Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Phytochemical Evaluation, Physicochemical Standardization, and Antioxidant Potential of a Polyherbal Formulation: A Comprehensive Assessment

Garima Yadav[1], Isha Arora[2], Pooja Rani[2], Rimmy Nandal[1*]

[1]Shri Baba Mastnath Institute of Pharmaceutical Sciences and Research, Baba Mastnath University, Rohtak-124021

[2]Chandigarh Group of Colleges Jhajeri, Mohali, Chandigarh Pharmacy College-Jhanjeri, Mohali (Punjab), India, 140307

***Corresponding Author:**

Dr. Rimmy Nandal

[1]Shri Baba Mastnath Institute of Pharmaceutical Sciences and Research, Baba Mastnath University, Rohtak-124021

Email ID: nandalrimmy17@gmail.com

Cite this paper as: Garima Yadav, Isha Arora, Pooja Rani, Rimmy Nandal, (2025) Phytochemical Evaluation, Physicochemical Standardization, and Antioxidant Potential of a Polyherbal Formulation: A Comprehensive Assessment. *Journal of Neonatal Surgery*, 14 (32s), 1265-1277.

ABSTRACT

The present study focuses on the formulation, standardization, and evaluation of a traditional polyherbal churna comprising *Zingiber officinale*, *Piper nigrum*, *Plumbago zeylanica*, *Terminalia chebula*, *Trachyspermum ammi*, and *Argyreia nervosa*. The formulation was prepared in-house following classical Ayurvedic guidelines, and its quality was assessed through organoleptic, physicochemical, and phytochemical parameters. Physicochemical parameters such as foreign organic matter, loss on drying, ash values, and extractive values of individual ingredients were found within pharmacopoeial limits, ensuring purity and consistency. Organoleptic analysis showed a light brown powder with pungent taste and characteristic odour. The formulation demonstrated acceptable flow properties with bulk and tap densities of 0.382 and 0.462 g/ml, respectively. The sodium content was 8.5 ppm, and the pH ranged from neutral to mildly acidic (7.05 and 5.60 for 1% and 10% w/w, respectively). Phytochemical screening revealed a total phenolic content of 188 µg/ml, tannin content of 12%, piperine content of 0.552%, and gallic acid content of 0.382%. Antioxidant activity evaluated by DPPH, total antioxidant capacity, and reducing power assays revealed strong free radical scavenging potential, especially in methanolic extract, with IC_{50} values of 98 µg/ml (DPPH). The results validate the antioxidant efficacy and traditional therapeutic relevance of the polyherbal formulation, supporting its future use in clinical or preventive herbal applications.

.

Keywords: *Polyherbal formulation, antioxidant activity, piperine, gallic acid, DPPH, physicochemical evaluation, Ayurvedic churna*

1. INTRODUCTION

In recent decades, the world has witnessed a significant resurgence of interest in traditional medicine, particularly in herbal formulations, as global healthcare systems seek more holistic, safer, and cost-effective therapeutic options (Leonti et al., 2013;Pan et al., 2014; Li et al., 2017). According to a 2023 World Health Organization (WHO) report, nearly 80% of the global population in developing countries relies on traditional plant-based medicines for primary healthcare needs (Chaachouay et al., 2024; Ghosh et al., 2023). The global herbal medicine market, valued at approximately USD 151.91 billion in 2021, is projected to reach over USD 347.50 billion by 2029, growing at a CAGR of 11.2% during 2022–2029 (Bhattacharjee et al., 2024; Fayiah et al., 2023). This surge reflects both the growing consumer inclination toward natural health products and the increasing scientific validation of traditional knowledge systems like Ayurveda.

Among the various dosage forms in Ayurvedic pharmaceutics, polyherbal churna (finely powdered mixtures of multiple herbs) holds a central role due to its rapid absorption, synergistic phytotherapeutic potential, and ease of preparation and administration (Rani et al., 2024). The rationale behind polyherbalism lies in the concept of multi-targeted action, wherein each plant component contributes a unique pharmacological benefit, and together, the formulation enhances therapeutic efficacy while minimizing adverse effects. Despite widespread usage, there is a pressing need for scientific standardization,

safety profiling, and bioactivity evaluation of such formulations to meet global quality benchmarks and ensure clinical reliability (Ghosh et al., 2015).

A prominent health concern in modern society is oxidative stress, a condition caused by an imbalance between free radicals (such as reactive oxygen species—ROS) and antioxidants in the body (Rajini et al., 2023;Rani et al., 2024). Oxidative stress is implicated in the pathogenesis of several chronic and degenerative diseases including cancer, cardiovascular disorders, neurodegeneration (like Alzheimer's and Parkinson's), diabetes, and premature aging. A growing body of research emphasizes the role of natural antioxidants in neutralizing free radicals and protecting against cellular damage. Plant-derived antioxidants, particularly phenolics, flavonoids, tannins, and alkaloids, have shown promising potential due to their efficacy, bioavailability, and safety profile (Kumari et al., 2025; Leyane et al., 2022)

In this context, the present study focuses on developing and evaluating a polyherbal churna comprising six widely recognized medicinal plants: *Zingiber officinale* (Sunthi), *Piper nigrum* (Marica), *Plumbago zeylanica* (Citraka), *Terminalia chebula* (Haritaki), *Trachyspermum ammi* (Ajmoda), and *Argyreia nervosa* (Vriddhadaruka). Each of these herbs is traditionally used in Ayurveda for gastrointestinal, respiratory, metabolic, and antioxidant applications. For example, *Zingiber officinale* is well-known for its anti-inflammatory and digestive stimulant effects; Piper nigrum enhances bioavailability of other herbs; *Terminalia chebula* is rich in tannins and phenolics with rejuvenating properties; and *Argyreia nervosa* is reported to have adaptogenic and immunomodulatory effects (Liguori et al., 2022; Liguori et al., 2018;Singh 2019). However, there exists a paucity of comprehensive research evaluating their combined pharmacognostic and phytochemical attributes in a standardized churna format.

The rationale behind this research is to bridge the gap between traditional polyherbal wisdom and modern pharmacological validation by investigating the physicochemical stability, flow properties, organoleptic characteristics, and phytochemical profile of the formulation. Additionally, the study evaluates its antioxidant activity using well-established models such as DPPH scavenging, total antioxidant capacity, and reducing power assays. These parameters provide vital insight into the formulation's therapeutic potential and contribute to establishing it as a scientifically credible herbal antioxidant supplement.

The objectives of this study are four-fold:

- To develop a standardized polyherbal churna based on Ayurvedic texts and modern formulation protocols.
- To assess the physicochemical parameters such as ash values, extractive values, pH, sodium content, and flow properties.
- To quantify bioactive compounds including total phenolics, tannins, piperine, and gallic acid through validated chromatographic and spectrophotometric methods.
- To evaluate the antioxidant potential of aqueous and methanolic extracts through DPPH, total antioxidant capacity, and reducing power assays, with comparative analysis using ascorbic acid as a standard.

This study is intended not only to provide pharmacognostic validation of a classical Ayurvedic churna but also to serve as a model for integrating traditional formulations with contemporary scientific methodologies to ensure efficacy, safety, and reproducibility

2. MATERIAL AND METHODS

Collection and Identification of Plant Materials

Plant material

All these ingredients (Trachyspermum ammi, Piper nigrum, Plumbago zeylanica, Terminalia chebula, Zingiber officinale and Argyreia nervosa) were procured from the local market and were authenticated by botanist.

Determination of Physico-Chemical Constants

Foreign organic matter- 250 g i.e. quantity specified in the individual monograph, of the original sample was weighed accurately and spread out in a thin layer. The samples were inspected with the unaided eye or with the use of a magnifying lens (6X or 10X) and the foreign organic matter were separated manually as completely as possible and weighed. The percentage of foreign organic matter was weighed and determined with reference to the weight of the drug taken.

Loss on drying (LOD) - About 2-5 g of the prepared air-dried individual materials were accurately weighed in a previously dried and tared flat weighing bottle. The samples were distributed evenly and were placed in the drying chamber (Oven). Drying was carried out by heating to 100-105°C, the bottle was removed from the oven and the bottle was closed promptly and allowed to cool to room temperature and then weighed. The experiment was repeated till two consecutive weighing and the results did not differ by more than 5 mg, unless otherwise stated in the test procedure. The loss in weight on drying was then calculated.

Ash value- Ash content of the crude drug is generally taken to be the residue remaining after incineration. It represents the inorganic salts naturally occurring in the drug and adhering to it, but may also include inorganic matter added for the purpose of adulteration.

Total ash is the residue remaining after incineration. Acid insoluble ash is the part of the total ash, which is insoluble in dilute hydrochloric acid. Water-soluble ash is the part of total ash, which is soluble in hot water.

a. Total ash- About 2g of the individual powdered ingredients of churna were accurately weighed in a tared silica crucible. The powdered drug was spread as a fine layer at the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed. The procedure was repeated till a constant weight was observed. The percentage of the total ash was calculated in triplicate with reference to the air-dried drug.

b. Acid insoluble ash- The ash obtained as described in the determination of total ash was boiled with 25 ml of hydrochloric acid for 5 min. The insoluble ash was collected on an ashless filter paper by filtration and it was washed with hot water. The insoluble ash was transferred into a tared silica crucible, ignited, cooled and weighed. The procedure was repeated till a constant weight was observed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Extractive values- Extractive value is a measure of the content of the drug extracted by solvents. Extractive value can be water soluble, ethanol soluble and ether soluble extractives. Extractive value is unless and otherwise prescribed, carried out by maceration.

Water soluble extractive- 4 g of previously weighed air-dried powdered individual ingredients of churna were taken in a glass stoppered flask and macerated with 100 ml of chloroform water (1:99). It was shaken frequently for 6 h and then allowed to stand for 18 h. It was filtered rapidly taking precautions against loss of the solvent. 25 ml of filtrate was evaporated to dryness in a tared flat-bottomed petri dish, dried at 105°C, cooled in a dessicator and weighed. The percentage of water-soluble extractive was calculated with reference to air-dried drug.

Note - Ethanol soluble extractive, follow the same procedure of water-soluble extractive excepting the solvent (water) which is replaced by ethanol.

Fluorescence analysis - The powdered samples were exposed to Ultraviolet light at wavelength of 254 nm and 366 nm. One mg of powdered drug was placed on a micro slide and observed under UV 366, UV 254 and in day light to observe the fluorescent characteristics of powder, if any. One mg powdered drug was placed on a micro slide and treated with one ml 1N HCl and observed under UV 366, UV 254 and in day light while wet. One mg powdered drug was placed on a micro slide and treated with one ml 1N NaOH and observed after a few minutes in day light, under UV 366, UV 254. One mg powdered drug was placed on a micro slide and treated with one ml 1N NaOH in one ml methanol and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with one ml 50% KOH and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with 1 ml of 50% sulphuric acid and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with 1 ml of conc. sulphuric acid and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with one ml of 50% HNO_3 and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with one ml of Conc. HNO_3 and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with one ml of acetic and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with 1 ml of iodine and observed under UV 366, UV 254 and in day light while still wet (Kokate et al., 2014; Khandelwal et al., 2013; Kumari, et al., 2024)

Preparation of Churna

*The In-house formulation was prepared as per the procedure given in Ayurvedic Formulary of India. All the ingredients were powdered separately, passed through 80 # sieve and then mixed together in specified proportions to get uniformly blended churna. Formulation was formulated according to The Ayurvedic Formulary of India, 2003 (*Mukherjee et al., 2002; Singleton et al., 1956; Patel et al., 2011)

Table 1: Formular of herbal preparation

S. No.	***Ingredients***	***Quantity (gm)***
1.	*Trachyspermum ammi*	*12*
2.	*Piper nigrum*	*12*
3.	*Plumbago zeylanica*	*12*

4.	*Terminalia chebula*	*60*
5.	*Zingiber officinale*	*120*
6.	*Argyreia nervosa*	*120*

***Preparation of* extracts**

Methanolic extract: The powdered *preparation* about 100 g was extracted with methanol by hot extraction process (soxhlet) for 72 h. After completion of the extraction, the solvent was recovered by distillation and concentrated in vacuo.

Aqueous extract (AJM): (Chloroform:water taken in 1:99) The powdered *preparation* ws macerated with chloroform water for seven days. After the completion of maceration residue was removed by filtration followed by the evaporation of solvent and extract was concentrated *in vacuo* (Sharma et al., 2013)

Determination of Physico-Chemical Parameters

1. Organoleptic evaluation - Organoleptic evaluation refers to evaluation of formulation by color, odour, taste, texture etc. The organoleptic characters of the samples were carried out based on the method as described.

2. Ash values and *extractive values* were determined as described earlier.

3. *Determination of pH* - The pH of different formulations in 1% w/v and 10% w/v of water-soluble portions were determined using pH paper (range 3.5-6) and (6.5-1.4) with standard glass electrode at 24^0 C.

4. *Estimation of sodium content* - Sodium content was estimated by flame photometer by using a flame photometer. A stock solution 100µg/ml of NaCl was prepared in distilled water and further dilutions were made to get 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml, 12µg/ml respectively for preparing the standard graph shown in the table. Sodium content of the formulations was estimated by flame photometric method based on the measurement of emission intensity in nanometer. The method was validated for linearity, precision, and accuracy. The method obeyed Beer's law in the concentration range 2-12 µl/ml. The standard drug solution was assayed repeatedly (n=3) and mean error and relative standard deviation (precision) was calculated.

5. *Determination of physical characteristics of formulations* Physical characteristics like bulk density, tap density, angle of repose, Haussner ratio and Carr's index were determined for different formulations. The term bulk density refers to method used to indicate a packing of particles or granules. The equation for determining bulk density (D_b) is $D_b=M/V_b$ where M is the mass of particles and V_b is the total volume of packing. The volume of packing can be determined in an apparatus consisting of graduated cylinder mounted on mechanical tapping device (Jolting Volumeter) that has a specially cut rotating can. Hundred gm of weighed formulation powder was taken and carefully added to cylinder with the aid of a funnel.

The initial volume was noted and sample was then tapped until no further reduction in volume was noted. The initial volume gave the bulk density value and after tapping the volume reduced, giving the value of tapped density.

7. *Angle of repose* has been used as an indirect method quantifying powder flowability, because of its relationship with interparticle cohesion. The fixed funnel and the free-standing cone method employs a method that is secured with its tip at a given height (H), above the glass paper that is placed on a flat horizontal surface. Powder or granules were carefully poured through the funnel until the apex of the conical pile just touched the tip of funnel. Thus, with R being the radius of the conical pile. Tan α = H/R or α = arc tan H/R, where α is the angle of repose.

Haussner's ratio is related to interparticle friction and as such can be used to predict the powder flow properties. The equation for measuring the Hausner's ratio is D_f/D_o

Where, D_f = Tapped density and D_0 = Bulk density.

Carr's compressibility index is another indirect method of measuring the powder flow from bulk density [32-36]. The equation for measuring Carr's index is

$$\% \text{ Compressibility} = \frac{D_f - D_0}{D_f} \times 100$$

Where D_f = tapped density, D_0 = Bulk density.

Total Phenolic Content Reagents used for total phenolic content were Folin Ciocalteu Reagent and Sodium Carbonate (20 % w/v).

Procedure: Total soluble phenolics in the extracts were determined with Folin-Ciocalteu reagent using gallic acid as a

standard phenolic compound. 1.0 ml of extract solution containing 1.0 mg extract in a volumetric flask was diluted with 46 ml of distilled water. 1.0 ml of Folin-Ciocalteu reagent was added and the content of the flask mixed thoroughly. 3 min later 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm.

Tannin Content (WHO,1998)- Tannins are substances capable of turning animal hide into leather by binding with proteins to form water soluble substance which are resistant to proteolytic enzymes. This process, when applied to living tissue it is known as astringent and therefore used as a therapeutic agent.

Procedure: About 2 g of the *Preparation A and Preparation B* were weighed and transferred into a conical flask. 150 ml water was added and boiled for 30 min. It was cooled and transferred to a 250-ml volumetric flask with water and the volume was made up with water. The solution was filtered and the total amount of material extractable into water was determined by evaporating 50 ml of the extract to dryness, drying was continued at 105º C till constant weight was obtained as follows (T_1). The amount of plant material remains unbound to the hide powder after its addition and which is extractable into water was determined. About 6.0g of hide powder was added to about 80 ml of the above extract. The mixture was shaken for 60 min and was filtered. 50 ml of the filtrate was evaporated to dryness, drying was continued at 105º C till a constant weight (T_2). The solubility of hide powder was determined by taking 6.0 g of hide powder in 80 ml of water and the mixture was shaken for 60 min and filtered. 50 ml of clear filtrate was evaporated to dryness as described earlier and was noted (T_0). The quantity of total tannins was thus calculated by the following formula.

Quantity of tannins (%) = $[T_1 – (T_2 + T_0)] \times 500 / w$

Where w, is the weight of leaf powder in grams [37-39]

Estimation of Piperine in Preparation

Preparation of piperine standard solution- A stock solution of standard *Piperine* (1 mg/ml) was prepared by transferring 5 mg of *Piperine*, accurately weighed, into a 5 ml volumetric flask, dissolving in 2 ml methanol. It was then sonicated for 10 min and the final volume of the solutions was made up to 5 ml with methanol to get stock solutions containing 1 mg/ml.

Preparation of sample solution- Accurately weighed 100 mg of dried methanolic extract of *Preparation A* was transferred to a 10 ml volumetric flask dissolving in 5 ml of methanol. It was then sonicated for 10 min and the contents of the flask were filtered through Whatman No. 1 paper (Merck, Mumbai, India). The final volume of the solution was made up to 5 ml with methanol to get stock solution of 20 mg/ml.

Mobile phase- benzene: ethyl acetate (2:1)

Instrumentation And Chromatographic Conditions- HPTLC was performed on 20 cm × 10 cm aluminum backed plates coated with silica gel $60F_{254}$ (Merck, Mumbai, India). Standard solution of *Piperine* and sample solution were applied to the plates as bands on the same chromatographic plate by use of a Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100 µl Hamilton (USA) syringe. Ascending development was performed at room temperature (28 ± 2°C) using mobile phase in a Camag glass twin-trough chamber previously saturated with mobile phase vapour for 20 min. After development, the plates were dried and then scanned at 254 nm with a Camag TLC Scanner- 3 (Srivastava et al., 2010; Blois et al., 1958)

DPPH Radical Scavenging Assay

Principle:

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1- Diphenyl-2- Picryl Hydrazine. The ability to scavenge the free radical, DPPH was measured in the absorbance at 517 nm.

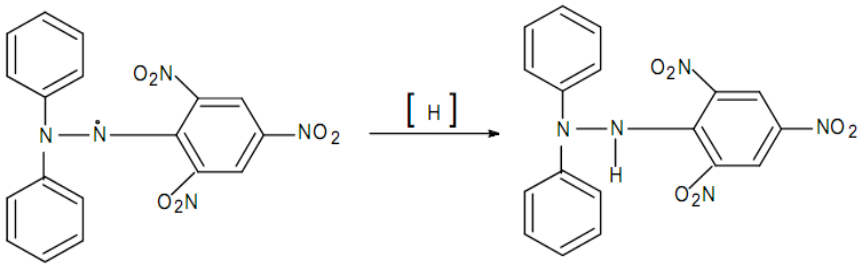

Figure 1: Reduction of N-nitroso-diphenylamine derivative to N,N-diphenylhydrazine via hydrogenation

Procedure- The antiradical activity for the churna extracts was assessed on the basis of the radical-scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The concentration of DPPH was kept at 300 µM in methanol. The extracts were dissolved in methanol. 10 µl of each extract solution was allowed to react with 200 µl DPPH at 37 °C for 30 min in a 96-well microliter plate. After incubation, decrease in absorption for each solution was measured at 490 nm using a microplate reader. Ascorbic acid was used as reference (Braca et al., 2001; Prieto et al., 1999).

$$\% \text{ Scavenging} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

Total Antioxidant Capacity

Total antioxidant capacity was measured according to the method reported by Prieto *et al.* with slight modifications. In brief, 100 µg of extract and 100 µg of ascorbic acid (as standard) were taken in 0.1 ml of ethanol, combined separately in an eppendroff tube with 1.9 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance was measured at 695 nm against a blank. A typical blank solution contained 1.9 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the samples. For samples of unknown composition, water-soluble antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicate and values are expressed as ascorbic acid equivalents in µg per ml of extract (Akinmoladun et al., 2015; Umamaheswari et al., 2007)

Determination of Reducing Power of Churna Extract

The reducing power of extract was determined according to the method of Oyaizu (Oyaizu, 1986). 10 mg of extract in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50^0C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm (Oyaizu et al., 1986; Verma et al., 2016)

3. RESULTS AND DISCUSSION

Physico-Chemical Evaluation of Individual Ingredients

All six herbal ingredients used in the formulation—*Zingiber officinale* (Sunthi), *Piper nigrum* (Marica), *Plumbago zeylanica* (Citraka), *Terminalia chebula* (Haritaki), *Trachyspermum ammi* (Ajmode), and *Argyreia nervosa* (Vrddhadaruka)—were evaluated for key physicochemical parameters including foreign organic matter (FOM), loss on drying (LOD), total ash, acid-insoluble ash, and extractive values (alcohol and water soluble).

The foreign organic matter for all samples was within prescribed limits, indicating good purity. Notably, *Trachyspermum ammi* had the highest FOM at 0.275%, while the rest ranged between 0–0.125%, conforming to acceptable pharmacopoeial standards. The LOD values, an indicator of moisture content, ranged from 1.23 ± 0.152% (*Z. officinale*) to 5.56 ± 0.251% (A. nervosa). These values were within pharmacopeial limits and confirmed the air-dried nature of the materials.

Table 2: FOM and LOD of ingredients

S. No	Botanical name	FOM %	LOD %
1	*Zingiber officinale*	0	1.23±0.152
2	*Piper nigrum*	0	3.9±0.458
3	*Plumbago zeylanica*	0.125	2.76±1.05
4	*Terminalia chebula*	0	4.53±0.251
5	*Trachyspermum ammi*	0.275	2.73±0.404
6	*Argyreia nervosa*	0	5.56±0.251

Ash values assess the total inorganic content. The total ash ranged from 2.46 ± 0.361% (P. zeylanica) to 7.623 ± 0.243% (A.

nervosa), and the acid-insoluble ash, which measures siliceous matter, remained below 0.709% for all ingredients, indicating minimal contamination.

Extractive values provide information about the presence of soluble constituents. The alcohol-soluble extractive was highest in *T. chebula* (85.536 ± 3.129%), while *Z. officinale* showed the highest water-soluble extractive (27.678 ± 0.202%), reflecting the presence of polar and semi-polar phytoconstituents(Nandal et al., 2024; Kumar et al., 2025).

Table 3: Ash and extractive values of ingredients

S. No.	Botanical name	Total Ash %	Acid Insoluble Ash %	Water Soluble Extractive %	Alcohol Soluble Extractive %
1	*Zingiber officinale*	5.823± 0.329	0.260± 0.33	27.678 ± 0.202	29.025 ± 1.532
2	*Piper nigrum*	5.288 ± 0.224	0.390 ± 0.060	11.427 ± 0.087	10.343 ± 0.173
3	*Plumbago zeylanica*	2.460 ± 0.361	0.150 ± 0.027	13.367 ± 0.348	15.334 ± 0.285
4	*Terminalia chebula*	2.832± 0.428	0.267 ± 0.022	41.923 ± 0.768	85.536 ± 3.129
5	*Trachyspermum ammi*	7.328 ± 0.111	0.709± 0.068	17.711 ± 0.336	22.298 ± 0.134
6	*Argyreia nervosa*	7.623 ± 0.243	0.256± 0.033	12.721±0.242	6.417 ± 0.428

Organoleptic and Physicochemical Analysis of the Polyherbal Formulation

The prepared formulation appeared as a light brown powder with a characteristic pungent odor and taste. These organoleptic features are consistent with the presence of *Zingiber officinale* and *Piper nigrum*.

Table 4: Organoleptic Properties of prepared formulation

Characteristics	Remarks
Colour	Light brown
Taste	Pungent
Odour	Characteristic

Ash values for the formulation were 7.114 ± 0.767% (total ash) and 0.596 ± 0.067% (acid-insoluble ash), within acceptable limits, indicating controlled inorganic residue.

The extractive values revealed 14.180 ± 0.163% (water-soluble) and 9.551 ± 0.386% (alcohol-soluble) content, supporting the presence of a wide range of phytoconstituents including alkaloids, flavonoids, and glycosides.

pH values of 7.05 (1% solution) and 5.60 (10% solution) suggested that the formulation is mildly acidic to neutral, appropriate for oral administration.

Table 5: Ash Values, Extractive values and pH of prepared formulation

Parameters	(Mean±SD)
% Total Ash	7.114 ± 0.767
% Acid Insoluble Ash	0.596 ± 0.067
Alcohol Soluble Extractive	9.551 ± 0.386
Water Soluble Extractive	14.180± 0.163
pH of 1% w/w Soln	7.05 ± 0.055

pH of 10% w/w Soln	5.60 ± 0.055

Sodium Content

The sodium content of the formulation was found to be 8.5 ppm, which is within a safe range for herbal products intended for regular consumption.

Flow Properties and Physical Parameters

The bulk density (0.382 ± 0.013 g/ml) and tapped density (0.462 ± 0.017 g/ml) showed moderate packing efficiency. Angle of repose (43.38°) indicated fair flow properties. The Hausner's ratio (1.578) and Carr's Index (34.66%) suggested moderate cohesiveness, implying that granule flow can be optimized for formulation processing.

Table 6: Physical Characteristics of prepared formulation

S. No.	**Parameters**	Mean(n=3)±SD
1	Bulk density	0.382 ± 0.013
2	Tap density	0.462 ± 0.017
3	Angle of repose	43.380 ± 0.020
4	Hausner's ratio	1.578 ± 0.003
5	Carr's Index	34.66 ± 1.73

Phytochemical Quantification

The total phenolic content of the formulation was 188 µg/ml (gallic acid equivalents), and tannin content was 12%, indicating strong astringent and antioxidant properties.

HPTLC estimation revealed gallic acid content at 0.382% and piperine content at 0.552%. These compounds are known for their therapeutic properties including anti-inflammatory and digestive benefits.

Table 7: Estimation of Total Phenol Content of Different Formulations

S. No.	**Sample**	**Concentration(µg/ml)**	**Absorbance**
1	Gallic Acid	50	0.231
		100	0.472
		150	0.645
		200	0.866
		250	1.047
		300	1.265
2	Formulation	1000	0.825

Table 8: Total Phenol Content and Tannin Content of Prepared Formulations

Content	**Quantity**
Total phenol content (µg/ml)	**188**
Tannin content %	**12**

Antioxidant Activity

The formulation exhibited concentration-dependent DPPH radical scavenging activity, with the methanolic extract (80.98%) showing slightly higher efficacy than the aqueous extract (78.91%) at 200 µg/ml. The standard ascorbic acid achieved 95.22% inhibition, highlighting comparable antioxidant potential.

Total antioxidant capacity of the aqueous and methanolic extracts was equivalent to 120 and 150 µg/ml of ascorbic acid respectively, affirming substantial antioxidant richness.

In the reducing power assay, both extracts showed a dose-dependent increase in absorbance, with methanolic extract exhibiting slightly higher reducing capacity than aqueous.

The IC_{50} values for DPPH scavenging were 100 µg/ml (aqueous), 98 µg/ml (methanolic), and 11 µg/ml (ascorbic acid), further confirming the antioxidant potential of the formulation.

Table 9: Quantitative Estimation of Gallic Acid and Piperine

S. No.	Active constituents	Amount
1	Gallic acid	0.382
2	Piperine	0.552

Antioxidant Studies

Table 10: Scavenging of DPPH Free Radicals (Preparation A) ????????

S. No.	Conc. (µg/ml)	Aqueous % scavenging	Methanolic %scavenging	Ascorbic acid %scavenging
1.	5	3.86	4.1	22.22
2.	10	8.52	10.9	45.78
3.	25	12.23	16.04	65.56
4.	50	25.43	27.09	78.43
5.	100	44.65	48.11	91.78
6.	200	78.91	80.98	95.22

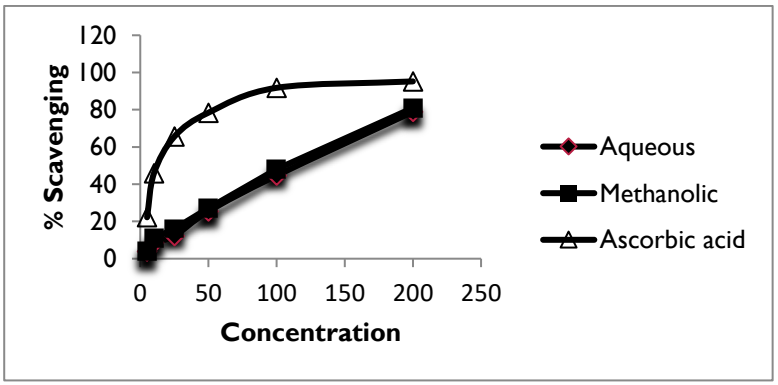

Figure 2: DPPH scavenging activity

Table 11: Total Antioxidant Capacity of preparation

S.No.	Sample	Conc. (µg/ml)	Absorbance
1	Aqueous	1000	0.852
2	Methanolic	1000	0.770
3	Ascorbic acid	25	0.167
		50	0.324
		100	0.604
		200	1.084
		400	2.005
		800	3.489

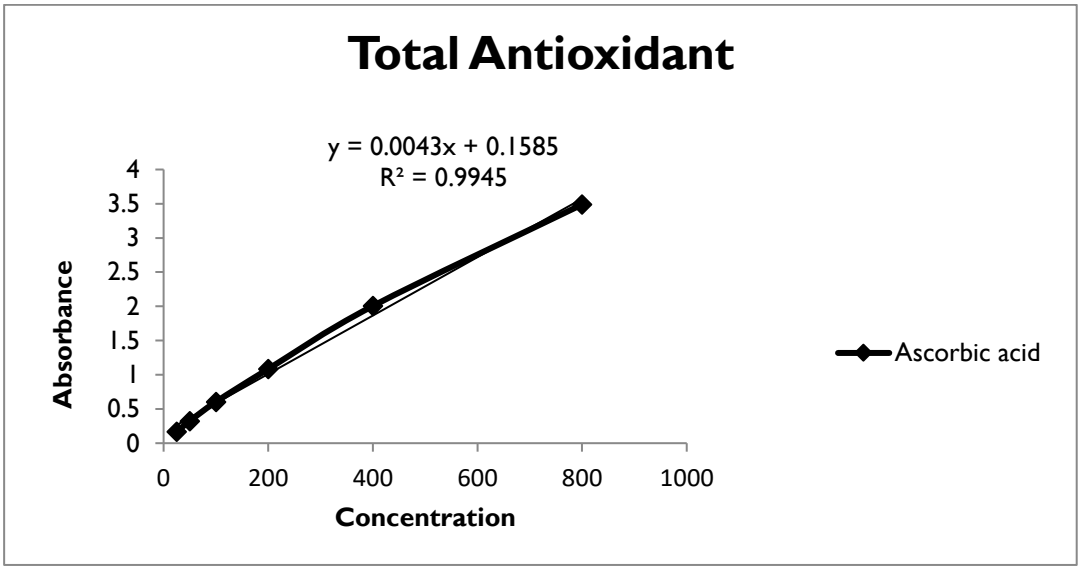

Figure 3: Total antioxidant capacity standard curve

- 1000 m µg/ml Aqueous extract of Preparation A is equivalent to 120 µg/ml of Ascorbic acid
- 1000 µg/ml Methanolic extract of Preparation A is equivalent to 150 µg/ml of Ascorbic acid.

Table 12: Reducing Power Assay of Preparation

S. No.	Conc.	Aqueous	Methanolic	Ascorbic acid
1.	**(µg/ml)**	**Absorbance**	**Absorbance**	**Absorbance**
2.	100	0.212	0.219	0.558
3.	200	0.409	0.412	0.925
4.	400	0.666	0.776	1.121
5.	600	0.895	1.07	1.328
6.	800	1.013	1.115	1.521
7.	1000	1.238	1.272	1.786

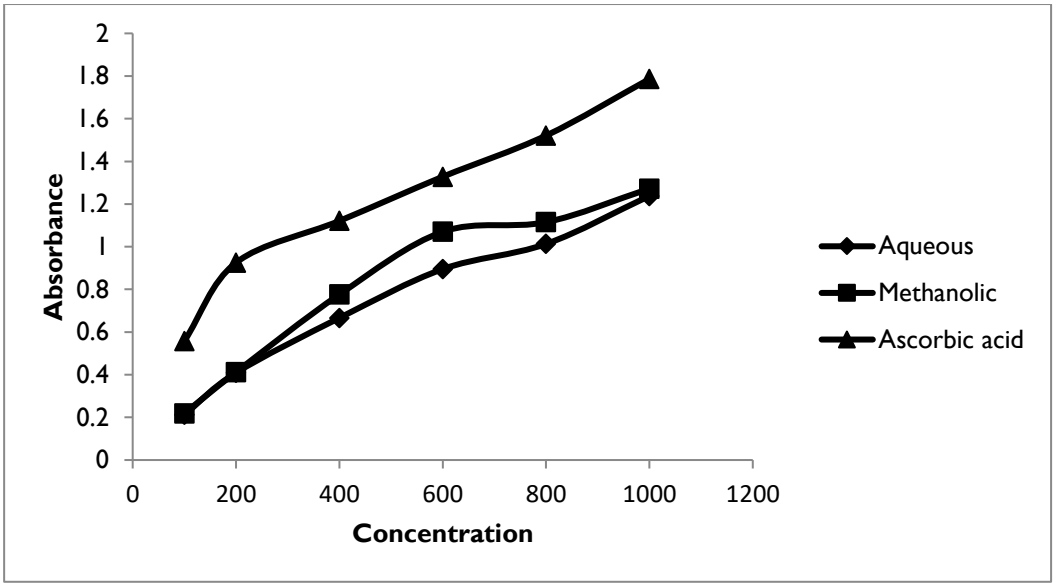

Figure 4: Reducing Power Assay standard cureves

- Methanolic extract of Preparation A exhibits more reducing power than aqueous extract when compared with the Ascorbic acid.

Table 13: IC_{50} values of different Antioxidant models of preparation

Models	Aqueous extract (µg/ml)	Methanolic extract (µg/ml)	Ascorbic acid (µg/ml)
DPPH scavenging activity	100	98	11
ABTS scavenging activity	310	145	14
Nitric oxide scavenging activity	620	600	220

4. CONCLUSION

The polyherbal formulation demonstrated promising physicochemical stability, acceptable flow properties, potent antioxidant capacity, and a significant presence of phytochemicals like phenolics, tannins, piperine, and gallic acid. These findings support its traditional use and provide a strong foundation for its further therapeutic and clinical evaluation.

CONTRIBUTIONS FROM AUTHORS

Authors contributed to analysis of data, drafting or revising the article, agreed on journal to be submitted, provided final approval to the version published and agreed to accept responsibility for all elements of work.

DISCLOSURE

There is no conflict of interest, according to the authors.

REFERENCES

[1] Akinmoladun FO, Akinrinlola BL, Komolafe MA. In vitro antioxidant and antibacterial activities of extracts of selected Nigerian vegetables. Afr J Biomed Res. 2015;18(3):159–168.

[2] Bhattacharjee B, Sandhanam K, Ghose S, Barman D, Sahu RK. Market overview of herbal medicines for lifestyle diseases. InRole of Herbal Medicines: Management of Lifestyle Diseases 2024 Feb 27 (pp. 597-614). Singapore: Springer Nature Singapore.

[3] Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199–1200.

[4] Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from Bupleurum fruticosum. J Nat Prod. 2001;64(7):892–895. doi:10.1021/np010084+

[5] Chaachouay N, Zidane L. Plant-derived natural products: a source for drug discovery and development. Drugs and Drug Candidates. 2024 Feb 19;3(1):184-207.

[6] Fayiah M, Fayiah MS, Saccoh S, Kallon MK. Value of herbal medicine to sustainable development. InHerbal medicine Phytochemistry: Applications and trends 2023 Jul 18 (pp. 1-28). Cham: Springer International Publishing.

[7] Ghosh S, Bishal A, Ghosh SK, Jana K, Gayen B, Sahu S, Debnath B. Herbal medicines: A potent approach to human diseases, their chief compounds, formulations, present status, and future aspects. Int. J. Membr. Sci. Technol. 2023 Oct 11;10:442-64.

[8] Ghosh S, Pradhan P, Bhateja P, Sharma YK. A recent approach for development and standardization of ayurvedic polyherbal formulation (Churna) for antioxidant activity. Am. Res J Pharm. 2015;1(1):5-12.

[9] Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 23rd ed. Pune: Nirali Prakashan; 2013.

[10] Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 49th ed. Pune: Nirali Prakashan; 2014.

[11] Kumari P, Khatkar A, Rani P, Khatkar S, & Nandal, R. Optimizing the Microwave-Assisted Extraction Method to Enhance Extraction Yield and Total Phenolic Content from Moringa Leaves (Moringa oleifera Lam.). Cuestiones de Fisioterapia, 54(3), 1941-1959. (2025)

[12] Kumari, P., Nandal, R., Rani, P., Rathi, P., Khatkar, S., & Khatkar, A. In Silico Screening of Phytoconstituents and Medicinal Plants as Antidiabetic Drug Discovery. Corrosion Management ISSN: 1355-5243, 34(2), 227-245.2024

[13] Leonti M, Casu L. Traditional medicines and globalization: current and future perspectives in ethnopharmacology. Frontiers in pharmacology. 2013 Jul 25;4:92.

[14] Leyane TS, Jere SW, Houreld NN. Oxidative stress in ageing and chronic degenerative pathologies: molecular mechanisms involved in counteracting oxidative stress and chronic inflammation. International journal of molecular sciences. 2022 Jun 30;23(13):7273.

[15] Li FS, Weng JK. Demystifying traditional herbal medicine with modern approach. Nature plants. 2017 Jul 31;3(8):1-7.

[16] Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. Oxidative stress, aging, and diseases. Clinical interventions in aging. 2018 Apr 26:757-72.

[17] Mukherjee PK. Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. New Delhi: Business Horizons; 2002. p. 184–186.

[18] Oyaizu M. Studies on products of browning reaction—antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet. 1986;44(6):307–315.

[19] Pan SY, Litscher G, Gao SH, Zhou SF, Yu ZL, Chen HQ, Zhang SF, Tang MK, Sun JN, Ko KM. Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. Evidence-Based Complementary and Alternative Medicine. 2014;2014(1):525340.

[20] Patel RP, Patel MP, Patel JK. Development and validation of HPTLC method for estimation of piperine in herbal formulations. Int J Pharm Sci Res. 2011;2(10):2520–2523.

[21] Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999;269(2):337–34.

[22] Rajini PS, Muralidhara M. Therapeutic efficacy of ayurvedic polyherbal formulations (PHF): Interactive mechanisms and broad-spectrum activities against neurological disorders. InAyurvedic herbal preparations in neurological disorders 2023 Jan 1 (pp. 89-111). Academic Press.

[23] Rani, P., Pahwa, R., Verma, V., & Bhatia, M. Preparation, characterization, and evaluation of ketoconazole-loaded pineapple cellulose green nanofiber gel. International journal of biological macromolecules, 262, 130221.2024

[24] Rani, P., Verma, V., Kumar, S., & Bhatia, M. Isolation, characterization and evaluation of pineapple crown waste nanofiber gel entrapping ampicillin in topical bacterial infections. Iranian Polymer Journal, 33(5), 687-698.2024

[25] Sharma V, Paliwal R, Sharma S. HPTLC method for quantitative estimation of piperine in Piper nigrum L. and its formulation. Asian J Pharm Clin Res. 2013;6(4):139–141.

[26] Singh R, Singh K. Zingiber officinale: a spice with multiple roles. Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences. 2019 Oct;5(2):113-25.

[27] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3):144–158.

[28] Srivastava P, Viswanathaswamy AHM, Mohan GK. HPTLC method for estimation of piperine in Trikatu Churna and Piper longum fruit. Pharmacognosy Res. 2010;2(2):113–116. doi:10.4103/0974-8490.62955

[29] Umamaheswari M, Chatterjee TK. In vitro antioxidant activities of the fractions of Coccinia grandis L. leaf extract. Afr J Tradit Complement Altern Med. 2007;4(4):231–238. doi:10.4314/ajtcam.v4i4.31204

[30] Verma, M., Deep, A., Nandal, R., Shinmar, P., & Kaushik, D. Novel drug delivery system for cancer management: A review. Current Cancer Therapy Reviews, 12(4), 253-272.2016

[31] Nandal, R., Kumar, D., Aggarwal, N., Kumar, V., Narasimhan, B., Marwaha, R.K., Sharma, P.C., Kumar, S., Bansal, N., Chopra, H. and Deep, A., 2024. Recent advances, challenges and updates on the development of therapeutics for malaria. EXCLI journal, 23, p.672.

[32] Kumar, D., Gupta, V., Tanwar, R. et al. From skin to bone: sun avoidance and osteoarthritis risk. Rheumatol Int 45, 146 (2025). https://doi.org/10.1007/s00296-025-05904-5.