g) to give an aqueous solution of alkaloid chlorides.[23]

2.5.2. Estimation of Total Tannins. A 2 g sample was defatted with 25 mL of petroleum ether for 12 h, and then the marc was boiled for 2 h with 300 mL of double-distilled water. It was cooled, diluted to 500 mL, and filtered.

A 25 mL portion of this infusion was poured into a 2 L porcelain dish, and 20 mL of Indigo solution was added along with 750 mL of double-distilled water. It was titrated with 0.1 N potassium permanganate solution until the blue solution was changed to green. Thereafter, it was added until the solution was turned golden yellow in color. Similarly, the mixture of 20 mL of Indigo solution and 750 mL of double-distilled water was titrated. The difference between the two titrations was calculated in mL. Each mL of 0.1 N potassium permanganate solution was equivalent to 0.004157 g of total tannins.[23]

2.5.3. Estimation of Total Saponin Content. Standard saponin solution was prepared by dissolving 10 mg of diosgenin, and 16 mL of methanol was added along with 4 mL of distilled water. To the aliquots for each tube was added vanillin reagent (8% w/v, 0.25 mL). Besides this, sulfuric acid (72% v/v, 2.5 mL) was also added slowly on the inner side of the wall. The solutions were mixed well and the tubes were transferred to a 60 °C water bath. After 10 min of incubation, the tubes were cooled in an ice-cold water bath for 3−4 min. The absorbance was measured at 544 nm against the reagent blank. A 0.1 g sample of freeze-dried solution was dissolved in aqueous methanol (80%, 0.1 mL). A 0.25 ml of aliquot was taken for the spectrophotometric determination of total saponins at 544 nm.[24]

2.5.4. Estimation of Total Flavonoids. Total flavonoid contents were measured as quercetin equivalent (QE) per gram of dried samples by comparison with quercetin. Standard curve of quercetin was drawn to compare and calculate the QE of the samples. Standard or sample solution (0.2−1 mg/mL) was made up to 1 mL volume, containing 160 μL of distilled water, 15 μL of 5% $NaNO_2$, and 100 μL sample solution. After 5 min, 15 μL of 10% $AlCl_3$ was added to the mixture. At the 6th min, 100 μL of 1M NaOH was added, and the volume was made up to 1 mL with distilled water. The absorbance was measured using a UV−visible spectrophotometer at 510 nm and the corresponding concentration of flavonoids in samples was determined.[10]

2.6. Estimation of Powder and Granules of *Dhatryadi Rasayana* for *In Vitro* Antioxidant Activity. *2.6.1. DPPH Radical Scavenging Activity.* A 1 g sample was extracted from methanol (50%, 50 mL) in a conical flask. On the next day, the mixtures were filtered via Whatman filter paper (No. 1) and the content was made up to 100 mL with 50% methanol (Standard solution). 1 mL solution was taken from the standard solution in another volumetric flask, and the volume was diluted with 50% methanol. From this stock solution, working solutions of 10, 20, 30, and 40 μg/mL concentrations were prepared in the case of the *Dhatryadi Rasayana* churna sample. The antioxidant activity was determined from these stock solutions. Similarly, from the stock solution of *Dhatryadi Rasayana* granules, the working solutions of 2, 4, 6, 8, 10, and 12 μg/mL concentrations were prepared. These working solutions were used for the estimation of antioxidant activities.[25]

2.6.2. Ferric Oxide Reducing Antioxidant Power Assay. A 1 g sample was taken and dissolved in 25 mL of water in a conical flask. The conical flask was shaken in a rotary shaker for 6 h and kept for 18 h. On the next day, the sample was filtered with Whatman filter paper (No. 1) in a crucible and evaporated until dryness. Afterwards, the residue was dissolved in 100 mL of water. A 1 mL portion of this stock solution was taken in a volumetric flask, and the volume was made upto 100 mL with water. The working solution was used for the antioxidant property estimation. The working solutions of ascorbic acid were prepared with different concentrations of 100, 200, 400, 600, 800, and 1000 μmol/L and used for calibration.[26]

2.7. Immunomodulatory Activity of Powder and Granules Formulations of *Dhatryadi Rasayana*. The approval by the institutional animal ethics committee (Ref. DMIHER/IAEC/22-23/19) was used for the proposed protocols. In this study, cyclophosphamide injections of IP 1000 mg and Levamisole 50 mg were used. Cyclophosphamide was used as an immunosuppressant drug (negative control). Levamisole hydrochloride (50 mg) was used as a positive reference standard immunomodulatory drug (positive control). In this study, healthy male and female Wistar rats, aged between 4 and 6 weeks and weighing between 150 and 200 g, were taken. The animals were acclimatized to the laboratory prior to starting the experiments and were fed with a chow diet and regular drinking water. The rats were kept in cages in groups of six animals per cage, in a temperature-controlled environment at 23 °C (±2). *Dhatryadi Rasayana churna* and its granules were administered in the rats orally for 2 weeks. Rats were divided randomly into 5 groups (n=6/group). The treatment period for the study was 2 months.

2.7.1. Antigen Preparation. Sheep blood was obtained from a local slaughter house in a sterile bottle containing anticoagulation agent (Alsever's solution) (1:2, sheep blood: Alsever's solution) to prevent the coagulation of blood. Blood was centrifuged at 2000 rpm for 10 min to enable RBCs to settle down (sedimented, SRBCs). The supernatant was discarded, leaving SRBCs pellets. The SRBCs were washed three times with phosphate buffer saline (Pyrogen free, pH 7.2) and then kept in refrigeration for use in the challenge and immunization study.

2.7.2. Group Treatment and Dosing of Animals. Five groups of animals were used in this immunomodulatory experimental study. In control group A, the normal saline (10 mL/kg) body weight (bw) was administered orally. In negative control group B, cyclophosphamide (100 mg/kg bw.) was administered subcutaneously, and in standard group C, cyclophosphamide (100 mg/kg bw) and levamisole (50 mg/kg) were administered. In experimental group 1 (Test 1, D), cyclophosphamide (100 mg/kg bw) and *Dhatryadi Rasayana* churna (500 mg/kg bw) were administered orally. However, in experimental group 2 (Test 2, E), cyclophosphamide (100 mg/kg bw) and *Dhatryadi Rasayana* granules (500 mg/kg bw) were given.

2.7.3. Determination of Complete Blood Count. A 2 mL amount of fresh blood was collected by retro-orbital puncture from each of the animals from groups B (negative control), A (control), C (standard), E (Test 2), and D (Test 1) on day

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14th into the EDTA-containing containers. The blood sample was analyzed by hematological analyzer for the complete blood count.

2.7.4. Determination of Delayed-Type Hypersensitivity Responses. On the 7th day of the study, groups C (Standard), D (Test 1), and E (Test 2) were injected subcutaneously with 0.1 mL of 10% SRBCs in NS (normal saline) into the right hind footpad. The contralateral paw was similarly given an identical amount of 0.1% phosphate-buffered saline (PBS). The administration of *Dhatryadi Rasayana* churna and its granules was continued until the 14th day. On the 14th day, animals were injected subcutaneously with 0.1 mL of 10% SRBCs in NS into the left hind footpad of the rats. A vernier calliper was used to measure the footpad thickness after 4, 8, and 24 h in order to assess the degree of delayed-type hypersensitivity (DTH) reaction in the rats.[27] The change in the thickness of the left hind paw and right hind paw was used as an extent of DTH reaction by the following expression (8) and was expressed as a mean percent increment in thickness/edema:

$$\frac{\text{Left foot pad challenged with antigen} - \text{Right foot pad control}}{\text{Left foot pad challenged with antigen}} \times 100 \quad (8)$$

2.7.5. Hemagglutination Antibody Titre Test. The animal's immunization was done by injecting 0.1 mL of SRBCs intraperitoneally (ip) on the 7th day of the experiment. The administration of *Dhatryadi Rasayana* churna and its granules was continued for another 7 days until day 14 and blood samples were collected by retro-orbital puncture. Blood was centrifuged at 2000 rpm for 10 min to obtain the serum. Antibody titers were determined by the hemagglutination technique. For antibody titer determination, serial 2-fold dilutions were made in normal saline in microtiter plates of 96-well capacity. The SRBCs (25 μL, 1% SRBC in normal saline) were added to these dilutions. The titer plate was incubated at 37 °C for 1 h and examined for hemagglutination. The reciprocal of the highest dilution of test serum giving agglutination was taken as the hemagglutination antibody titer (HA units/μL).[28]

2.8. Statistical Analysis. In cases wherever necessary, the investigations were employed in triplicate (n=3). Statistical analysis was performed by ANOVA to assess the significance. Further, Student's t test (p<0.05) was also performed using Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1. Preparation of *Dhatryadi Rasayana* Churna and Its Granules. *Dhatryadi Rasayana* acts as a rejuvenating agent for the promotion of health, prevention, and cure from diseases in all ages and stages of life and consists of three ingredients *Dhatri* (*Emblica officinalis* Gaertn.), *Bhringraj* (*Eclipta alba* Linn.), and *Tila* (*Sesamum indicum* Linn.). *Dhatryadi Rasayana* churna is a polyherbal formulation prepared using powders of all three ingredients in equal amounts and sieved through the sieve (#80). Firstly, *Dhatri* and *Bhringraj* were dried in sunlight and shady area, respectively, in order to make them free of moisture. Thenceforth, the grinding was done by a mixer grinder and sieved through mesh (#80). *Tila* was also dried in sunlight to obliterate the moisture and were grinded using a mixer grinder, but cannot be sieved as they contain oil in them. For the preparation of *Dhatryadi Rasayana* granules, *Dhatryadi Rasayana Kwatha* (decoction) was prepared using all three ingredients in equal amounts. On adding *Sharkara* (sugar) to *Kwatha,* excessive frothing was seen that required continued stirring. As the moisture content reduced in sugar syrup, cohesive force increased, and further application of heat imparted kinetic movement to the sugar molecules, whereas when it was cooled, the loss of kinetic movement made the sugar molecules to coalesce. The average time required for the preparation of granules was upto 5 h. After adding *Sharkara* (sugar) to the *Kwatha*, it took an average of upto 2 h to attain the consistency and an additional 20−30 min for the formation of desired consistency required for the preparation of granules. The temperature during the whole process was maintained at 100 °C for optimum preservation of active constituents in the product.

3.2. Physicochemical Characterization of *Dhatryadi Rasayana* Churna and Its Granules. If the moisture content is above the permissible limit, then the formulation is more likely to get infected by fungal growth. Loss on drying at 105 °C signified the amount of moisture present in the formulation. In prepared *Dhatryadi Rasayana* churna and its granules, the moisture content was 4.14 ± 0.612% w/w and 2.23 ± 0.321% w/w, respectively, which indicated that the granules had more stability than churna. It was consistent with the data of loss on drying for which the results of churna and granules were 2.35 ± 0.111% and 0.05 ± 0.014% w/w, respectively. Acid-insoluble ash represented the presence of inorganic content, which is not expected in the herbal formulation. The obtained value of acid-insoluble ash in churna was 1.28 ± 0.075% w/w and for granules, it was 0.17 ± 0.020% w/w. The total ash-value was assessed to determine the authenticity and purity of the medicine. Higher the ash value, more is the contamination, adulteration, or substitution. The total ash value was found to be 7.48 ± 0.085% w/w for churna and 2.73 ± 0.015% w/w for granules. The extractive value, namely, water soluble and alcohol soluble indicated the content of active constituents present in the given amount of formulation. Water-soluble extractive for churna was 0.35 ± 0.071% w/w and for granules it was found to be 91.77 ± 1.293% w/w. The soluble contents are also related with dissolution/solubilization of formulation in gastrointestinal tract and assimilation along with other liquid media. The active constituents soluble in water are easily soluble in a medium like saliva, which in turn helps in faster absorption from the oral cavity itself. It also makes it combatively more soluble in water. The pH of the aqueous dispersions of these preparations was also determined. The results of pH estimation indicated the slightly acidic pH of both preparations. The pH of *Dhatryadi Rasayana* churna was found to be 4.10 ± 0.985 and for its granules was 4.47 ± 0.112. The preparations were also assessed for micromeritic properties, namely, tapped density, bulk density, porosity, flow property, compressibility, etc. The tapped density for churna was 0.54 ± 0.021 g/mL and for granules, it was 0.50 ± 0.066 g/mL. The bulk density for churna was 0.38 ± 0.021 g/mL and for granules, it was 0.48 ± 0.01 g/mL. The angle of repose, Carr's index, and Hausner's ratio indicated the flowability of powder and granules. The Carr's index for churna was 27.33 ± 1.527 and for granules, it was 6.04 ± 0.613. Hausner's ratio for churna was 1.37 ± 0.020 and for granules, it was observed as 1.09 ± 0.063. Both the parameters indicated the poor flow property of churna and the excellent flowability of granules. The angle of repose for churna

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was 41.67 ± 1.154 indicating the passable flow property of churna and for granules, it was 26.97 ± 0.578 indicating the excellent flow property of granules. The results of all parameters for churna and granules are summarized in Table 3. The results of the parameters revealed that the data obtained

Table 3. Characterization of *Dhatryadi Rasayana* Churna and Its Granules[a]

s. no.	parameters	*Dhatryadi Rasayana* churna (mean ± SD)	*Dhatryadi Rasayana* granules (mean ± SD)
1	total ash content (% w/w)	7.48 ± 0.085	2.73 ± 0.015
2	acid-insoluble ash content (% w/w)	1.28 ± 0.075	0.17 ± 0.020
3	water-soluble extractive	0.35 ± 0.071	91.77 ± 1.293
4	alcohol-soluble extractive (% w/w)	0.36 ± 0.023	41.45 ± 1.095
5	loss on drying (% w/w)	2.35 ± 0.111	0.05 ± 0.014
6	pH (1% w/v aqueous dispersion)	4.10 ± 0.985	4.47 ± 0.112
7	bulk density (g/mL)	0.38 ± 0.021	0.48 ± 0.010
8	tapped density (g/mL)	0.54 ± 0.021	0.50 ± 0.066
9	angle of repose (°)	41.67 ± 1.154	26.97 ± 0.578
10	Carr's index	27.33 ± 1.527	6.04 ± 0.613
11	Hausner's ratio	1.37 ± 0.020	1.09 ± 0.063
12	total sugar (% w/w)	.....	78.13 ± 0.041
13	reducing sugar (% w/w)	.....	23.64 ± 0.965
14	non-reducing sugar (% w/w)	.....	53.72 ± 0.421
15	particle size by sieve method		
	20 mesh (pass%)	48.90 ± 0.877	0.79 ± 0.025
	40 mesh (pass%)	27.04 ± 1.010	0.65 ± 0.025
	60 mesh (pass%)	13.07 ± 0.572	0.52 ± 0.016
	80 mesh (pass%)	4.48 ± 0.562	0.37 ± 0.025
	100 mesh (pass%)	3.15 ± 0.975	0.34 ± 0.014

[a]Mean = Average of three successive results (n=3), SD = standard deviation, Student's t test ($p < 0.05$, $p = 0.115$, two tailed).

for *Dhatryadi Rasayana* churna was statistically different from *Dhatryadi Rasayana* granules (p<0.05). The presence of total sugar, reducing sugar, and non-reducing sugar was observed in granules due to addition of sugar as the binding agent during the formation. Comparatively higher bulk density of granules could be due to the presence of void space. The higher values of water-soluble and alcohol-soluble extractives for granules might be due to the addition of decoction of churna components during the formation of granules and these water-soluble components would be released during the extraction process in aqueous and alcoholic media. The particle size analysis by the sieving method revealed comparatively larger size of granules than churna.

The total sugar for granules was found to be 78.13 ± 0.041% w/w (reducing sugar 23.64 ± 0.965% w/w, non-reducing sugar 53.72 ± 0.421% w/w). The heavy metals, namely, mercury, cadmium, lead, and arsenic in churna and granules were found within the permissible limits. In *Dhatryadi Rasayana* churna, mercury and cadmium were found as 0.701 and 0.112 ppm, respectively. The amount of arsenic and lead was found as 0.370 and 0.491 ppm, respectively. In *Dhatryadi Rasayana* granules, mercury and cadmium were found as 0.223 and 0.047 ppm, respectively. The amount of arsenic and lead in *Dhatryadi Rasayana* granules was 0.205 and 0.292 ppm, respectively. The permissible limits of mercury and cadmium have been reported as NMT 1 ppm and NMT 0.3 ppm, respectively, and for arsenic and lead, the permissible limits are NMT 3 ppm and NMT 10 ppm, respectively.[23]

The total microbial plate count and total yeast and mold count were also found within permissible limits for both the formulations. Specific pathogens such as *E. coli*,*Salmonella*spp., *S. aureus*, and *Pseudomonas aureus* were absent in both the preparations.

3.3. Phytochemical Analysis of *Dhatryadi Rasayana* Churna and Its Granules. Secondary metabolites are key drivers of the pharmacological actions of medicinal plants. The phytochemical analysis and estimation of active components of *Dhatryadi Rasayana* demonstrated that the granules contained higher amounts of total alkaloid, tannin, saponin, and flavonoids than churna as shown in Table 4. The comparatively

Table 4. Phytochemical Analysis for *Dhatryadi Rasayana* Churna and Granules[a]

phytochemical constituents	*Dhatryadi Rasayana* granules (% w/w ± SD)	*Dhatryadi Rasayana* churna (% w/w ± SD)
total alkaloids	2.40 ± 1.132	0.50 ± 0.298
total tannins	11.08 ± 2.468	9.84 ± 1.527
total saponins	7.34 ± 1.598	4.18 ± 2.126
total flavonoids	12.46 ± 2.645	9.34 ± 1.026

[a]Student's t test, two tailed ($p < 0.05$, $p = 0.015$).

higher content of the phytochemicals was obtained in granules than churna (p<0.05). The granules were developed from the *Kwatha* of crude drugs of churna. The phytochemicals of granules would be released easily in comparison to churna during analysis resulting in their higher content.

These important classes of secondary metabolites, such as tannins, saponins, flavonoids, and alkaloids, have been found to exhibit many important biological properties. Alkaloids constitute an important class of structurally diversified compounds that have a nitrogen atom in the heterocyclic ring and are derived from amino acids. They are known to possess antioxidant activities due to their ability to act as scavengers of free radicals, metal chelating activity, or electron or hydrogen donation ability.[29] Saponins are the compounds that possess a polycyclic aglycone moiety with either a steroid (steroidal saponins) or triterpenoid (triterpenoidal saponins) attached to a carbohydrate unit (a monosaccharide or oligosaccharide chain).[30] Saponins have demonstrated numerous pharmacological properties, such as antitumor, anti-inflammatory, spermicidal, sedative, expectorant, and analgesic properties.[31] Tannins are polyphenols that have the ability to precipitate protein. Tannin molecules cross-link the protein and make it more resistant to bacterial and fungal attacks. Flavonoids are the largest group of naturally occurring phenols. They may be divided into various classes according to the oxidation level of the central ring (ring C). The most common of these are anthocyanins, flavones, and flavonols. Tannins and flavonoids are polyphenols that are effective free radical scavengers and play an important role as antioxidant agents.[32] Amalaki (*Emblica officinalis* Gaertn.), especially fruit, contains numerous phytoconstituents, namely, polyphenols like gallic

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acid, ellagic acid, different tannins, minerals, vitamins, amino acids, fixed oils, and flavonoids like rutin and quercetin.[33] The extract or plant has been identified to be efficacious against various ailments like inflammation, cancer, osteoporosis, neurological disorders, hypertension, lifestyle diseases, parasitic, infectious disorders, etc.[34] *Eclipta alba* (L.) Hassak, (Family Asteraceae), an important medicinal plant in the tropical and subtropical regions, is widely used in treating various diseases of skin, liver, and stomach in India, Nepal, Bangladesh, and other countries. It has shown a wide range of biological activities, such as antimicrobial, anticancer, hepatoprotective, neuroprotective, and hair growth-promoting activities. The active phytochemicals present in *Eclipta alba* are coumestan derivatives, phenolic acid derivatives, flavonoids, triterpenoid and steroid saponins, substituted thiophenes, etc.[11] Sesame (*Sesamum indicum* L.) of the family Pedaliaceae is one of the first oil crops used in humans. Numbers of *in vitro* and *in vivo* studies and clinical trials have found sesame seeds to be rich in lignan-like active ingredients. The phytochemical constituents of sesame have antioxidant, cholesterol reduction, blood lipid regulation, liver and kidney protection, cardiovascular system protection, anti-inflammatory, antitumor, and other effects that have great benefits to human health.[12]

3.4. Antioxidant Potential of *Dhatryadi Rasayana* Churna and Granules. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron transfer that produces a violet solution in methanol.[25] This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to a colorless methanol solution. The odd electron of the nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine. On mixing DPPH solution with a substance that can donate a hydrogen atom, it gives rise to the reduced form with the loss of violet color. DPPH shows a strong absorption band at 517 nm due to its odd electron and the solution appears deep violet in color, the absorption vanishes as the electron pairs off. The DPPH value is determined by IC_{50} (half maximal inhibitory concentration), which indicates the concentration of the sample required to inhibit 50% of the radical. The lower the IC_{50} value, the higher the antioxidant activity of the sample. The IC_{50} values for churna and granules were obtained from a linear regression analysis. The linear equation for churna was $y = 2.0987x - 0.156$, $R^2 = 0.9914$ and for granules, the equation was $y = 5.8389x - 4.507$, $R^2 = 0.9882$. The IC_{50} value for churna was 23.89 μg/mL and for granules, it was found to be 9.33 μg/mL, which indicated comparatively higher antioxidant potential of *Dhatryadi Rasayana* granules in comparison to churna. DPPH scavenging effect of *Dhatryadi Rasayana* granules at different concentrations has been shown in Table 5 and for churna, it has been shown in Table 6. In the preparation of granules, the components of churna were treated for getting the *Kwatha* (decoction), and the concentrated decoction was added to sugar. The decoction could have an extracted amount of active principles of churna components. The radial availability of active principles in granules might be responsible for comparatively more antioxidant potential in comparison to churna. In churna formulation, the extraction of active principles might be slow resulting in comparatively higher IC_{50} value.

In the FRAP assay, the antioxidant activity of the sample is evaluated by oxidation−reduction potential. In this method,

Table 5. DPPH Scavenging Effect of *Dhatryadi Rasayana* Granules

concentration (μg/mL)	DPPH scavenging effect (%) (±SD)
2	2.34 ± 0.721
4	17.97 ± 2.347
6	30.86 ± 1.530
8	41.80 ± 1.180
10	54.30 ± 0.895
12	66.41 ± 1.365

Table 6. DPPH Scavenging Effect of *Dhatryadi Rasayana* Churna

concentration (μg/mL)	DPPH scavenging effect (%) (±SD)
10	17.79 ± 0.023
20	44.27 ± 0.077
30	66.40 ± 0.111
40	80.63 ± 0.018

antioxidants react with the ferric tripyridyl-triazine complex (Fe(III)−TPTZ) and produce intense blue color of ferrous tripyridyl-triazine complex (Fe(II)−TPTZ). The antioxidant activities are expressed as the concentrations of antioxidants having a ferric-reducing ability.[35] The FRAP reaction is conducted at an acidic pH to maintain iron solubility. The reaction at a low pH decreases the ionization potential that drives hydrogen atom transfer and increases the redox potential. During reduction of Fe^{+++} to Fe^{++} occurring in the presence of 2,4,6-trypyridyl-s-triazine, the formation of a colored complex with Fe^{++} occurs showing the absorbance at 593 nm. The reducing power is related to the degree of hydroxylation and the extent of conjugation. In this study, *Dhatryadi Rasayana* granules showed 50.0 mmol/100 g of ascorbic acid and for churna, the result was found as 77 mmol/100 g of ascorbic acid. The results of the study reflected the antioxidant potential of both the preparations. The results of FRAP assay indicated that the active components of churna and granules reacted with ferric tripyridyl-triazine complex and developed the intense color of ferrous tri-pyridyl-triazine complex. In this study, the antioxidant potential of granules was more in comparison to churna as less amount of granules was effective to generate the color complex in comparison to churna and it would be possible due to ready availability of antioxidant principles of granules during the chemical process of the assay. In both of the assays, the antioxidant potential of granules was more than that of churna, and it would be due to the presence of water-soluble active principles of churna components in granules as decoction of churna components was added during the formation of granules.

3.5. Immunomodulatory Effect of *Dhatryadi Rasayana* Churna and Its Granules. In Ayurveda, "Rasayana" are claimed to possess immunomodulatory activity.[36] "Rasayana" seems to operate through immunostimulant or immunoadjuvant by affecting the effector arm of the immune response. The present study was conducted to explore the immunomodulatory activity of *Dhatryadi Rasayana* churna and its granules by evaluating its effect on the complete blood count, DTH (delayed-type hypersensitivity) reactions, and hemagglutination antibody titers. DTH provides a functional *in vivo* assessment of cell-mediated immunity.[37,38] In this assay, antigen (SRBC) was injected subcutaneously in the foot pad of animals on days 7 and 14, which activated specific memory

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Table 7. Delayed-Type Hypersensitivity: Mean Percent Increment in Thickness/Edema on the 14th Day of the Study[a]

DTH study time duration	group A (normal control) (mean ± SEM)	group B (negative control) (mean ± SEM)	group C (standard) (mean ± SEM)	group D (test-1) (mean ± SEM)	group E (test-2) (mean ± SEM)
4th hour	4.23 ± 0.145	37.92 ± 0.613	24.04 ± 0.580	27.96 ± 0.826	30.73 ± 0.529
8th hour	5.93 ± 0.306	41.62 ± 0.639	19.66 ± 0.634	24.47 ± 0.693	27.58 ± 0.522
24th hour	3.97 ± 0.209	43.89 ± 0.807	13.69 ± 0.359	20.95 ± 0.629	24.24 ± 0.438

[a]ANOVA (single factor), $p<0.05$ (f=43.76, p = 0.000026).

Table 8. Hemagglutination Antibody Titre Test Done on the 14th Day of Study

group A (normal control) (mean ± SEM)	group B (negative control) (mean ± SEM)	group C (standard) (mean ± SEM)	group D (test-1) (mean ± SEM)	group E (test-2) (mean ± SEM)
24.19 ± 2.235	5.08 ± 0.0737	49.88 ± 1.903	35.93 ± 1.728	32.77 ± 1.692

Table 9. Blood Investigations on the 14th Day of the Study[a]

blood investigation parameters	group 1 (normal control) (mean ± SEM)	group 2 (negative control) (mean ± SEM)	group 3 (standard) (mean ± SEM)	group 4 (test-1) (mean ± SEM)	group 5 (test-2) (mean ± SEM)
WBC count ($10^3/mm^3$)	5.43 ± 0.209	2.86 ± 0.102	6.18 ± 0.254	4.45 ± 0.192	4.0 ± 0.131
RBC ($10^6/mm^3$)	5.14 ± 0.103	2.57 ± 0.121	6.42 ± 0.042	5.13 ± 0.039	4.95 ± 0.048
HGB (g/dLl)	14.6 ± 0.167	8.3 ± 0.369	16.7 ± 0.278	14.0 ± 0.185	14.4 ± 0.269
platelet count ($10^3/mm^3$)	458.3 ± 5.314	219.3 ± 7.342	517.5 ± 5.977	546.5 ± 4.681	527 ± 5.174
neutrophil count (%)	43.76 ± 2.736	15.45 ± 0.289	41.59 ± 3.448	31.02 ± 2.182	28.78 ± 1.227
lymphocyte count (%)	67.23 ± 2.527	32.73 ± 1.960	57.28 ± 2.002	50.63 ± 3.998	52.45 ± 1.502
monocyte count (%)	7.9 ± 0.129	3.01 ± 0.162	6.8 ± 0.279	6.9 ± 0.102	6.6 ± 0.235
eosinophil count (%)	4.8 ± 0.311	2.01 ± 0.116	3.73 ± 0.111	4.21 ± 0.262	3.78 ± 0.130
basophil count (%)	0.6 ± 0.05	0.3 ± 0.030	0.7 ± 0.077	0.5 ± 0.087	0.6 ± 0.05

[a]ANOVA (singe factor), $p>0.005$ (p=0.973, f=0.123).

T-cells and the observed results were due to the recruitment of mononuclear cells and neutrophils. The activation of T cells leads to the release of lymphokines, which causes the activation and accumulation of macrophages, increases vascular permeability, induces vasodilatation, and produces inflammation. It also produces a boost in phagocytic activity and increases the concentration of lytic enzymes for more effective killing of microorganisms. This results in the increase in the thickness of the foot pad, erythema, and indurations at the site of antigen injection in previously immunized animals.[39] The effect of *Dhatryadi Rasayana* churna and its granules on cell-mediated immune response was studied by a delayed-type hypersensitivity (DTH) reaction. The results obtained in delayed-type hypersensitivity reaction indicated that there was a significant decrease in foot paw edema of rats of standard group, *Dhatryadi Rasayana* churna treated group (Test 1), and *Dhatryadi Rasayana* granules treated group (Test 2) when compared against negative control (consuming cyclophosphamide) ($p<0.05$, p = 0.00065, f = 17.94). The successive suppression of the immunological edema after 4, 8, and 24 h has been shown in Table 7. The decrement in paw edema in *Dhatryadi Rasayana* churna and its granules treated animal groups might be due to the presence of phytoconstituents having the anti-inflammatory effect.

A hemagglutination antibody titer test provides a functional *in vivo* assessment of humoral immune response, which is mediated by antibody molecules secreted by plasma cells. Humoral immunity is also called an antibody-mediated immunity. The hemagglutination titer is a measure of the antibodies generated against the SRBC antigen. Humoral immunity involves the interaction of B cells with the antigen and their subsequent proliferation and differentiation into plasma cells that secrete antibodies. This study is based on the principle that if sufficient antibodies are present to agglutinate and form cross-linking with the antigen, the antibody−antigen complex forms a mat at the bottom of the well. If insufficient antibodies are present, then the cells roll down the sloping sides of the well to form a red pellet or "button" at the bottom of the well. The hemagglutination antibody titer value for granules was very close to churna but less than the standard group. It indicates that *Dhatryadi Rasayana* in the form of churna and granules contains compounds that can stimulate the production of antibodies in an immunocompromised animal. The results of hemagglutination antibody titer are shown in Table 8.

Besides this, the complete blood count was also assessed at 14th day of the study as shown in Table 9. The results of various hematological parameters, such as WBC, RBC, HGB, platelet, neutrophil, lymphocytes, monocytes, eosinophils, and basophils, indicated no significant difference in results obtained for control, standard, and experimental group ($p>0.05$). When the blood investigation parameters observed in the animal group consuming churna (Test 1) were compared with the data obtained from Test 2 consuming granules, the data were also non-significant statistically ($p>0.05$, p = 0.318). It was revealed that the phytochemicals present in both churna and granule preparations could not affect the blood profile of the animals. However, the blood investigation parameters obtained from animals of group 2 (negative control) consuming immunosuppressing agent, cyclophosphamide were comparatively lower in comparison to blood profile data obtained from group 1 (consuming normal saline), group 3 (consuming

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clyclophosphamide and levamisole), group 4 (consuming churna), and group 5 (consuming granules) but these changes were non-significant (p>0.05) as shown in Table 9. In a reported study elsewhere, the intra-peritoneal administration of cyclophosphamide in mice has revealed a reduction in platelets, white blood cells, hemoglobin, and red blood cells in comparison to control group consuming physiological saline.[40]

Dhatryadi Rasayana churna is a polyherbal formulation consisting of Amalaki, Bhringraj, and Tila and its granules are the modified dosage form of *Dhatryadi Rasayana* churna. Various secondary metabolites, such as alkaloids, flavonoids, terpenoids, and tannins, contribute to the immunomodulatory activity of *Dhatryadi Rasayana*. *E. Officinalis* fruits contain ellagic acid, gallic acid, quercetin, kaempferol, emblicanin, flavonoids, glycosides, and proanthocyanins. Vitamin C, tannins (emblicanin A and B), and flavonoids present in *E. officinalis* are responsible for immunomodulatory, antioxidant, and anticancer activities. A large variety of chemical compounds including coumestans, alkaloids, thiophens, flavonoids, polyacetylenes, triterpenes, and their glycosides have been derived from *E. alba*. Wedelolactone (Coumestan) and other chemical compounds are accountable for the immunomodulatory activity of *E. alba*. Sesamin, Sesamol, Sesamolin, and Sesaminol are the main active lignans found in *S. indicum* and are used for combating a wide range of immune-related problems. Thus, *Dhatryadi Rasayana* tends to have a competent immunomodulatory activity and may protect the body against various deleterious immune-related diseases.

4. CONCLUSION

Dhatryadi Rasayana cannot be consumed as such as mentioned in classical texts due to palatability issues as it sticks to the throat and causes uneasiness as well as it can easily acquire moisture content. Hence, it can be preserved in the form of granules that overcome palatability issues, have a better shelf-life, convenient in handling, storage, and are easy to administer. In a contemporary study, *Dhatryadi Rasayana* granules have been found to have acceptable product features. The analytical data in the form of micromeritics analysis and phytochemical investigations of the formulation have provided a baseline analytical quality profile for the granules. The results of the present study revealed that *Dhatryadi Rasayana* churna and its granules both exhibited the presence of active components such as alkaloids, tannins, saponin, and flavonoids. The DPPH and FRAP antioxidants assays have revealed the remarkable antioxidant activity of both the churna and the granules, which may be useful against different infections and diseases. Delayed-type hypersensitivity and hemagglutinin antibody titer tests performed for immunomodulatory effect of churna and granules have also indicated the remarkable immunomodulatory impacts of both of the formulations. The blood investigation at the 14th day of the study has also reflected the safety of the formulations in terms of blood profile for oral administration. Moreover, *Dhatryadi Rasayana* in the form of granules and churna may have potential in therapeutics of different diseases and may be useful in improving the vigor and vitality of the body.

AUTHOR INFORMATION

Corresponding Author

Sheenam Rani − *P.G. Department of Rasa Shastra and Bhaishajya Kalpana, Rishikul Campus, Uttarakhand Ayurved University, Dehradun, Uttarakhand 249404, India*; Email: joshansheenam416@gmail.com

Authors

Usha Sharma − *P.G. Department of Rasa Shastra and Bhaishajya Kalpana, Rishikul Campus, Uttarakhand Ayurved University, Dehradun, Uttarakhand 249404, India*

Manish Purushottam Deshmukh − *Deputy Director (Interdisciplinary Research), Datta Meghe institute of Higher Education & Research, Wardha, Maharashtra 442001, India*

Vipin Kumar − *Department of Pharmaceutical Sciences, Gurukul Kangri (Deemed to be University), Haridwar, Uttarakhand 249404, India*; orcid.org/0000-0002-7557-6720

Khem Chand Sharma − *P.G. Department of Rasa Shastra and Bhaishajya Kalpana, Rishikul Campus, Uttarakhand Ayurved University, Dehradun, Uttarakhand 249404, India*

Mayank Kumar Malik − *Department of Chemistry, Gurukul Kangri (Deemed to be University), Haridwar, Uttarakhand 249404, India*

Vetriselvan Subramaniyan − *Pharmacology Unit, Jeffrey Cheah School of Medicine and Health Sciences, Monash University, Subang Jaya, Selangor 47500, Malaysia; School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab 144001, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c06784

Author Contributions

Conceptualization, V.K. and K.C.S.; methodology, V.K. and V.S.; software, M.K.M. and S.R.; validation, V. K. and M.K.M.; investigation, V.K. and M.P.D.; resources, V.S. and M.K.M.; data curation, U.S.; writing, S.R., M.K.M. and V.S.; writing—review and editing, S.R., M.K.M., V.K. and V.S.; visualization, M.P.D.; supervision, U.S.

Notes

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