

COMPACTION BEHAVIOR (KAWAKITA, HECKEL AND LEUENBERGER ANALYSIS), ANTIMICROBIAL AND *IN VITRO* ANTI-INFLAMMATORY EVALUATION OF A POLYHERBAL ETHYL ACETATE EXTRACT TABLET FORMULATION

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ABSTRACT

Objectives: The ethyl acetate extract fraction of plant extracts of *Cayratia trifolia* (roots), *Sesbania grandiflora* (flowers), *Cordia dichotoma* (barks) and *Tephrosia purpurea* (seeds) have excellent effects for the treatment of inflammation, oxidative damage to hepatic cells and microbial infection. There is a need to develop a stable and scalable dosage form like tablet.

Methods: The extract powders were mixed in equal proportion and compressed into tablets by the wet granulation method. The extracts and granules were evaluated for micromeritic properties, microbiological assay and *in vitro* anti-inflammatory potential of individual extracts and their mixture was evaluated.

Results: Kawakita and Heckel analysis demonstrated that the granules exhibited reduced cohesiveness, increased plasticity, that is, compressibility ($K=0.0178$), and low yield pressure ($P_y=56.17$). Leuenberger analysis for granules exhibited higher maximum tensile strength ($\sigma_{\max}=0.05328$) and lower compression susceptibility ($\gamma=0.2903$). Both the granules and chloramphenicol demonstrated significant antimicrobial activity against *Escherichia coli*, with zones of inhibition measuring 45.1 ± 2.85 mm and 47.0 ± 2.99 mm, respectively. The IC_{50} of the mixture of extracts ($171.67 \mu\text{g/mL}$) is substantially lower than that of the most potent individual extract, *C. dichotoma* ($255.0 \mu\text{g/mL}$). The tablets passed all the quality control tests, with a release 85% of quercetin release in 45 min.

Conclusion: The final formulation with a mixture of extracts passed quality control parameters, demonstrating potent synergistic anti-inflammatory and antimicrobial properties.

Keywords: Kawakita analysis, Heckel Analysis, Leuenberger analysis, Protein denaturation inhibition study.

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INTRODUCTION

Polyherbal formulations have been widely used in traditional systems of medicine and continue to gain global recognition due to their perceived safety, efficacy, and holistic health benefits. Polyherbal formulations, which combine multiple medicinal plants, are a cornerstone of ayurvedic and traditional medicine systems, aiming to enhance therapeutic efficacy through synergistic action and reduce potential toxicity associated with individual plant extracts [1]. *Cayratia trifolia* (L.) Domin is traditionally used for its anti-inflammatory, antioxidant, and antidiabetic properties. The root extracts have shown promising pharmacological activities attributed to their rich phytochemical composition, including flavonoids and phenolic compounds [2]. *Sesbania grandiflora* (L.) Poir. is widely utilized for its antimicrobial, antiulcer, and hepatoprotective properties. The flowers are known to possess bioactive constituents such as saponins and polyphenols [3]. *Cordia dichotoma* G. Forst. has been traditionally used for treating cough, ulcers, and skin diseases. Its bark is particularly valued for its anti-inflammatory antimicrobial, and antioxidant effects, linked to the presence of tannins, flavonoids, and alkaloids [4]. *Tephrosia purpurea* (L.) Pers. is an important plant in Ayurvedic formulations, used for its hepatoprotective, antidiabetic, and antitumor activities. The seeds are a rich source of flavonoids and terpenoids that contribute to its diverse pharmacological effects [5].

Combining the ethyl acetate extracts of the four plant parts into a tablet dosage form could provide dose uniformity. Moreover, incorporating these extracts into a conventional tablet system would impart several desirable attributes associated with modern solid dosage forms [6]. Examples of such advantages include patient-friendly administration,

improved acceptability owing to dosage form presentation, extended shelf life, assured product quality, enhanced dosing accuracy, and reduced transportation costs resulting from the conversion of bulky powders into compact tablets [7].

Reported literature suggests that ethyl acetate fraction of plant extracts *C. trifolia* (roots) [8], *S. grandiflora* (flowers) [9], *C. dichotoma* (barks) [10], and *T. purpurea* (seeds) [11] have excellent effect for the treatment of oxidative damage to hepatic cells and microbial infection. As the above plant extracts have excellent potential to treat various diseases in combination, there is a need to develop a stable and scalable dosage form like tablet. In the present study, ethyl acetate extracts of all the above four plant parts will be scientifically evaluated for flowability, compressibility, antimicrobial activity and In-Vitro antioxidant activity individually as well as in combination.

MATERIALS AND METHODS

Materials

The following plant parts, such as *C. trifolia* (roots), *S. grandiflora* (flowers), *Cordia dichotoma* (barks), and *T. purpurea* (seeds), were identified, authenticated and collected from Daringibadi and Amapani hill areas of Kandhamal district, Odisha, India, during July 2017–August 2017. *Streptococcus viridans*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Staphylococcus epidermis* (Gram +ve bacteria) were procured. *Salmonella* Typhi, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Escherichia coli* (Gram--ve bacteria) were also selected and procured from National Collection of Industrial Microorganisms, CSIR-National Chemical Laboratory, Pune.

Methods

Qualitative phytochemical tests for extracts

Ethyl acetate extracts of all four selected plant parts were subjected to qualitative phytochemical tests to detect the presence of all important phytoconstituents such as flavonoids, saponins, tannins, triterpenoids, alkaloids, and glycosides by following various qualitative tests as mentioned in literature [12] (Table 1).

Preparation of granules

Herbal extract powders were mixed in the ratio of 1:1:1:1, that is, extract of *C. trifolia* (roots), *S. grandiflora* (flowers), *C. dichotoma* (barks) and *T. purpurea* (seeds). The dry powder extracts of the four plant parts and other excipients were dry mixed in rapid mixer granulator for 12 min in the mixing unit with the help of an impeller blade. After that non-aqueous binder solution (10% W/V PVPK30) was added to the mixing bowl, and wet mixing was continued for 15 min with impeller blade and chopper blade. The wet mass passed through (#12) and the granules were dried at 60°C for 25 min fluidized bed dryer. The dried granules were passed through (#14) to calibrate the granules. The dried granules were mixed with lubricants and the remaining portion of disintegrating agent in hexagonal blender for 10 min. The composition is given in Table 2.

Characterization of extracts and granules

Micromeritic properties

The herbal extract powders and granules (10 g) were poured into a 100 mL measuring cylinder, and the volume was noted to determine bulk density (BD). Subsequently, the filled measuring cylinder was tapped for 100 tapings repeatedly till a constant volume was obtained for determination of tapped density (TD) [13].

$$\rho_r = \frac{\rho_A}{\rho_T}$$

$$\text{Hausner's ratio (H.R)} = \frac{TD}{BD}$$

True density of extracts and granules was determined by pycnometry [14]. The porosity was calculated by using the following equation

$$\text{Porosity(\%)} = 1 - \frac{BD}{\text{True Density}} \times 100$$

Angle of repose and coefficient of friction were determined using the fixed height method using funnel [15]. The extract powders were poured into the funnel by closing the outlet through fingers. Then the extract powders were allowed to flow, and the radius and height of the

formed heap were determined. The angle of repose and coefficient of friction were determined.

$$\tan \theta = \frac{h}{r}$$

$$\theta = \tan^{-1} \frac{h}{r}$$

Granular friability index

Sample of the granules retained on #60 was selected to separate the fines from granules [16]. A 10 g sample of granules was introduced into the friabilator drum and rotated at 25 revolutions/min for 4 min. Following this, the material was passed through a #60 sieve, and the weights of the granules retained on the sieve as well as the powder that passed through were recorded. Amount of fines should not be more than 1% of the initial weight of granule, that is, <1 % of 10 g (Not more than 100 mg of fines i.e., amount passed through #60 after friability test). The experiment was repeated 10 times, and an average reading was recorded.

Kawakita analysis

Flow ability, cohesiveness and compactibility of the herbal extract powders and granules were determined using the Kawakita equation [17]. TD was measured by transferring 10 g of powder, introduced at a 45° angle, into a graduated cylinder (50 mL). The initial bulk volume (V_0) was recorded with precision. After successive tapings numbers (n=10, 30, 60, 100, 150, 200, 250, and 300), the corresponding volume (V_n) was noted. The Kawakita equation, applied to the tapping experiment, served as a tool for assessing the flow of extracts and granules.

$$\frac{N}{C} = \frac{N}{a} + \frac{1}{ab}$$

In this equation, a and b are constants, where a represents compactibility, while $1/b$ corresponds cohesiveness.

$$C = \frac{V_0 - V}{V}$$

Compaction studies

Heckel equation

Herbal extract powders and granules were compressed into compacts at applied pressures ranging from 10 to 50 kg/cm². The compaction behavior of the powders was analyzed using the Heckel equation [18].

$$\ln \frac{1}{1 - \rho_r} = KP + A$$

Table 1: Phytochemical analysis of ethyl acetate extracts of plant parts

Tests	Ethyl acetate extract			
	Roots of <i>Cayratia trifolia</i>	Flowers of <i>Sesbania grandiflora</i>	Barks of <i>Cordia dichotoma</i>	Seeds of <i>Tephrosia purpurea</i>
Carbohydrate	–	–	–	–
Glycoside	–	–	–	–
Gum and mucilage	–	–	–	–
Protein and amino acid	–	–	–	–
Tannin and phenolic compound	+	+	+	+
Steroid and sterol	–	–	–	–
Triterpenoid	+	+	+	+
Saponin	–	–	–	–
Flavone and flavonoid	+	+	+	+

(+) stands for present and (–) stands for absent

Table 2: Composition of tablet

S. No.	Ingredients	Quantity per tablet (mg)	Quantity for 100 tablets (g)
1	Ethylacetate extract of Roots of <i>Cayratia tri folia</i>	50	5
2	Flowers of <i>Sesbania grandiflora</i>	50	5
3	Barks of <i>Cordia dichotoma</i>	50	5
4	Seeds of <i>Tephrosia purpurea</i> .	50	5
5	Lactose	185	18.5
	Starch		
6	Intra-granular	25	25
	Extra-granular	25	25
7	PVP K30 (10% w/v alcoholic solution)	45	45
8	Talc	10	10
9	Magnesium stearate	5	5
10	Aerosil	5	5
	Total	500	50

$$\rho_r = \frac{\rho_A}{\rho_T}$$

Where ρ_r denotes relative density, ρ_A apparent density and ρ_T true density, P applied pressure, K degree of plasticity, P_y yield pressure and A initial volume of compact.

Leuenberger analysis

Radial crushing strength (Tensile strength)

Tablets were compressed from individual extracts, their mixture, and granules at varying compaction pressures of 10, 20, 30, 40, 50, 75, and 100 kg/cm², maintaining a constant dwell time of 5 s [19].

$$\sigma_T = \frac{2F_B}{\pi D_T h_r}$$

Where σ_T denotes the radial crushing strength, F_B the crushing strength, D_T the diameter of the tablet and h_r the thickness of the tablet.

Compression susceptibility γ and σT_{max} using leuenberger equation

The value of σT_{max} and the compression susceptibility γ was calculated from this equation [20] using statistical software (Graph Pad Prism 5).

$$\sigma T = \sigma T_{max} (1 - e^{-\rho^r \gamma^p})$$

Compression of granules into tablets

The prepared granules were compressed into herbal tablets of 12 mm diameter using circular flat punches in a multistation tablet press (Karnavati Mini Press II). The batch size for the herbal tablets was 200 tablets. The compression force was adjusted to get tablet hardness of 5 kg/cm² or more.

Quality control tests for tablets (Q.C tests)

The prepared tablets from the mixture of herbal extract powder were subjected to tests such as drug content, weight variation, hardness, disintegration, and friability as per the standard procedure [21]. Tablets containing the mixed herbal extract powder (10 tablets) was crushed, sonicated in 0.1 N HCl for 5 min, filter, diluted and analyzed for quercetin (marker for polyphenol) content by taking absorbance at 256 nm [22]. Quercetin release from the tablets containing the mixed herbal extract powder was assessed using a USP six-stage dissolution apparatus I (Electrolab®) operated at 50 rpm. At 15, 30, 45, and 60 min, 5-mL samples were collected, filtered, appropriately diluted, and analyzed for quercetin content with a ultraviolet (UV)/visible double-

beam spectrophotometer at 256 nm. The amount of quercetin was calculated from the calibration curve of standard quercetin.

Antimicrobial assay

Preparation of stock solution

Standard stock solutions of chloramphenicol were prepared at concentrations of 100 and 1000 µg/L. Twelve sterilized; capped test tubes were arranged in two rows on a rack. From a freshly prepared stock solution stored in a 30 mL universal bottle, 8 mL of broth containing the required drug concentration was prepared and mixed thoroughly. From this mixture, 2 mL was transferred to the first tube of each row, while 4 mL was added to the test bottle. Subsequently, 4 mL of fresh broth was added and mixed using a sterile pipette. From this mixture, 2 mL was transferred to the next tube in the same row. This stepwise dilution process was continued for all tubes. The final tube in each row received 2 mL of fresh broth only. One row of tubes was inoculated with a single drop of overnight-grown microbial culture, adjusted to approximately 10⁶ microorganisms/mL, while the second row served as the control and was inoculated in the same manner. The size of the inoculum significantly influenced the test outcome. All tubes were incubated at 37°C for 18 h. A separate tube containing 2 mL of broth with the test microorganism was incubated and then stored at 4°C for 12 h to serve as the reference standard for complete growth inhibition.

Zone of inhibition (ZOI) study

The antimicrobial activity of extracts obtained from selected plant parts was evaluated using the disc diffusion technique. Fresh 24-h cultures of the microbial strains were uniformly inoculated onto sterile Petri plates containing solidified agar medium. Sterilized Whatman filter paper discs of 6 mm diameter were individually impregnated with different concentrations of the plant extract (50–250 µg/mL) and carefully placed on the surface of the inoculated agar plates. The inoculum density was standardized to deliver a final concentration (10⁹ CFU/mL) with incubation at 37°C for 24 h to allow microbial growth, followed by measurement of zones of inhibition. Chloramphenicol (50–250 µg/mL) was employed as a positive control.

In vitro anti-inflammatory study

Protein denaturation inhibition study

The *in vitro* anti-inflammatory potential of individual plant extracts and their mixture was assessed via an *in vitro* protein denaturation assay. 1 mL of the extract (at concentrations between 100 and 500 µg/mL) was mixed with 1 mL of 1 mM egg albumin solution at pH 6.8. This mixture was initially incubated at 37°C for 15 min, and then subjected to a 70°C for 10 min to activate denaturation. After the samples were cooled under running water for 10 min, turbidity was quantified using a UV-visible spectrophotometer at 660 nm [23]. The efficacy of the extracts was then evaluated by calculating the percentage of protein denaturation inhibition in comparison to a control group (Ibuprofen).

% Denaturation Inhibition

$$= \frac{(\text{Control absorbance} - \text{Sample absorbance})}{\text{Control absorbance}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical investigation

Ethyl acetate extracts of *C. trifolia* (roots), *S. grandiflora* (flowers), *C. dichotoma* (barks) and *T. purpurea* (seeds) confirmed the presence of tannins, phenolic compounds and triterpenoids (Table 1).

Preparation of granules

The initial physical and micromeritic properties of all the four extract powders and their mixture exhibited poor flowability and poor compressibility. Hence, it was not suitable for processing into a tablet dosage form. Literature reports suggest that modifying particle

size can enhance flow properties, as larger particles tend to exhibit lower cohesiveness compared to finer ones. To achieve porous and free-flowing granules, the wet granulation technique was employed, facilitating the preparation of tablets with high mechanical strength even at relatively low compression pressures. The use of PVPK30 as a wet non-aqueous binder was found to be suitable for obtaining granules with desirable physical strength.

Micromeritic properties

The study of micromeritic properties for extracts (Table 3) revealed that the lowest and highest BD was observed for the ethyl acetate extracts of the flowers of *S. grandiflora* and barks of *C. dichotoma*, respectively. This suggests that the extract of flower was fluffier and occupies more volume, whereas the extract of the bark was denser and occupies less volume. Subsequently, the TD for extracts was determined. The increase in density after subjecting to tapping for all extracts suggests that there was a decrease in volume with the application pressure (tapping) [24]. The granules prepared from the mixture all four extracts exhibited the highest BD as well as the extent of increase in density after tapping. Granules showed substantial compressibility and considerable inter-particle void volume in the loose state. During tapping, particles settle into a more efficient packing arrangement, collapsing voids and raising the density. Another reason for the increase TD could be porous granules can deform or fracture under tapping, filling adjacent voids and elevating the TD. The true density value for extracts was in the range of 1.3061–1.558 g/cc. These granules exhibited true density within the similar range as that of extracts.

The angle of repose, coefficient of friction, Carr's index and Hausner's ratio for all the extracts and their mixture suggest that they are poorly flowable and not suitable for processing into a tablet dosage form. The prepared granules exhibited all the above parameters in the desirable range for processing into a tablet dosage form [25]. The porosity of granules was more than double the porosity of extracts, suggesting the suitability of the granules for tableting [26]. Granules with elevated initial porosity undergo greater plastic deformation, which can be substantiated further from heckle analysis [27].

Granular friability index

A granular friability index below 1% indicates that <1% of the granule mass is lost during various steps of granulation, such as dry mixing, wet mixing, and drying, reflecting excellent mechanical robustness. PVPK30 (10% W/V) non-aqueous binder uniformly distributed and promoted inter-particle cohesion, enhanced granule strength and reduced weight loss [28]. Moisture content of the granules was 4% with optimum drying conditions. Over-drying (which can embrittle granules) and under-drying (which can cause sticking and weak inter-granular bonds). Controlled drying in FBD at 60°C ensured balanced plasticity and hardness.

Kawakita analysis

Superior flow performance was evident in the #12 granules, as reflected by their decreased "a" value compared to the extracts. Among all extracts, the order of flowability was as follows barks of *C. dichotoma*>seeds of *T. purpurea*>roots of *C. trifolia*>flowers of *S. grandiflora*. Whereas, the lowest value of 1/b was observed for seeds of *T. purpurea*, suggesting that it was less cohesive among all other extracts. The granules were less cohesive because dense particles are generally less cohesive; hence, flowability was improved [29] (Table 4 and Fig. 1).

Heckel analysis

An increased a value of the granules suggests stronger fragmentation behavior, better packing density, and accelerated rearrangement during compression. Among the extracts, barks of *C. dichotoma* and seeds of *T. purpurea* showed higher value for A, augmenting better packing and faster rearrangement. Greater slopes for granules indicated a greater degree of plasticity (K), hence better compressibility [30]. Lower value of yield pressure for granules than extracts exhibited higher resistance to compaction pressure (Table 5 and Fig. 2).

Leuenberger analysis

The compression susceptibility (γ) value of the granules revealed that maximum crushing strength was achieved more rapidly at a lower compaction pressure (30 kg/cm²), indicating strong bonding characteristics of the granules. Similarly barks of *C. Dichotoma* exhibited maximum crushing strength at 40 kg/cm², suggesting good bonding properties among all other extracts. In all other cases,

Table 3: Physical and micromeritic properties of extracts and granules

Parameters	Ethyl extract					
	Roots of <i>Cayratia trifolia</i>	Flowers of <i>Sesbania grandiflora</i>	Barks of <i>Cordia dichotoma</i>	Seeds of <i>Tephrosia purpurea</i>	Mixture of all four extracts (1:1:1:1)	Granules of powder mixture
Bulk density (g/cc)	0.2601±0.101 ^a	0.218±0.02 ^a	0.345±0.07 ^a	0.263±0.04 ^a	0.255±0.03 ^a	0.437±0.05 ^b
Tapped density (g/cc)	0.3678±0.089 ^a	0.304±0.06 ^a	0.452±0.07 ^a	0.342±0.07 ^a	0.345±0.07 ^a	0.538±0.04 ^a
True density (g/cc)	1.3061±0.045 ^a	1.412±0.08 ^a	1.558±0.02 ^b	1.233±0.07 ^a	1.347±0.07 ^a	1.354±0.08 ^a
Angle of repose (°)	38±3.4 ^a	41.05±0.23 ^a	38.81±2.6 ^a	46.67±4.61 ^b	39.65±2.65 ^a	23.166±1.608 ^b
Coefficient of Friction	0.71±0.02 ^a	0.64±0.08 ^a	0.67±0.04 ^a	0.75±0.08 ^a	0.69±0.02 ^a	0.41±0.03 ^b
Carr's index (%)	29.31±2.25 ^a	35.37±1.72 ^b	23.91±1.8 ^b	25.52±1.62 ^a	27.63±1.35 ^a	18.88±1.5 ^b
Hausner's ratio	1.414±0.02 ^a	1.39±0.03 ^a	1.31±0.08 ^a	1.30±0.078 ^a	1.35±0.05 ^a	1.23±0.08 ^b
Porosity	13.84±1.34 ^a	15.68±1.27 ^a	12.10±1.27 ^a	14.98±1.27 ^a	16.54±1.27 ^a	37.59±3.54 ^b

Mean±standard deviation, n=10, "a" indicates no significant difference (p>0.05) and "b" indicates a significant difference (p<0.05)

Table 4: Kawakita analysis of extracts and granules

Kawakita	Compactibility (a)	Cohesiveness (1/b)	Coefficient of determination (r ²)
Roots of <i>Cayratia trifolia</i>	0.334352312	18.21424022	0.981843712
Flowers of <i>Sesbania grandiflora</i>	0.549105742	17.67597532	0.975137762
Barks of <i>Cordia dichotoma</i>	0.158874262	18.54289605	0.991005315
Seeds of <i>Tephrosia purpurea</i>	0.180115274	13.86887608	0.993632651
Mixture of all four extracts (1:1:1:1)	0.278485041	18.605453	0.99037545
Granules of powder mixture	0.088737894	15.01360648	0.9929854

Table 5: Heckel analysis of extracts and granules

Heckel	Slope (K)	Intercept (A)	Yield pressure (Py)	Coefficient of determination (r^2)
Roots of <i>Cayratia trifolia</i>	0.0065	0.3578	152.207	0.995
Flowers of <i>Sesbania grandiflora</i>	0.0014	0.3086	694.54	0.994
Barks of <i>Cordia dichotoma</i>	0.0095	0.5568	105.15	0.996
Seeds of <i>Tephrosia purpurea</i>	0.0107	0.5365	92.85	0.993
Mixture of all four extracts (1:1:1:1)	0.0092	0.3924	108.65	0.974
Granules of powder mixture	0.0178	0.4984	56.17	0.998

Table 6: Leuenberger analysis of extracts and granules

Leuenberger	Compression susceptibility (γ) (1/kg/cm ²)	Maximum tensile strength, $\sigma_{t_{max}}$ (kg/cm ²)	Coefficient of determination (r^2)
Roots of <i>Cayratia trifolia</i>	0.1473	0.00853	0.9869
Flowers of <i>Sesbania grandiflora</i>	0.07471	0.00441	0.9863
Barks of <i>Cordia dichotoma</i>	0.2086	0.01973	0.9952
Seeds of <i>Tephrosia purpurea</i>	0.1929	0.00928	0.9909
Mixture of all four extracts (1:1:1:1)	0.1282	0.00668	0.9881
Granules of powder mixture	0.2903	0.05328	0.9995

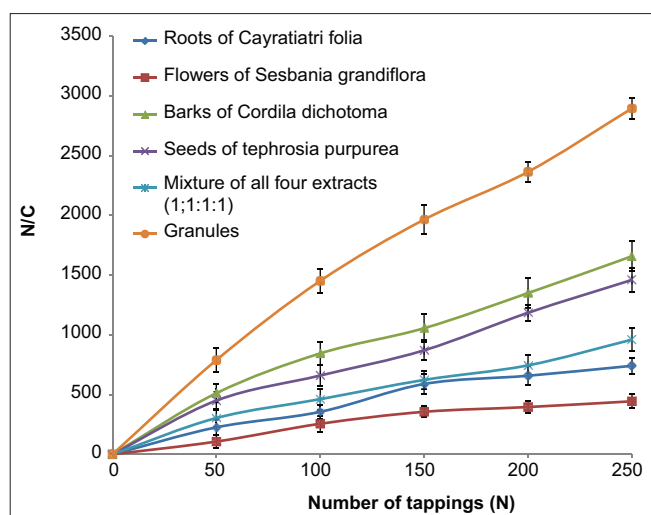


Fig. 1: Kawakita analysis for extracts and granules

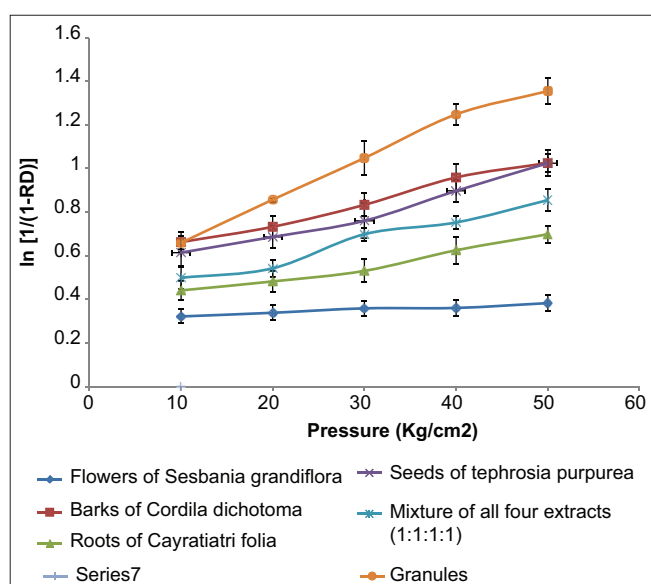


Fig. 2: Heckel analysis for extracts and granules

the maximum tensile strength was achieved at a higher pressure of 50 kg/cm² or more.

Compared with the extracts, the granules displayed an elevated $\sigma_{t_{max}}$, suggesting that they develop compacts with enhanced strength [31]. The comparatively low compression susceptibility of *S. grandiflora* flowers implied that higher compaction pressure was necessary for the gradual development of $\sigma_{t_{max}}$ (Table 6).

Quality control tests

The prepared poly-herbal tablets passed all the Q. C tests for tablets (Table 7). The quercetin content in tablets prepared with the mixed herbal extract powder was nearly 17%. The hardness was more than 5 kg/cm² suggests that optimum concentration of binder solution was incorporated during granulation. These results indicate that the compaction pressure applied during the compaction of granules was optimal to produce tablets with hardness values exceeding 5 kg/cm².

Moreover, the observed friability (weight loss <1%) can be ascribed to the binder properties, granule size distribution, drying conditions, and applied compression force. The tablets disintegrated in around 8 min, which can ascribed to the addition of starch as disintegrants in two proportions, that is, 50% as intra-granular and 50% as extra-granular. As shown in Fig. 3, the tablets prepared with the mixed herbal extract powder released over 85% of their quercetin in 45 min.

Microbiological assay

ZOI of ethyl acetate extracts of *C. trifolia* (roots), *S. grandiflora* (flowers), *C. dichotoma* (barks), *T. purpurea* (seeds) and their physical mixtures (1:1:1:1) in granulated form were determined to ascertain their antimicrobial effects in reference to the reported literatures at different concentrations using eight numbers of human pathogenic micro-organisms, including four numbers g (+) and four numbers of g (-) bacteria. The ZOI data demonstrated pronounced antimicrobial activity for the tested samples. Notably, the granulated mixture of all four plant extracts in an equal ratio (1:1:1:1) exhibited broad-spectrum inhibition against both Gram-positive and Gram-negative bacterial strains. The inhibitory zones produced by the combined formulation increased in a concentration-dependent manner and were found to be comparable with those of the standard antibiotic chloramphenicol at higher concentrations. Combined extract exhibited synergistic interaction among bioactive phytoconstituents. Overall, these findings suggest that the polyherbal granulated formulation

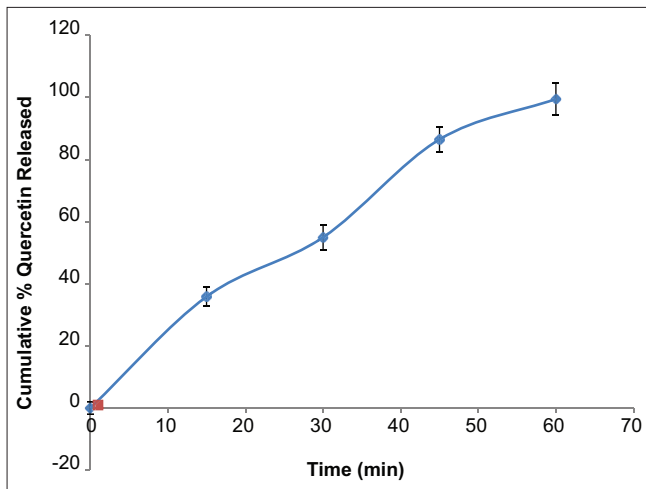


Fig. 3: *In vitro* dissolution study for tablets prepared with the mixed herbal extract powder

Table 7: Quality control tests for tablets

QC parameters	Results
*Drug content	17.4±0.25
*Thickness (mm)	4.1±0.08
*Diameter (mm)	12±0.04
**Hardness (kg/cm ²)	5.2±0.21
***Friability (%)	0.47±0.02
**Disintegration time (min)	8.5±0.8

Mean±standard deviation, *n=10, **n=6 and ***n=20

possesses significant antimicrobial potential comparable to that of a conventional antibiotic (Table 8).

In vitro anti-inflammatory study

Protein denaturation inhibition study

The anti-inflammatory potential of various ethyl acetate extracts and their mixture was evaluated using the bovine serum albumin denaturation assay [32]. A concentration-dependent inhibition of protein denaturation was observed for all samples (100–500 µg/mL). Among the individual plant extracts, the bark extract of *C. Dichotoma* demonstrated the highest efficacy with an IC₅₀ of 255.0 µg/mL, followed by the roots of *C. trifolia* (IC₅₀=322.2 µg/mL), seeds of *T. purpurea* (IC₅₀=351.0 µg/mL), and flowers of *S. grandiflora* (IC₅₀=376.5 µg/mL). Notably, the mixture of all four extracts displayed superior anti-inflammatory activity compared to the individual extracts. The mixture achieved 76.81±3.51% inhibitions at the maximum concentration of 500 µg/mL. The calculated IC₅₀ value for the mixture was 171.67 µg/mL, indicating significantly higher potency than any single plant extract used in the study. The results are given in Table 9. The IC₅₀ of the mixture of extracts (171.67 µg/mL) is substantially lower than that of the most potent individual extract, *C. dichotoma* (255.0 µg/mL). This suggests a synergistic interaction between the diverse phytochemical constituents present in the four different plants. Such synergy in polyherbal formulations often occurs when different bioactive compounds – such as polyphenols or flavonoids – act on multiple molecular targets simultaneously to inhibit the denaturation process. The observed inhibition is likely due to the presence of secondary metabolites in the ethyl acetate fraction that interact with the albumin molecules, increasing their thermal stability. These findings support the traditional use of these plants in treating inflammatory disorders and highlight the potential of the combined extract as a more effective therapeutic agent than single-plant preparations.

Table 8: Zone of inhibition of extracts and their mixture

Sample	Concn. (µg/mL)	<i>Staphyloco. epidermidis</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus viridans</i>	<i>Bacillus subtilis</i>	<i>Salmonella Typhi</i>	<i>Escherichia coli</i>	<i>Proteus vulgaris</i>	<i>Pseudomonas aeruginosa</i>
Roots of <i>Cayratia trifolia</i>	50	0.6±0.02	*	*	0.7±0.02	*	2.4±0.15	*	0.7±0.05
	100	1.8±0.15	1.4±0.31	0.6±0.08	2.0±0.16	0.9±0.24	6.5±0.42	1.0±0.06	3.1±0.38
	150	4.9±0.28	3.9±0.18	1.5±0.25	6.4±0.32	5.2±0.38	9.8±0.74	3.5±0.15	6.7±0.36
	200	13.0±1.25	9.9±0.51	4.9±0.28	9.1±0.80	9.8±0.61	13.6±1.28	8.1±0.72	10.4±0.71
	250	21.4±1.68	13.3±1.29	8.6±0.22	18.2±1.58	13.9±1.28	19.6±1.18	15.0±1.04	13.8±1.21
Flowers of <i>Sesbania grandiflora</i>	50	0.3±0.06	*	*	*	*	1.1±0.16	*	*
	100	2.7±0.18	0.7±0.05	*	0.8±0.15	0.4±0.03	4.7±0.22	0.4±0.07	1.4±0.11
	150	4.3±0.36	2.7±0.18	0.6±0.14	4.2±0.33	4.3±0.28	8.1±0.31	2.9±0.21	3.9±0.19
	200	9.8±0.82	8.4±0.31	4.3±0.31	8.1±0.63	8.6±0.48	13.1±0.51	6.5±0.44	8.7±0.35
	250	18.2±1.28	11.5±0.94	9.1±0.61	15.3±1.05	12.4±1.02	19.4±0.18	13.7±0.92	11.5±0.78
Barks of <i>Cordia dichotoma</i>	50	0.6±0.03	4.7±0.16	*	0.9±0.18	*	1.4±0.18	*	0.8±0.08
	100	2.6±0.16	9.2±0.71	*	3.5±0.29	0.9±0.18	4.3±0.32	0.8±0.16	3.2±0.31
	150	6.4±0.32	16.1±0.75	*	7.4±0.42	3.1±0.31	7.9±0.41	2.8±0.29	7.1±0.44
	200	12.3±0.58	20.5±1.18	0.4±0.07	11.9±0.61	6.3±0.41	11.6±0.48	9.8±0.38	10.9±0.42
	250	18.0±1.12	23.8±1.92	2.7±0.18	14.6±0.85	11.9±0.42	20.1±0.28	16.4±1.18	16.6±0.42
Seeds of <i>Tephrosia purpurea</i>	50	1.0±0.21	4.8±0.21	*	0.9±0.08	*	1.4±0.15	*	0.8±0.08
	100	2.8±0.19	9.4±0.48	*	3.5±0.26	0.8±0.15	4.3±0.31	0.8±0.14	3.2±0.31
	150	7.3±0.28	15.6±0.34	*	8.5±0.06	3.2±0.28	7.9±0.44	2.8±0.72	7.1±0.44
	200	11.8±0.51	19.9±1.18	0.6±0.32	13.0±0.63	6.9±0.75	11.6±0.36	9.8±0.39	10.9±0.42
	250	17.4±1.12	23.8±1.92	3.0±0.21	15.6±0.22	11.4±0.04	19.9±0.28	15.5±1.24	16.5±0.07
Mixture of all four extracts (1:1:1:1) as Granules	50	5.0±0.42	15.8±0.34	9.2±1.12	17.6±1.26	22.6±1.28	20.9±2.36	*	11.3±1.48
	100	10.6±0.24	26.4±1.69	16.5±1.08	24.6±1.94	28.3±1.36	28.7±2.44	0.2±0.18	16.8±1.36
	150	17.4±1.47	31.4±2.18	24.1±1.82	29.3±2.36	34.8±2.19	35.2±2.31	5.5±0.27	25.3±1.70
	200	21.6±1.74	36.4±1.34	29.6±2.84	34.7±2.15	36.2±2.46	41.3±3.42	8.9±0.30	29.4±2.28
	250	29.2±2.80	40.6±1.14	36.1±2.21	41.1±2.42	44.2±3.62	45.1±2.85	15.7±1.12	32.4±2.55
Chloramphenicol	50	5.6±0.48	16.4±0.36	9.8±1.18	18.5±1.33	23.9±1.33	21.7±2.44	*	11.8±1.56
	100	11.3±0.26	27.2±1.78	17.1±1.14	25.4±2.01	29.2±1.41	29.6±2.57	0.2±0.19	17.4±1.42
	150	18.2±1.54	32.3±2.30	25.2±1.89	30.4±2.49	36.2±2.28	36.9±2.39	5.8±0.28	26.3±1.78
	200	22.5±1.81	37.6±1.39	30.7±2.95	36.1±2.24	37.8±2.56	42.8±3.56	9.2±0.31	30.6±2.39
	250	30.7±2.95	42.1±1.19	37.9±2.32	43.0±2.55	46.0±3.80	47.0±2.99	16.4±1.17	33.7±2.67

Mean±Standard deviation, n=3, All results above are mean of 3 times recurrent study after every 24 h for bacteria at 37° centigrade, *No inhibition

Table 9: *In vitro* anti-inflammatory by protein denaturation method

Concentration (µg/ml)	Standard	Roots of <i>Cayratia trifolia</i>	Flowers of <i>Sesbania grandiflora</i>	Barks of <i>Cordia dichotoma</i>	Seeds of <i>Tephrosia purpurea</i>	Mixture of all four extracts
0	0	0	0	0	0	0
100	72.26±0.12	36.12±0.78	0.35±0.62	39.5±1.25	34.5±1.25	44.47±1.18
200		42.62±0.56	41.59±1.24	45.67±0.38	39.67±0.38	52.12±3.12
300		49.08±0.24	46.37±0.46	53.44±1.14	45.44±1.14	60.17±0.39
400		57.7±0.38	56.32±1.16	61.36±1.52	54.36±1.52	68.34±0.65
500		64.78±0.54	62.18±0.82	69.59±1.46	61.59±1.46	76.81±3.51
IC ₅₀		322.2	376.5	255.0	351.0	171.67

CONCLUSION

The ethyl acetate extracts of *C. trifolia* (roots), *S. grandiflora* (flowers), *C. dichotoma* (bark), and *T. purpurea* (seeds) were successfully formulated into a stable herbal tablet dosage form using a non-aqueous wet granulation technique. Systematic evaluation of micromeritic properties demonstrated that granulation significantly optimized flow and compression characteristics of the herbal extracts. The resulting formulation exhibited potent synergistic *in vitro* anti-inflammatory and anti-microbial activity. Furthermore, the polyherbal tablet complied with all standard pharmacopoeial quality control parameters. These findings validate the potential of the combined extracts and demonstrate a viable pathway for the standardization and scalable production of high-quality herbal tablets.

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CONTRIBUTION BY EACH AUTHOR

Avilash Bhatta: Performed the practical experimental work. Sambit Parida: Guided the candidate while executing the work.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest in the manuscript.

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