DEVELOPMENT AND EVALUATION OF POLYHERBAL TABLET FOR ANTI-MICROBIAL RESISTANCE

By

THAKOR PREYASH DEVANG

Enrolment No. 21P1P058

Under the guidance of

Ms. Krutika Dixit

[Assistant Professor, Pharmacognosy]

A Project Report Submitted to School of Pharmacy, ITM SLS Baroda University in Partial Fulfilment of the Requirements for the Semester VIII Project Work (BP812PW) of Bachelor of Pharmacy

SCHOOL OF PHARMACY

ITM SLS BARODA UNIVERSITY

April 2025

DECLARATION

I declare that the Project work titled **"Development and evaluation of polyherbal tablet for anti-microbial resistance"** carried out by me during the period of semester VIII, B.Pharm 2023-24 is as per prescribed guidelines under the guidance of Ms. Krutika Dixit**.**

Details of project work premise: **School of Pharmacy, ITMBU**

The co-team members of the project work include,

1. Bhatt Aditi Maheshbhai, 21P1P004,
2. Patel Kesha Hitesh, 21P1P035,
3. Prajapati Harsh Sandip Kumar, 21P1P044,
4. Varia Ayushi Dilipbhai, 21P1P060,

Thakor Preyash Devang
21P1P058

Date: Place:

CERTIFICATE

This is to certify that project work embodied in this report titled **"Development and evaluation of polyherbal tablet for anti-microbial resistance"** was carried out by **Thakor Preyash Devang** (**21P1P058)** as per prescribed guidelines for partial fulfilment of the **Semester VIII** Project work (BP812PW) of **Bachelor of Pharmacy** degree to be awarded by ITM SLS Baroda University. This project work has been carried out under my guidance and supervision comprises of the bonafide work done by him to my full satisfaction.

Date:

Place:

Ms. Krutika Dixit
Assistant Professor (Guide)

Ms. Prajakta Kulkarni
Associate Professor (Co-Guide)

Dr. Jaswandi Mehetre
HOD-Pharmaceutics

Dr. Vimal Kumar
Dean-School of Pharmacy

Seal of Institute

PROJECT APPROVAL CERTIFICATE

This is to certify that project work embodied in this report titled **"Development and evaluation of polyherbal tablet for anti-microbial resistance"** was carried out by **Thakor Preyash Devang** (**21P1P058)** of School of Pharmacy, Vadodara is approved for partial fulfilment of semester VIII Project Work (BP812PW) of degree of Bachelor of Pharmacy by ITM SLS Baroda University.

Date:

Place:

Examiner's Signature and Name: _____

( )

ACKNOWLEDGEMENTS

I take this opportunity to sincerely thank all those who played a vital role in the successful completion of my thesis. This journey has been both challenging and rewarding, and it would not have been possible without the support, guidance, and encouragement of many individuals.

First and foremost, I would like to express my deepest gratitude to my guide Ms. Krutika Dixit ma'am, whose guidance, patience, and valuable insights were instrumental throughout the research process. From the very beginning, they believed in my potential and continuously motivated me to aim higher.

I would also like to extend my heartfelt thanks to the faculty members and staff of the ITM School of Pharmacy, who provided us with the resources and encouragement necessary to carry out my research. Their support has meant a great deal to me.

I would like to thank Dr. Jaswandi Mehetre mam to help us in various aspects of Project. Also, some special thanks to Ms. Maanika Menon ma'am, Ms. Ritika Gajre ma'am, Ms. Jigisha Panchal ma'am and Ms. Nidhi Patel ma'am to keep us motivating and giving us guidance whenever necessary during the project.

To my friends, I am truly grateful. Your constant motivation, emotional support, and willingness to help in times of stress kept me grounded and focused. Special thanks to Jainil Mistry, Adarsh Yadav, Het Bhatia, Dharmesh Prajapati, for always being there.

I would also like to acknowledge the contributions of my team members Harsh Prajapati, Kesha Patel, Ayushi Varia and Aditi Bhatt by their support we have successfully completed project.

Finally, to my family, thank you for your unwavering belief in me. Your love, sacrifices, and constant support have been my foundation.

Thank You…

Preyash Thakor

TABLE OF CONTENTS

DEVELOPMENT AND EVALUATION OF POLYHERBAL TABLET FOR ANTI-MICROBIAL RESISTANCE

Submitted By

Thakor Preyash Devang

Supervised By

Ms. Krutika Dixit

Pharmacognosy

Assistant Professor

ABSTRACT

Antimicrobial resistance (AMR) is a growing global health crisis, contributing to over 1.27 million deaths annually, with projections reaching 10 million deaths per year by 2050 if effective interventions are not implemented. The increasing prevalence of multidrug-resistant (MDR) pathogens necessitates novel adjunct therapies to enhance antibiotic efficacy. This study focuses on the development and evaluation of an enteric-coated herbal tablet as an antimicrobial adjuvant therapy, incorporating *Berberis aristata* extract (berberine) and *Punica granatum* extract (punicalagin). Berberine inhibits bacterial efflux pumps, increasing intracellular antibiotic accumulation, and disrupts membrane integrity, enhancing bacterial susceptibility. Punicalagin exhibits biofilm inhibition, preventing bacterial persistence and reducing resistance development. The synergistic antimicrobial properties of these bioactive compounds improve the efficacy of conventional antibiotics against MDR pathogens. The tablet formulation is developed using pharmaceutical excipients to ensure stability, controlled release, and patient compliance. An enteric coating is applied to facilitate targeted intestinal delivery, optimizing bioavailability and minimizing gastric degradation. The formulation process involves wet granulation, followed by enteric coating with polymers for controlled drug release. The herbal tablet is standardized and validated using Thin Layer Chromatography. This herbal-based adjuvant therapy presents a promising natural strategy to restore antibiotic potency and mitigate the escalating AMR crisis.

Keywords: Antimicrobial resistance, polyherbal adjuvant therapy, berberine, punicalagin, efflux pump inhibition, biofilm disruption, enteric-coated tablet.

Appendix I List of Figures

Appendix II List of Tables

1. Introduction

1.1. Antimicrobial resistance

Antimicrobial resistance is a frightening global public health issue in the 21st century. Antimicrobial resistance occurs when bacteria, fungi, viruses and pathogens stop responding to the antimicrobial medications which makes antibiotics and other antimicrobial agents ineffective making the infection more difficult to treat which increases the risk of disease spread, severe illness and deaths. With the time microbes evolve mechanisms to withstand with the antimicrobial agents which affects the conventional therapy plan for treatment of infections which leads to increased transmission and higher mortality. [1-3]

According to World Health Organization (WHO), AMR is one of the top ten global public health threats facing humanity. The resistance can be intrinsic or acquired through mutation or horizontal gene transfer. The spectrum of resistance ranges from multi-drug resistance (MDR) to extensively drug resistance (XRD) particularly in bacteria such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus.* Recent studies shows that AMR is responsible for over 1.27 million deaths annually, with potential projections of reaching 10 million deaths per year by 2050 if left unaddressed. In addition, AMR has significant economic costs. The World Bank estimates that AMR could result in US$ 1 trillion additional healthcare cost by 2050, and US$ 1 trillion to US$ 3.4 trillion gross domestic product (GDP) losses per year by 2030. Data from Centers for Disease Control and Prevention and WHO reveals regional disparities, with low and middle-income countries bearing the highest burden due to inadequate healthcare infrastructure, unregulated antibiotic usage, and poor sanitization. [1-6]

1.2. Etiology

Antimicrobial resistance can be caused due to multiple factors which includes following causes:

- **Overuse and Misuse of Antimicrobials**: Inappropriate prescribing of antibiotics for viral infections.
- **Poor Infection Control in Healthcare premises:** Inadequate hygiene and sterilization can facilitate the spread of resistant organisms.
- **Agricultural Practices:** Use of antibiotics in livestock as growth promoters or prophylaxis select for resistant strains that can be transmitted to humans.
- **Environmental factors:** Pharmaceutical waste, hospital effluents, and agricultural runoff introduce antimicrobials into ecosystems, exerting selective pressure.[7,8]

1.3. Mechanisms of Resistance

- **Active Efflux of Antibiotics:** Bacterial cells contain efflux pumps which are transport proteins that expels toxic substances from the cells. These efflux pumps lower the intracellular concentration of antibiotics by their active removal which increases the bacterial survival. Overexpression of these efflux pumps significantly contributes to the multidrug resistance by expelling various antibiotics like tetracyclines and fluoroquinolones.
- **Alteration of Membrane Permeation:** the membrane of bacteria works as a barrier to the antibiotics. reduction in membrane permeability leads to less entry of antibiotics in bacterial cell and decreased intracellular concentration of the drug. Alterations such as reduced porin expression and altered porin structure limits the antibiotics entry in bacterial cell.
- **Biofilm formation:** The bacterial communities encapsulate themselves in a matrix of extracellular polymeric substances (EPS) which often adheres to the surfaces like tissues of lungs, catheters. These matrix or biofilms functions as a physical barrier and limit the antibiotic diffusion. Also, in biofilms due to the less oxygen and nutrient gradients bacteria are in slow growing phase or dormant phase that are less susceptible to the antibiotics.
- **Enzymatic Degradation:** Bacteria produce some enzymes that inactivates antibiotics by breaking them down or modifying their structure, rendering them ineffective. For example, ß-lactamase enzyme hydrolyzes the ß-lactam ring of penicillins and cephalosporins which leads to develop resistance.[9-12]

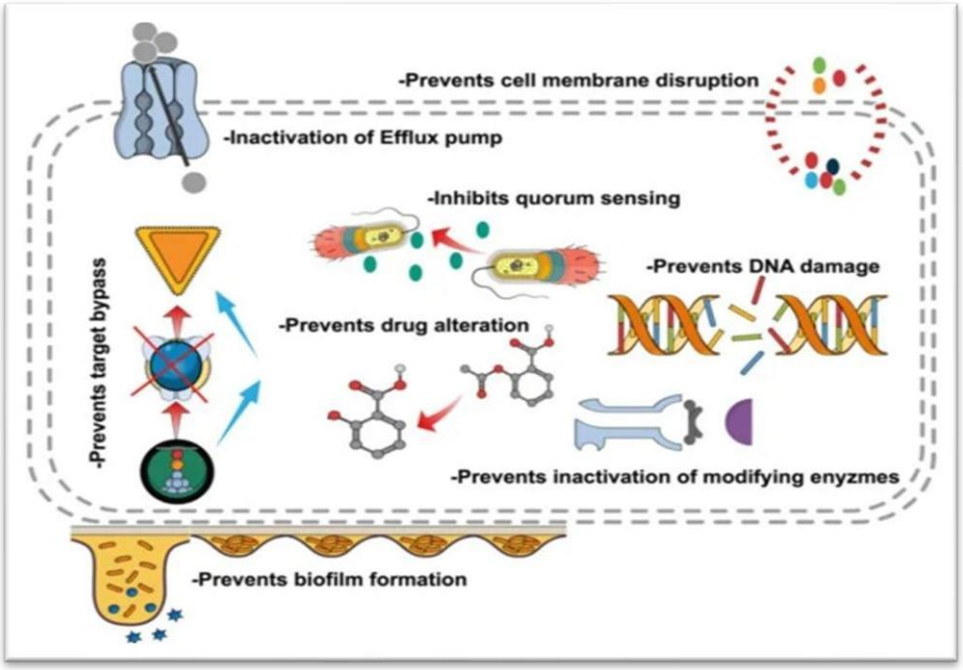


Fig. No. 1.1: Mechanisms of Resistance

1.4. Antibiotic Adjuvants

Antibiotic adjuvants are the agents which enhances the effectiveness of antibiotics when used in combination while they themselves may possess minimal or no inherent antimicrobial activity. These adjuvants work by targeting the mechanisms of resistance or by modulating host responses which helps to restore the effectiveness of conventional antibiotics against resistant bacteria.[13]

There are basically two classes of adjuvants based on their mechanism of action:

1. Class I Adjuvants: this type of adjuvant work by either inhibiting the active resistance by directly inhibiting the enzymes responsible for resistance or by inhibiting passive mechanisms like efflux pump inhibition, inhibition of biofilm formation, membrane permeation of antibiotics.
2. Class II Adjuvants: They modulate the host's immune response to enhance the outcomes of the therapy.[14,15]

These adjuvants can be synthetic or herbal, Clavulanic acid, Sulbactam, EDTA are some of the examples of synthetic adjuvants whereas Berberine, Curcumin, Eugenol, Epigallocatechin gallate are some of the examples of herbal adjuvants.

2. Aim, Objective and Rationale

Aim: To formulate and evaluate enteric coated polyherbal tablet for minimizing the antimicrobial resistance.

Objective:

- To formulate a polyherbal tablet for AMR containing *Berberis aristata*, *Punica granatum*, *Piper nigrum* extracts along with appropriate excipients.
- To conduct pre-formulation studies on extracts in compliance with WHO guidelines.
- To perform physicochemical properties of formulated tablets.

Rationale:

1. The rise of AMR has reduced the effectiveness of conventional antibiotics, necessitating alternative therapeutic approaches to combat resistant pathogens.
2. A polyherbal formulation, integrating various medicinal plant extracts i.e. *Berberis aristata*, *Punica granatum*, *Piper nigrum*, enhances antimicrobial potency through the synergistic action of diverse phytochemicals.
3. Bioactive compounds i.e. berberine, punicalagin act as efflux pump inhibitors, biofilm disruptors, and quorum sensing inhibitors, effectively counteracting microbial resistance mechanisms.
4. The inclusion of herbal extracts with antimicrobial properties serves as an adjuvant to conventional therapy, potentially reducing dependence on synthetic antibiotics and mitigating resistance development.
5. The utilization of plant-derived alternatives fosters a natural and sustainable approach in addressing AMR.

3. Literature Review and Drug profile

3.1. Literature Review

Table. No. 3.1: Review of Literature

Plant	Description	Reference
Berberis aristata	• Strong antibacterial activity • Efflux Pump Biofilm Disruption • Synergistic Effect with Antibiotics. • Low Resistance Development	➢ Zhou H, Wang W, Cai L, Yang T. Potentiation and mechanism of berberine as an antibiotic adjuvant against multidrug-resistant bacteria. Infection and Drug Resistance. 2023 Dec 31:7313-26.
Punica granatum	• Rich in Punicalagin & Ellagic Acid • Broad-Spectrum Antimicrobial • Anti-Biofilm Activity • Synergy with Antibiotics • Immune-Boosting Effects	➢ Xu Y, Shi C, Wu Q, Zheng Z, Liu P, Li G, Peng X, Xia X. Antimicrobial activity of punicalagin against Staphylococcus aureus and its effect on biofilm formation. Foodborne pathogens and disease. 2017 May 1;14(5):282-7.
Piper nigrum	• Inhibits Drug-Metabolizing Enzymes • Enhances Absorption • Antibacterial action	➢ Db M, Sreedharan S, Mahadik K. Role of piperine as an effective bioenhancer in drug absorption. Pharm Anal Acta. 2018;9(7):1-4.

3.2. DRUG PROFILE

3.2.1. Drug Profile of *Berberis aristata*

Table. No: 3.2: Drug Profile of *Berberis aristata*

Drug Profile	**Description**
Synonyms	Indian Berberry, Daruharidra, Chutro, Sumba
Biological source	Obtained from Stem, bark, root *of Berberis aristata, Berberis vulgaris*
Family	Berberidaceae
Geographical location	It is native to the himalayan region, specially found in hilly areas of northen India, nepal, bhutan, and nilgiris hills of south india, as well as in sri lanka.
Morphology	 Fig. No. 3.1: *Berberis aristata*

	Height: shrub, 1–3 meters tall. Leaves: simple, ovate or elliptical, with serrated edges, and usually dark green. Flowers: yellow, small, in clusters, blooming in spring. Fruits: red or orange berries, elongated, and contain multiple seeds. Stems: thorny, branched, with a woody texture.
Chemical constituents	it consists of protoberberine and bis isoquinoline type of alkaloid. it's mainly containing alkaloids which are berbamine, berberine, oxycanthine, epiberberine, palmatine dehydrocaroline.
Mechanism of action	• strong antibacterial activity efflux pump • biofilm disruption • synergistic effect with antibiotics. • low resistance development
Side effects	Berberis aristata may cause side effects like gastrointestinal issues (nausea, diarrhea)
Medicinal Uses	Hepatoprotective, Antidiabetic, Anticancer, Antimalarial, Antimicrobial, Anti-inflammatory. (16-18)

3.2.2. Drug Profile of *Punica granatum*

Table. No: 3.3: Drug Profile of *Punica granatum*

Drug Profile	**Description**
Synonyms	Pomegranate, Anaar, Dadam
Biological source	Obtained from Fruit peel, seeds, leaves of *Punica granatum*

Family	Lythraceae
Sub Family	Punicaceae
Geographical location	It is native to Southwest Asia and the Mediterranean region. It is widely cultivated in India, Iran, Turkey, Spain, California (USA), and parts of North Africa. It thrives in subtropical and tropical climates.
Morphology	 Fig. No. 3.2: *Punica granatum* Punica granatum (Pomegranate) has the following key morphological features: Height: Small tree or shrub, 5–8 meters tall. Leaves: Simple, glossy, lance-shaped,and dark green. Flowers: Bright red or orange, trumpet-shaped, with a large central pistil. Fruits: Large, round, with a toughouter rind and filled with juicy red seeds (arils). Stems: Woody and spiny.

Chemical constituents	Peel mainly consists of Phenolics, flavonoids, ellagitannins and proanthocyanidin. The edible part of the fruit consists of arils and seeds. Arils consist of pectin, ascorbic acid, citric acid, malic acid etc. also consist of punicic acid which is mainly obtained from seed oil. Main peel constituents: Gallic acid, ellagic acid, punicalagin, ellagitannins, quercetin etc. main constituents of seeds are punicic acid, oleic acid, palmitic acid, stearic acid, sterols etc.
Mechanism of action	• Rich in Punicalagin & Ellagic Acid Broad-Spectrum Antimicrobial Anti-Biofilm Activity • Synergy with Antibiotics • Immune-Boosting Effects
Side effects	It may cause allergic reactions, gastrointestinal issues (like diarrhea or nausea), and interact with blood pressure or blood- thinning medications. Excessive intake, especially of the peel, should be avoided during pregnancy.
Medicinal Uses	Antidiabetic, Anticancer, Antimicrobial, Anti-inflammatory, Neuroprotective. [19-21]

3.2.3. Drug Profile of *Piper nigrum*

Table. No: 3.4: Drug Profile of *Piper nigrum*

Drug Profile	**Description**
Synonyms	Black pepper, Pepper, Kali Mirch, Pilpil
Biological source	It consists of dried unripe fruits of piper nigrum Linn.
Family	Piperaceae
Geographical location	It is native to south india and sri lanka. It thrives in tropical climates and is primarily grown in india, sri lanka, indonesia, vietnam, malaysia, and brazil.

Morphology	 Fig. No: 3.3: *Piper nigrum* Plant Type: Climbing vine that can grow up to 4 meters (13 feet) tall, using nearby structures or trees for support Stem: Woody at the base, becoming flexible towards the top. The stem has internodes and is glabrous (smooth, without hairs). Leaves: Simple, oval-shaped, and glossy. The leaves are dark green, about 10-15 cm long and 5-7 cm wide, with a pointed tip. They have a heart-shaped or acuminate apex. Flowers: Small, greenish-white in color. Flowers are arranged in spike-like inflorescences that are about 5-10 cm long. Each spike consists of numerous tiny flowers that bloom in dense clusters. Fruits (Peppercorns): Drupe-like, initially green, turning yellow and then red when fully ripe. The fruit contains a single seed, which is the peppercorn. After harvesting, the peppercorns are dried, turning black (for black pepper), green (for green pepper), or white (for white pepper, when the outer skin is removed). Roots: The plant has fibrous roots that spread out to absorb water and nutrients.

Chemical constituents	Primarily contains alkaloids such as piperine, chavicine, piperettine, and isopiperine. It also includes oleoresins and essential oils, with monoterpene hydrocarbons being the most abundant, followed by sesquiterpene hydrocarbons, monoterpenoids and sesquiterpenoids. Other components include starch, fat, and fatty acids like palmitic, oleic, linoleic, and linolenic acids. The phenolic content consists of glycosides with hydroxybenzoic and hydroxycinnamic acids as the most abundant phenolic compounds, along with significant amounts of quercetin and kaempferol.
Mechanism of action	• Inhibits drug-metabolizing enzyme • enhances absorption • antibacterial action
Side effects	It may cause stomach irritation, allergic reactions, and interact with medications by increasing their absorption. High doses should be avoided during pregnancy.
Medicinal Uses	Anticancer, Antimicrobial, Analgesic, Antipyretic, Hepatoprotective, Bio enhancing effect, Enzyme inhibitor, Antioxidant. [22-24]

4. Material and Methods

4.1. Chemical and equipment

The chemicals and equipment used in preparation of formulation are listed in Table 4.1 and 4.2 respectively. The glassware and distilled water were used from the lab of School of Pharmacy, ITM (SLS) Baroda University.

Table.no: 4.1: List of Chemicals

CHEMICALS	CATEGORY	SUPPLIERS
Berberis aristata extract	API	Prepared in Lab
Punica granatum peel extract	API	Prepared in Lab
Piper nigrum extract	Bioavailability enhancer	Prepared in Lab
Microcrystalline cellulose	Diluent	Loba Chemie Pvt. Ltd.
Carboxy methyl cellulose	Disintegrant	Loba Chemie Pvt. Ltd.
Polyvinyl pyrrolidone	Binder	Loba Chemie Pvt. Ltd.
Talc	Glidant	Loba Chemie Pvt. Ltd.
Magnesium Stearate	Lubricant	Loba Chemie Pvt. Ltd.
Eudragit	Coating polymer	Loba Chemie Pvt. Ltd.
Isopropyl Alcohol	Coating solvent	Loba Chemie Pvt. Ltd.
Ethanol	Extraction solvent	Loba Chemie Pvt. Ltd.

Table. No: 4.2: List of equipment

Equipment	Category	Suppliers
Digital Electronic Balance	For Weighing	Reptech
Mechanical Stirrer	For uniform mixing	REMI Elektrotechnik
UV- Visible Spectrophotometer	For determination of drug	Lab Intelligence Appliances
Disintegration apparatus	To determine disintegration time	Lab Intelligence Appliances
Tray dryer	For drying	Lab Intelligence Appliances
Friability tester	To check friability	Lab Intelligence Appliances

4.2. Preparation of extracts

4.2.1. Extraction of *Berberis aristata* :

- *Berberis aristata* root powder was obtained and was shed dried for 12 hrs.
- 20gms of the powder was weighted and was added to Soxhlet thimble with 200 ml of ethanol.
- The extraction process was carried out for about 8-10 hrs. at 60°C
- The obtained extract was filtered and concentrated it by evaporating ethanol on water bath at 60°C
- The extract was acidified by adding few drops of 1% HCL (ph-4) to form crystals.[25,26]

Fig. No. 4.1: Extraction assembly for *Berberis aristata*

4.2.2. Extraction of *Punica granatum*:

- *Punica granatum* peel was obtained and was shed dried for 24 hrs. and then crushed in powder
- 20gms of the powder was weighted and was added to Soxhlet thimble with 200 ml of ethanol.
- The extraction process was carried out for about 6-8 hrs. at 60°C
- The obtained extract was filtered and concentrated it by evaporating ethanol on water bath at 60°C
- The dry extract was obtained and percentage yield was calculated.[27,28]

Fig. No. 4.2: Extraction assembly for *Punica granatum*

4.2.3. Extraction of Piper nigrum:

- *Piper nigrum's* peppercorns was obtained and was shed dried for 24 hrs. and was crushed in powder.
- 20gms of the powder was weighted and was added to Soxhlet timbal with 200 ml of ethanol.
- The extraction process was carried out for about 6-8 hrs. at 60°C
- The obtained extract was filtered and concentrated it by evaporating ethanol on water bath at 60°C
- Add 20 ml of 10% alcoholic potassium hydroxide with constant stirring to the concentrated extract and filter.
- Allow the alcoholic solution to stand overnight. (excess water was added until precipitate comes out).
- needles of piperine separate out.
- collect the yellow needle shaped crystals of piperine.
- Calculate % yield.[29,30]

Fig. No. 4.3: Extraction assembly for *Piper nigrum*

4.3. Proximate Analysis of Extracts

4.3.1: Organoleptic Properties of Extracts: Organoleptic examination includes of colour, odour, taste examination.

4.3.2. pH determination of Extract: pH test was conducted using a pH meter. The pH readings were taken with the help of pH meter which was calibrated with the help of Standard buffer solution. 0.5gm extract was dissolved in 5ml water and pH reading were obtained.

4.3.3. Determination of Extractive Value for Extracts: To find out the extractive value of each extract the weight of sample taken (crude drug) for extraction and the weight of the solid extract are obtained first and the extractive value is calculated with the formula[31-35]:

$$\text{Extractive value(\%W/W)} = \frac{\text{Weight of solid extract (g)}}{\text{Weight of sample (g)}} \text{ X } 100$$

4.4. Phytochemical Screening

Table. No: 4.3: Phytochemical test Procedure[36]

Test	Procedure	Observation
Hager's test	Few mL filtrate + 1-2 mL Hager's reagents	A creamy white precipitate
Dragendroff's test	Few mL filtrate + 1-2 mL Dragendroff's reagents	A reddish-brown precipitate
Molisch's test	2mL filtrate + 2 drops of alcoholic α–naphthol + 1mL conc. H2SO4 (along the sides of test tube)	A violet ring
Benedict test	0.5mL filtrate + 0.5mL Benedict's reagent + Boiled for 2 min.	Green/yellow/red colour

Milon's test	2mL filtrate + few drops of Millon's reagent	A white precipitate
Baljet test	2mL extract + a drop of Baljet's reagent	A yellow-orange colour
Ferric chloride test	Extract aqueous solution + few drops 10% ferric chloride solution	A green precipitate
Ninhydrin test	2mL filtrate + 2 drops of Ninhydrin solution (10mg ninhydrin + 200mL acetone)	A purple-coloured sol. {Amino acids}
Lead acetate test	1mL plant extract + few drops of 10% lead acetate solution	A yellow precipitate
Braymer's test	1mL filtrate + 3mL distilled water + 3 drops 10% Ferric chloride solution	Blue-green colour
Salkowski's test	Filtrate + few drops of conc. H2SO4 (Shaken well and allowed to stand)	Red colour (in lower layer)

4.5. Thin Layer Chromatography of Extracts

Thin layer chromatography (TLC) is used widely in various fields to separate or purify mixtures of chemical and biological compounds. TLC often used for the direct analysis of crude samples with minimal purification procedures. The separation, the edge of the TLC plate is immersed in the mobile phase, which is developed through capillary force. The diversity of interactive forces among the analyte molecules, mobile phase, and stationary phase cause different analytes to move at different rates on the TLC plate.

The separated compounds on the plate are visualized and, in some instances, characterized. The sample spots are usually detected by spraying or dipping the TLC plate so that the analytes come into contact with chemical or biological reagents, which then react or interact with the functional groups of the analyte molecules. The separation

of the chemical compounds on a TLC plate is quantified in terms of the value of Rf (distance of analyte migration/distance of mobile phase migration).[37,38]

TLC of *Berberine aristata:*

- Sample preparation: methanolic extract of drug (drug extract dissolved in methanol).
- Stationary phase: silica gel G
- Detection Method: Under U.V 366 nm
- Mobile phase used:
  1. Toluene: Ethyl acetate: Diethyl amine (7:2:1)
  2. Toluene: Ethyl acetate: Formic acid (5:4:3)
  3. Formic acid: Ethyl acetate: Water (1:8:1). [39]

TLC of *Punica granatum:*

- Sample preparation: methanolic extract of drug (drug extract dissolved in methanol).
- Stationary phase: silica gel G
- Detection Method: Under U.V 366 nm
- Mobile phase used:
  1. Water: Acetic Acid (3:2)
  2. Chloroform: Ethyl acetate: Formic acid (5:4:1)
  3. Ethyl acetate: Methanol: Water (7.7:1.3:1). [40]

TLC of *Piper nigrum:*

- Sample preparation: methanolic extract of drug (drug extract dissolved in methanol).
- Stationary phase: silica gel G
- Detection Method: Under U.V 366 nm
- Mobile phase used: Toluene: Ethyl acetate (7:3). [41]

Mobile Phase Development

- Sample preparation: methanolic extract of all drugs (drugs extract dissolved in methanol).
- Stationary phase: silica gel G
- Detection Method: Under U.V 366 nm
- Mobile phase used:

Table. No: 4.4: List of Mobile phase used	
Mobile Phase	**Ratio**
Toluene: Ethyle acetate: Diethyl amine	7:2:1
Ethyl acetate: formic acid: glacial acetic acid: water	100:11:11:26
Ethyl acetate: methanol: water	77:13:10
Ethyl acetate: Methanol: Water: Formic acid	7:1:1:1
Ethyl acetate: Formic acid: Methanol	5:4:1
Ethyl acetate: Formic acid: Methanol	6:3:1
Toluene: Ethyl acetate: Formic acid: Methanol	**3:3:2:2**

4.6. Preformulation Tests

4.6.1. Bulk Density: 10gm of granules were weighed. These granules powder was poured in the 50 ml graduated measuring cylinder without tapping. The volume occupied by the powder was recorded as (V_0).

4.6.2. Tapped Density: 10gm of granules were weighed. These granules powder was poured in the 50 ml graduated measuring cylinder. The cylinder was tapped for 100 times. The final volume occupied by the powder after 100 taps was recorded as (V_f).

4.6.3. Carr's Index: with the help of bulk and tapped density values the Carr's Index was calculated using formula: Carr's Index (%) = [(Tapped Density - Bulk Density) / Tapped Density] × 100.

4.6.4. Hausner's ratio: with the help of bulk density and tapped density values the Hausner's ratio was calculated using formula: Hausner's Ratio = Tapped Density / Bulk Density.

4.6.5. Angle of Repose: A funnel was fixed at the height of 6 cm on a flat surface the paper was placed beneath it. The powder was allowed to flow through the funnel to

form a cone on the paper. The radius of **cone** was determined at the base. The Angle of repose was calculated using the formula: Angle of Repose (θ) = $\tan^{-1}(h/r)$**.** [42,43]

4.7. Preparation of Tablets

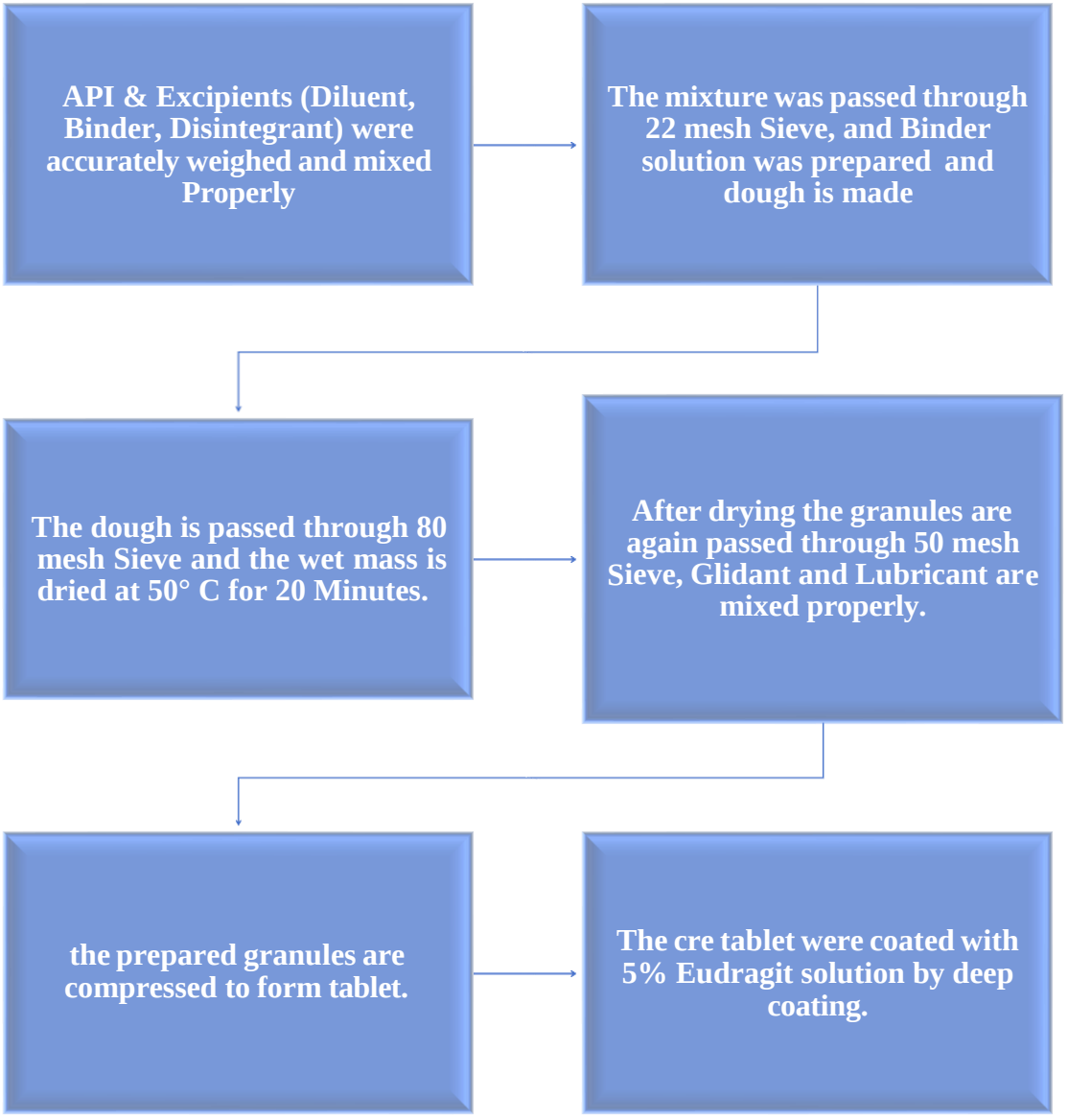


Fig. No. 4.4: Preparation of tablet. [44-46]

Different preliminary batches were formulated using different concentration of ingredients. [47,48]

Table. No: 4.5: Preliminary batches

Ingredients	**Use**	**F1(mg)**	**F2(mg)**	**F3(mg)**	**F4 (mg)**	**F5 (mg)**
Berberis aristata extract	API	300	300	300	300	300
Punica granatum extract	API	150	150	150	150	150
Piper nigrum extract	Bioenhancer	5	5	5	5	5
Microcrystalline cellulose	Diluent	100	100	100	100	100
Starch	Binder	35	70	-	-	-
Polyvinyl pyrrolidone	Binder	-	-	7	21	35
Carboxymethyl cellulose	Disintegrant	20	20	20	20	20
Magnesium stearate	Lubricant	10	10	10	10	10
Talc	Glidant	10	10	10	10	10

4.8. Evaluation of Tablets

4.8.1. Organoleptic evaluation: Organoleptic examination includes of colour, odour, examination.

4.8.2. Thickness and diameter: The thickness and diameter were measured with the help of vernier caliper. The vernier caliper was calibrated to ensure it reads zero when fully closed. The tablet was placed in the external jaws of the caliper. For diameter by flat face and for thickness by tablet's edges. The readings were recorded from the scale on vernier. The experiment was repeated for 5 tablets.

4.8.3. Weight variation: The weight variation test was performed by randomly selecting 20 tablets from each batch. Each tablet was individually weighed using an analytical balance. The average weight was calculated, and individual weights were compared against the average. The percentage deviation of each tablet was determined to ensure compliance with pharmacopeial limits.

4.8.4. Friability Testing: Friability testing was conducted using a Roche friabilator. A total of 10 tablets were accurately weighed and placed in the drum, which was rotated at 25 rpm for 4 minutes (100 revolutions). After the test, the tablets were dedusted and reweighed. The friability was calculated as the percentage weight loss and found to be within acceptable limits (<1%).

4.8.5. Hardness Testing: Tablet hardness was tested using a Monsanto hardness tester. Ten tablets from formulation were individually tested by placing them between the jaws of the tester and applying pressure until the tablet fractured. The force required to break each tablet was recorded in kg/cm², and the average hardness was calculated.

4.8.6. Disintegration: The disintegration test was performed using a USP disintegration test apparatus. 6 tablets were placed in individual tubes of the basket rack submerged in buffer solution (pH: 6.8) and 6 tablets were placed in individual tubes of the basket rack submerged in 0.1 N HCL maintained at 37 ± 2°. The basket-rack assembly should be placed in the disintegration medium and moved up and down at a frequency of 28 to 32 cycles per minute. The time taken for each tablet to completely disintegrate without leaving any residue was recorded, and the results complied with pharmacopeial standards. [49-51]

4.8.7. UV Spectroscopy

4.8.7.1. UV Spectroscopy of *Berberis aristata*

Accurately weighed 10 mg of *Berberis aristata extract* was dissolved in 100 ml methanol to get a stock solution of 100 µg/ml. From the stock solution, an aliquot of 1 ml was withdrawn and transferred to a 10 ml volumetric flask. It was diluted with methanol to obtain 10 µg/ml solution of *Berberis aristata extract* and spectra was recorded using UV Vis spectrophotometer between the range of 200-600 nm and the wavelength of maximum absorbance was determined. [52]

Preparation of standard calibration curve of drug:

The preparation of standard calibration curve was prepared using methanol for estimation of drug content and drug release. From the stock solution of 100 µg/ml, aliquots of0.5, 1.0, 1.5, 2.0, 2.5 ml were withdrawn and further diluted up to 10 ml with methanol to obtain a concentration range of 5 µg/ml-25 µg/ml. The absorbance of these solutions was measured at the λ_{max} obtained. A cumulative graph of concentration vs.

absorbance was plotted. The regression line equation and the square of the correlation coefficient (R^2) were calculated.

4.8.7.2. UV Spectroscopy of *Punica granatum*

Accurately weighed 10 mg of *Punica granatum extract* was dissolved in 100 ml of methanol to get a stock solution of 100 µg/ml. From the stock solution, an aliquot of 2 ml was withdrawn and transferred to a 10 ml volumetric flask. It was diluted with methanol to obtain 20 µg/ml solution of *Punica granatum extract* and spectra was recorded using UV Vis spectrophotometer between the range of 200-600 nm and the wavelength of maximum absorbance was determined. [53]

Preparation of standard calibration curve of drug:

The preparation of standard calibration curve was prepared using methanol for estimation of drug content and drug release. From the stock solution of 100 µg/ml, aliquots of 2.5, 2.6, 2.7, 2.8, 2.9 ml were withdrawn and further diluted up to 10 ml with methanol to obtain a concentration range of 25 µg/ml-29 µg/ml. The absorbance of these solutions was measured at the λ_{max} obtained. A cumulative graph of concentration vs. absorbance was plotted. The regression line equation and the square of the correlation coefficient (R^2) were calculated.

4.8.7.3. UV Spectroscopy of *Piper nigrum*

Accurately weighed 10 mg of *Piper nigrum extract* was dissolved in 10 ml of methanol in 100 ml to get a stock solution of 100 µg/ml. From the stock solution, an aliquot of 0.5 ml was withdrawn and transferred to a 10 ml volumetric flask. It was diluted with methanol to obtain 5 µg/ml solution of *Piper nigrum extract* and spectra was recorded using UV Vis spectrophotometer between the range of 200-600 nm and the wavelength of maximum absorbance was determined. [54,55]

Preparation of standard calibration curve of drug:

The preparation of standard calibration curve was prepared using methanol for estimation of drug content and drug release. From the stock solution of 100 µg/ml, aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 ml were withdrawn and further diluted up to 10 ml with methanol to obtain a concentration range of 1 µg/ml- 5 µg/ml. The absorbance of these solutions was measured at the λ_{max} obtained. A cumulative graph of concentration vs. absorbance was plotted. The regression line equation and the square of the correlation coefficient (R^2) were calculated.

5. Results and Discussion

5.1. Proximate evaluation of Extracts

Table. No. 5.1: Proximate analysis of Extracts

Tests	*Berberis aristata*	*Punica granatum*	*Piper nigrum*
Colour	Yellow	Brown	Yellowish, green
Odour	Odourless	Fruity	Pungent
Taste	Bitter	Sweet & Sour	Pungent
pH	4.3	4.7	5.8
Extractive value	7.8 % w/w	9.5 % w/w	1.8 % w/w

The extracts showed distinct organoleptic and physicochemical properties. The colours and odours were characteristic of each plant and within acceptable herbal standards. *Berberis aristata* was bitter and odourless, *Punica granatum* sweet-sour and fruity, while *Piper nigrum* was pungent in both taste and smell—all aligning with standard descriptions. The pH values (4.3–5.8) were within acceptable limits for plant extracts, indicating stability. Extractive values for *Berberis aristata* (7.8%) and *Punica granatum* (9.5%) complied with pharmacopeial standards, while *Piper nigrum* (1.8%) was lower, possibly due to its volatile content or extraction method.

5.2. Phytochemical Screening of Extracts

Table. No. 5.2: Phytochemical Screening of Extracts

Phytochemicals	*Berberis aristata*	*Punica granatum*	*Piper nigrum*
Alkaloid	+	+	+
Glycosides	+	+	+
Carbohydrates	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Proteins & AA	+	+	+
Phytosterols	-	+	-

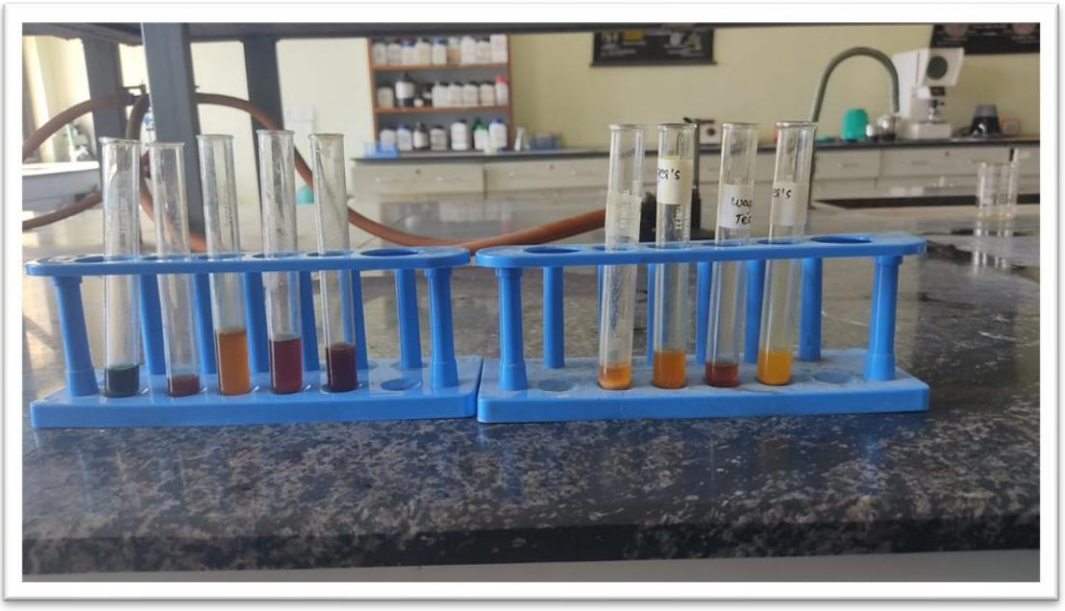


Fig. No. 5.1: Phytochemical Screening of Extracts

Phytochemical screening confirmed the presence of key bioactive compounds such as alkaloids, glycosides, carbohydrates, tannins, flavonoids, and proteins in all three extracts, supporting their therapeutic potential. Phytosterols were absent in *Berberis aristata* and *Piper nigrum*, which is consistent with some literature reports.

5.3. Thin Layer Chromatography

5.3.1. TLC of individual extracts:

Table. No. 5.3: TLC of *Berberine aristata, Punica granatum, Piper nigrum*

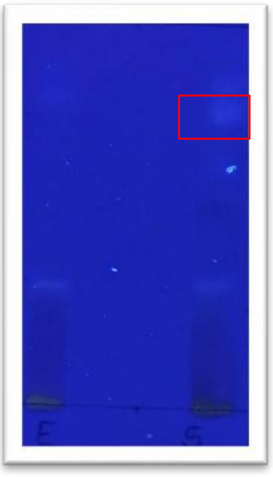
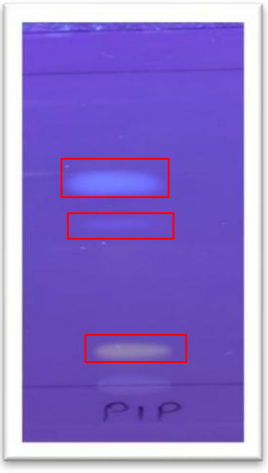
TLC Profile	***Berberis aristata***	***Punica granatum***	***Piper nigrum***
Sample preparation	1mg extract dissolved in 1ml ethanol	1mg extract dissolved in 1ml ethanol	1mg extract dissolved in 1ml ethanol
Stationary Phase	Silica gel G	Silica gel G	Silica gel G
Mobile Phase	Ethyl acetate: Methanol: Water (7.7:1.3:1)	Ethyl acetate: Methanol: Water (7.7:1.3:1)	Toluene: Ethyl acetate (7:3)
Detection Method	Under U.V 366 nm	Under U.V 366 nm	Under U.V 366 nm
Pictures			

	Fig. No. 5.2: TLC of *Berberine aristata*	Fig. No. 5.3: TLC of *Punica granatum*	Fig. No. 5.4: TLC of *Piper nigrum*
R_f Values	0.7	0.74	0.57, 0.45, 0.20

The TLC for *Berberine aristata, Punica granatum, Piper nigrum* in different mobile phases out of which in Ethyl acetate: Methanol: Water (7.7:1.3:1), Ethyl acetate: Methanol: Water (7.7:1.3:1), Toluene: Ethyl acetate (7:3) the R_f Values 0.7, 0.74, 0.57 were obtained respectively. These values comply with the Indian Ayurvedic Pharmacopoeia limits.

5.3.2. Single mobile phase development:

- Sample preparation: 1 mg of extract in 1 ml ethanol for each extract
- Stationary phase: silica gel G
- Detection Method: Under U.V 366 nm
- Mobile phase used: Toluene: Ethyl acetate: Formic acid: Methanol (3:3:2:2)

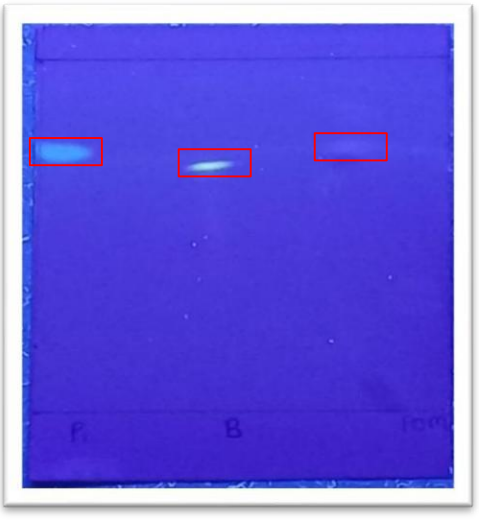


Fig. No. 5.5: Single mobile phase

Seven different mobile phases were used with different polarity and ratios in order to obtain the spots of every drug on TLC plate with the use of single mobile phase out of

which Ethyl acetate: Formic acid: Methanol (3:3:2:2) showed the best results which can be further developed for HPTLC purposes.

5.4. Preformulation evaluations:

Table. No. 5.4: Preformulation evaluations

Tests	Observation
Bulk density	17.5 g/ml
Tapped density	22.5 g/ml
Carr's index	22%
Hausner's ratio	0.77
Angle of repose	28°

The bulk density and tapped density indicate a moderately compressible powder. Carr's index suggests fair flow properties, while the Hausner's ratio indicates excellent flowability. An angle of repose of also supports Passable flow behavior. Overall, these parameters suggest that addition of glidant to the powder blend is suitable for further formulation and processing.

5.5. Preparation of preliminary batches

Table. No. 5.5: Preparation of preliminary batches

Batch	Observation
F1	Loose tablet was formed
F2	Hardness of 1.5 kg/cm^2 was obtained
F3	Hardness of 2.5 kg/cm^2 was obtained
F4	Hardness of 4 kg/cm^2 was obtained
F5	Hardness of 6.5 kg/cm^2 was obtained

Based on the observations the data suggested that the best hardness of the tablet was obtained in **F5 batch** with good friability in which PVP was used as binder.

5.6. Evaluation of Tablets

Table. No. 5.6: Basic Evaluations

Tests	Observation
Colour	Brown
Odour	Slight
Diameter	1.3 cm
Thickness	0.6 cm
Friability	0.01%

The formulation exhibited a brown colour with a slight odour, indicating acceptable organoleptic properties. The tablet dimensions1.3 cm in diameter and 0.6 cm in thickness are within standard limits for ease of handling. The extremely low friability value (0.01%) suggests excellent mechanical strength and minimal weight loss during handling, ensuring tablet durability.

5.7. Weight variation

Table. No. 5.7: Weight variation

Sr. No	Individual Weight (mg)	Average Weight	% Variation
1	594	600	-0.17%
2	599	600	-1.00%
3	604	600	+0.67%
4	597	600	-0.50%

5	597	600	-0.50%
6	615	600	+2.5%
7	605	600	+0.83%
8	600	600	0.00%
9	597	600	-0.50%
10	595	600	-0.83%
11	610	600	+1.67%
12	611	600	+1.83%
13	613	600	+2.17%
14	603	600	+0.50%
15	610	600	+1.67%
16	603	600	+0.50%
17	610	600	+1.67%
18	609	600	+1.50%
19	607	600	+1.17%
20	600	600	0.00%

The % variation for 20 tablets was find out and no tablet deviated from the specified range by Pharmacopoeia.

5.8. Hardness Testing

Table. No. 5.8: Hardness observation

Tablet No.	**Hardness Value (Kg/cm^2)**
1	6.5
2	6.75

3	6.5
4	7.0
5	7.0
6	6.25
7	6.5
8	6.5
9	6.75
10	7.0
Average	**6.67**

The Hardness of the tablets is within the range and suggests that it can with stand with mechanical stress during packaging and transport.

5.9. Disintegration Test

Table. No. 5.9: Disintegration time

Tablet No.	**Disintegration time (Min) (Buffer solution 6.8 pH)**	**Disintegration time (0.1 N HCL)**
1	32	Do not disintegrate for 2 hrs.
2	40	Do not disintegrate for 2 hrs.
3	45	Do not disintegrate for 2 hrs.
4	50	Do not disintegrate for 2 hrs.
5	45	Do not disintegrate for 2 hrs.
6	38	Do not disintegrate for 2 hrs.

The DT data suggest that the tablet does not disintegrate in acidic environment and disintegrated in the intestinal environment.

5.10. UV Spectroscopy

5.10.1. UV Spectroscopy of *Berberis aristata*

The wavelength (λmax) of *Berberis aristata extract* 10 µg/ml of c was measured using UV spectrophotometer and UV scan of which is showed in Fig. 5.5. Results are mentioned in the Table 5.9.

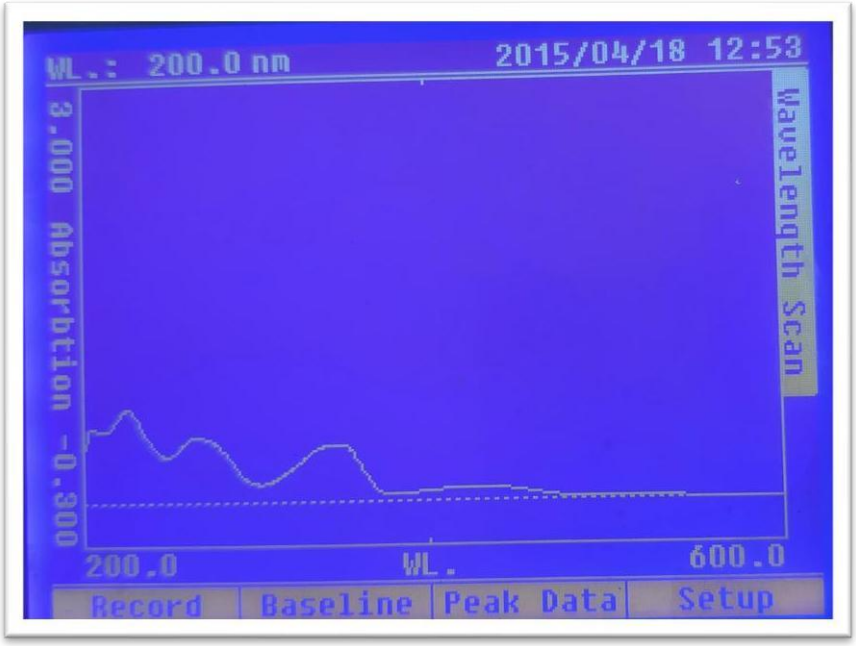


Fig. No. 5.6: UV scan of *Berberis aristata extract*

Table. No. 5.10: λmax of *Berberis aristata extract*		
Solvent	λmax (observed)	λmax (reported)
Methanol	432 nm	422 nm

Calibration curve of *Berberis aristata extract*

The concentration range studied and the absorbance obtained are mentioned in Table 5.9. and the standard plot of *Berberis aristata extract* in methanol is mentioned in Fig. 5.6.

Table. No. 5.11: Calibration range of *Berberis aristata extract*

Concentration (µg/ml)	**Absorbance ± SD**
0	0.000
5	0.059
10	0.110
15	0.172
20	0.223
25	0.294

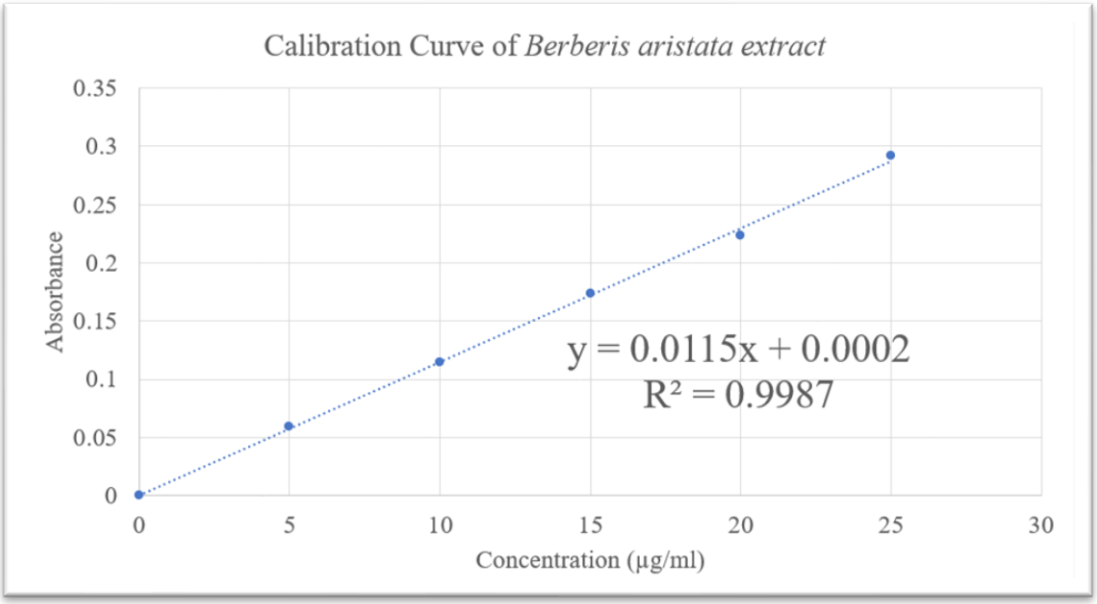


Fig. No. 5.7: Calibration curve of *Berberis aristata extract*

5.10.2. UV Spectroscopy of *Punica granatum*

The wavelength (λmax) of *Punica granatum* 20 µg/ml of c was measured using UV spectrophotometer and UV scan of which is showed in Fig. 5.7. Results are mentioned in the Table 5.11.

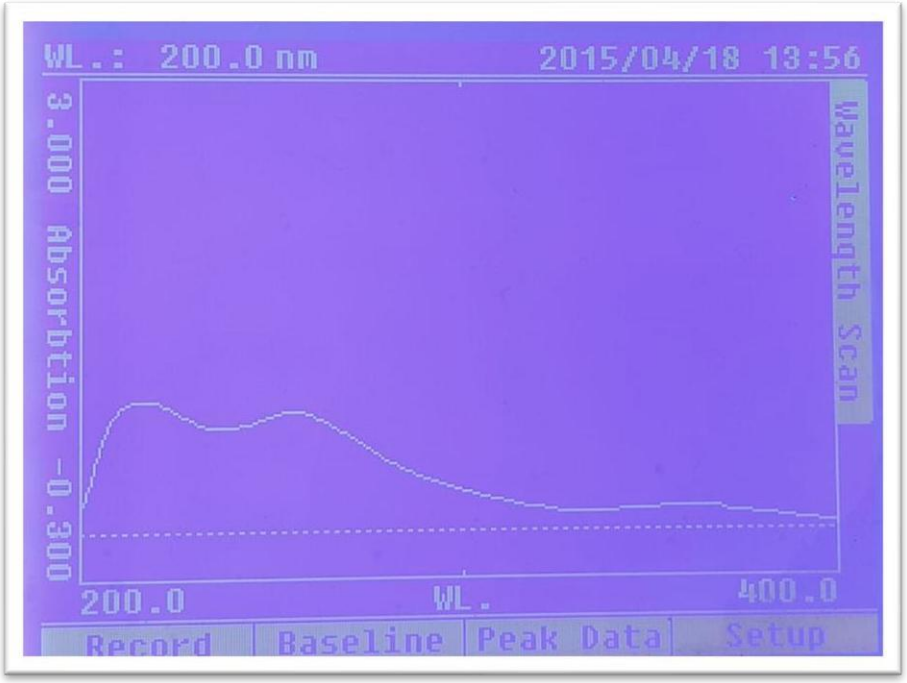


Fig. No. 5.8: UV scan of *Punica granatum*

Table. No. 5.12: λmax of *Punica granatum*

Solvent	λmax (observed)	λmax (reported)
Methanol	256 nm	260 nm

Calibration curve of *Punica granatum*

The concentration range studied and the absorbance obtained are mentioned in Table 5.12. and the standard plot of *Punica granatum extract* in methanol is mentioned in Fig. 5.8.

Table. No. 5.13: Calibration range of *Punica granatum*

Concentration (µg/ml)	**Absorbance ± SD**
0	0.000
25	0.790
26	0.820
27	0.878
28	0.902
29	0.870

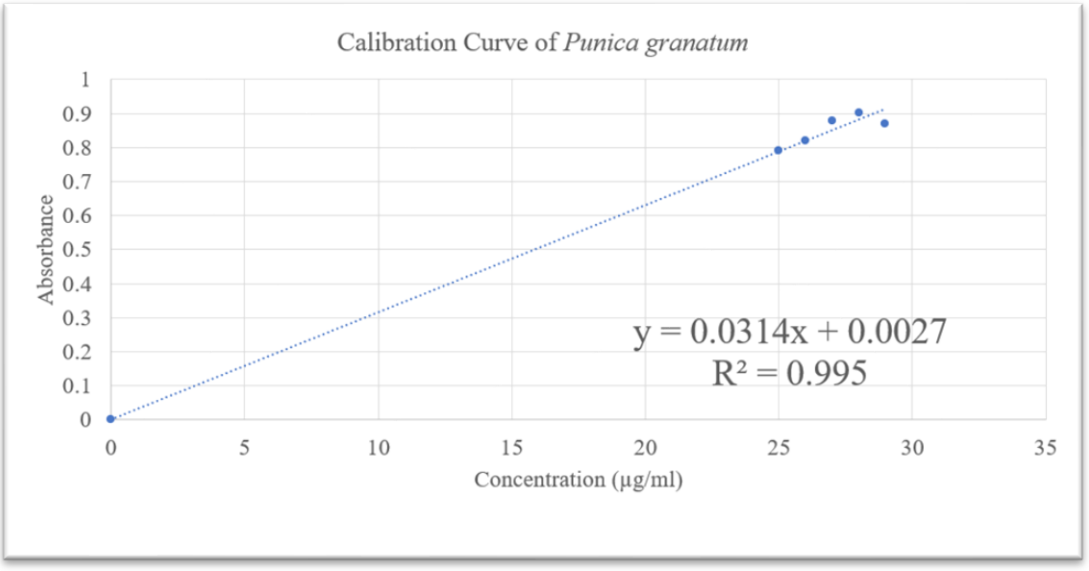


Fig. No. 5.9: Calibration curve of *Punica granatum extract*

5.10.3. UV Spectroscopy of *Piper nigrum extract*

The wavelength (λmax) of *Piper nigrum extract* 5 µg/ml of c was measured using UV spectrophotometer and UV scan of which is showed in Fig. 5.9. Results are mentioned in the Table 5.13.

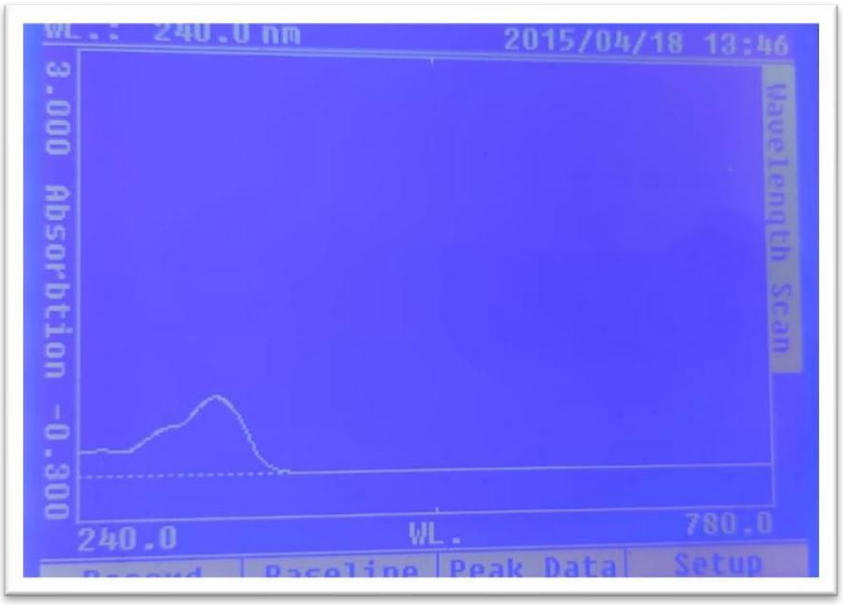


Fig. No. 5.10: UV scan of *Piper nigrum extract*

Table. No. 5.14: λmax of *Piper nigrum extract*

Solvent	λmax (observed)	λmax (reported)
Methanol	342 nm	342 nm

Calibration curve of *Piper nigrum extract*

The concentration range studied and the absorbance obtained are mentioned in Table 5.14. and the standard plot of *Berberis aristata extract* in methanol is mentioned in Fig. 5.10.

Table. No. 5.15: Calibration range of *Piper nigrum extract*

Concentration (µg/ml)	**Absorbance ± SD**
0	0.000
1	0.167
2	0.284
3	0.410
4	0.541
5	0.667

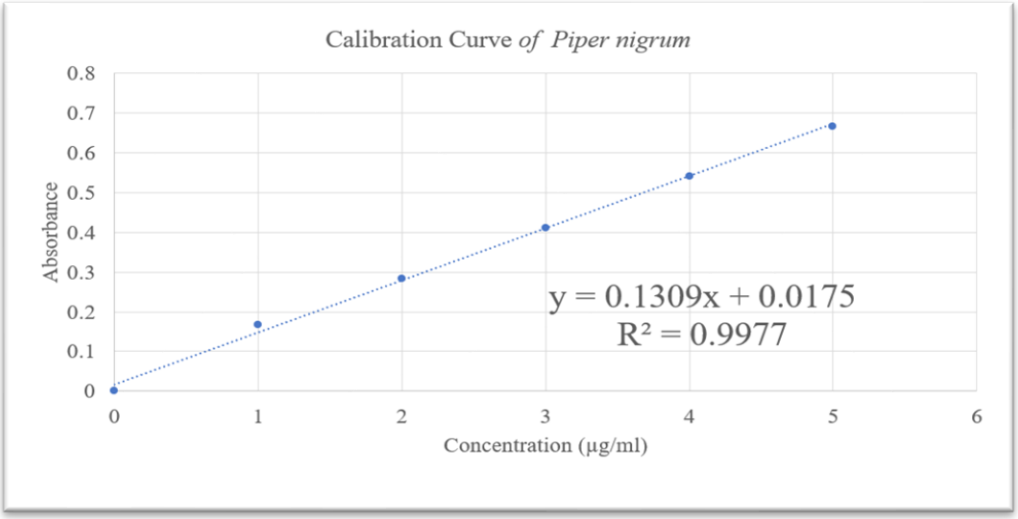


Fig. No. 5.11: Calibration curve of *Piper nigrum extract*

The λ_{max} of *Berberine aristata, Punica granatum, Piper nigrum* were found to be 432 nm, 256 nm and 342 nm which were found to be near of the reported data and can be considered in the range of ± 10 nm. The R^2 value for each drugs calibration curve 0btaineed was found to greater than 0.99 which indicates the data is significant.

5.11. Drug Content

The UV scan was done for the tablet and observed data are mentioned in table 5.15. and figure 5.11.

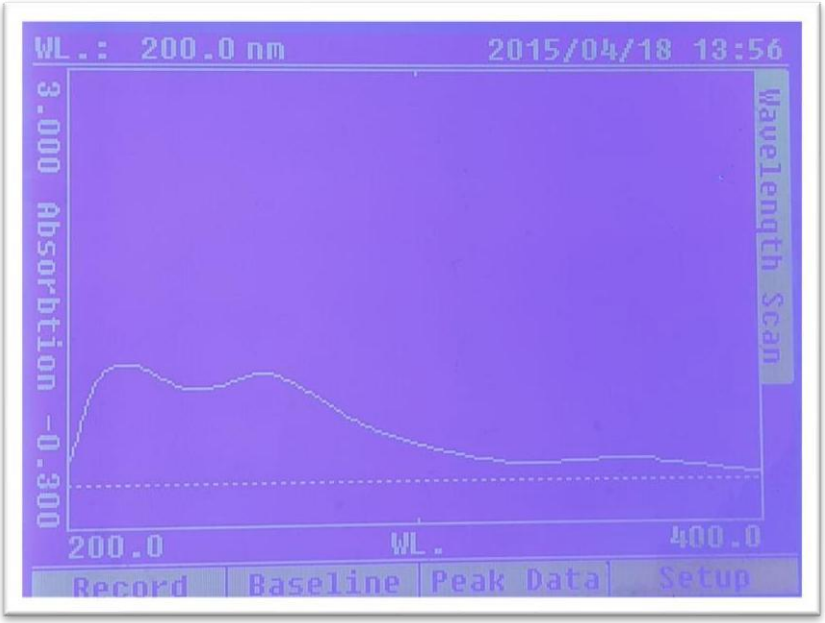


Fig. No. 5.12: UV scan of Tablet

Table. No. 5.16: UV Observations of Tablet		
Drug	Absorbance at 256nm	Absorbance at 432nm
Berberis aristata	-	0.115
Punica granatum	0.616	-

With the help of calibration curve and the UV observations obtained from the UV scan of tablet it was found out that the concentration of *Berberis aristata* was 294 mg and concentration of the *Punica granatum* was 148 mg. which reveals the concentrations as 98% and 98.6% respectively for the drugs.

5.12. Product and Label

Fig. No. 5.13: Final Product

Fig. No. 5.14: Label

6. Conclusion

Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi and parasites no longer respond to antimicrobial medicines. As a result of drug resistance, antibiotics and other antimicrobial medicines become ineffective and infections become difficult or impossible to treat, increasing the risk of disease spread, severe illness, disability and death. With potential projections of reaching 10 million deaths per year by 2050 due to AMR we can thus address this as silent pandemic.

The present study successfully formulated and evaluated herbal tablets containing *Berberis aristata*, *Punica granatum*, and *Piper nigrum*, targeting antimicrobial resistance (AMR).

As outlined in Chapter 1, AMR is a rising global health challenge, significantly diminishing the effectiveness of conventional antibiotics. This underscores the importance of alternative therapies like herbal adjuvant formulations, which exhibit broad-spectrum activities and lower tendencies to induce resistance.

Herbal adjuvant formulations can lead Reviving Antibiotics Potency which helps in Reducing Therapy Duration which ultimately helps in Minimizing Resistance Evolution.

Herbal adjuvant formulations can have Broad Spectrum Activity against bacteria and viruses which helps in Reducing Antibiotics Dosage & Toxicity which leads Enhancing Patient Outcomes and thus decrease mortality rate due to AMR.

Comprehensive phytochemical screening confirmed the presence of active constituents such as alkaloids, glycosides, tannins, and flavonoids in all three plant extracts, aligning with their traditional medicinal use. TLC analysis yielded Rf values of 0.7 for *Berberis aristata* with mobile phase of Formic acid: Ethyl acetate: Water (1:8:1), 0.72 for *Punica granatum* with mobile phase of Ethyl acetate: Methanol: Water (7.7:1.3:1), and 0.57,0.45,0.20 for *Piper nigrum* with mobile phase of Toluene: Ethyl acetate (7:3), validating the presence of targeted phytochemicals.

We also developed a single mobile phase for all three extracts (*Berberis aristata, Punica granatum, and Piper nigrum)* in Toluene: Ethyl acetate: Formic acid: Methanol (3:3:2:2).

Preformulation studies revealed good flow and compressibility: angle of repose ranged between 28, Carr's index between 22%, and Hausner's ratio from 0.77.

Among all formulations, **F5** demonstrated superior tablet properties with a hardness of **6.67 kg/cm²**, friability of **0.01**, disintegration time of **more than 40 minutes in intestinal pH** (6.8 phosphate buffer), and resistance to disintegration in acidic medium (0.1 N HCl), confirming enteric protection.

Spectrophotometric analysis provided high precision in drug content determination, showing. *Berberis aristata* was 294 mg and concentration of the *Punica granatum* was 148 mg. which reveals the concentrations as 98% and 98.6% respectively for the drugs.

Thus, this herbal tablet formulation does comply with pharmacopeial standards but also presents a promising, sustainable alternative in combating AMR. It offers a multi-targeted, stable, and effective approach suitable for further clinical evaluation and potential commercialization.

7. References

1. World Health Organization. Antimicrobial resistance [Internet]. Geneva: WHO; 2020 [cited 2025 Apr 4]. Available from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
2. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. (2016): 84-pp
3. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and therapeutics. 2015 Apr;40(4):277.
4. Collaborators AR. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022 Feb 12;399(10325):629-55.
5. Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2019 [Internet]. Atlanta: CDC; 2019 [cited 2025 Apr 4]. Available from: https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf
6. OECD. Stemming the Superbug Tide: Just a Few Dollars More [Internet]. Paris: OECD Publishing; 2018 [cited 2025 Apr 4]. Available from: https://www.oecd.org/health/stemming-the-superbug-tide-9789264307599-en.htm
7. Holmes AH, Moore LS, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ, Piddock LJ. Understanding the mechanisms and drivers of antimicrobial resistance. The Lancet. 2016 Jan 9;387(10014):176-87.
8. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H, Greko C. Antibiotic resistance—the need for global solutions. The Lancet infectious diseases. 2013 Dec 1;13(12):1057-98.
9. Verma P, Tiwari M, Tiwari V. Strategies to combat bacterial antimicrobial resistance: a focus on mechanism of the efflux pumps inhibitors. SN Comprehensive Clinical Medicine. 2021 Feb;3:510-27
10. Gillings MR. Lateral gene transfer, bacterial genome evolution, and the Anthropocene. Annals of the new York Academy of Sciences. 2017 Feb;1389(1):20-36.

11. Li XZ. Active efflux as a mechanism of resistance to antimicrobial drugs. Antimicrobial Drug Resistance: Mechanisms of Drug Resistance, Volume 1. 2017:131-48.
12. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. International journal of antimicrobial agents. 2010 Apr 1;35(4):322-32.
13. Kumar V, Yasmeen N, Pandey A, Ahmad Chaudhary A, Alawam AS, Ahmad Rudayni H, Islam A, Lakhawat SS, Sharma PK, Shahid M. Antibiotic adjuvants: synergistic tool to combat multi-drug resistant pathogens. Frontiers in Cellular and Infection Microbiology. 2023 Dec 20;13:1293633.
14. Liu Y, Li R, Xiao X, Wang Z. Antibiotic adjuvants: an alternative approach to overcome multi-drug resistant Gram-negative bacteria. Critical reviews in microbiology. 2019 May 4;45(3):301-14.
15. Kumar V, Yasmeen N, Pandey A, Ahmad Chaudhary A, Alawam AS, Ahmad Rudayni H, Islam A, Lakhawat SS, Sharma PK, Shahid M. Antibiotic adjuvants: synergistic tool to combat multi-drug resistant pathogens. Frontiers in Cellular and Infection Microbiology. 2023 Dec 20;13:1293633.
16. Komal S, Ranjan B, Neelam C, Birendra S, Kumar SN. Berberis aristata: A review. Int J Res Ayurveda Pharm. 2011;2(2):383-8.
17. Potdar D, Hirwani RR, Dhulap S. Phyto-chemical and pharmacological applications of Berberis aristata. Fitoterapia. 2012 Jul 1;83(5):817-30.
18. Choudhary S, Kaurav H, Madhusudan S, Chaudhary G. Daruharidra (Berberis aristata): review based upon its ayurvedic properties. International Journal for Research in Applied Sciences and Biotechnology. 2021;8(2):98-106.
19. Shaygannia E, Bahmani M, Zamanzad B, Rafieian-Kopaei M. A review study on Punica granatum L. Journal of evidence-based complementary & alternative medicine. 2016 Jul;21(3):221-7.
20. Jurenka J. Therapeutic applications of pomegranate (Punica granatum L.): a review. Alternative medicine review. 2008 Jun 1;13(2).

21. Al-Zoreky NS. Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. International journal of food microbiology. 2009 Sep 15;134(3):244-8.
22. Damanhouri ZA, Ahmad A. A review on therapeutic potential of Piper nigrum L. Black Pepper): The King of Spices. Med. Aromat. Plants. 2014;3(3):161.
23. Takooree H, Aumeeruddy MZ, Rengasamy KR, Venugopala KN, Jeewon R, Zengin G, Mahomoodally MF. A systematic review on black pepper (Piper nigrum L.): from folk uses to pharmacological applications. Critical reviews in food science and nutrition. 2019 Jun 27;59(sup1):S210-43.
24. Kumar-Sarangi M, Chandra-Joshi B, Ritchie B. Natural bioenhancers in drug delivery: An overview. Puerto Rico health sciences journal. 2018 Mar 14;37(1):12-8.
25. Neag MA, Mocan A, Echeverría J, Pop RM, Bocsan CI, Crişan G, Buzoianu AD. Berberine: Botanical occurrence, traditional uses, extraction methods, and relevance in cardiovascular, metabolic, hepatic, and renal disorders. Frontiers in pharmacology. 2018 Aug 21;9:557.
26. Ali Redha A, Siddiqui SA, Ibrahim SA. Advanced extraction techniques for Berberis species phytochemicals: A review. International Journal of Food Science & Technology. 2021 Nov;56(11):5485-96.-
27. Masci A, Coccia A, Lendaro E, Mosca L, Paolicelli P, Cesa S. Evaluation of different extraction methods from pomegranate whole fruit or peels and the antioxidant and antiproliferative activity of the polyphenolic fraction. Food chemistry. 2016 Jul 1;202:59-69.
28. Masci A, Coccia A, Lendaro E, Mosca L, Paolicelli P, Cesa S. Evaluation of different extraction methods from pomegranate whole fruit or peels and the antioxidant and antiproliferative activity of the polyphenolic fraction. Food chemistry. 2016 Jul 1;202:59-69.
29. Kolhe SR, Borole P, Patel U. Extraction and evaluation of piperine from Piper nigrum Linn. International Journal of Applied Biology and Pharmaceutical Technology. 2011;2(2):144-9.

30. Shityakov S, Bigdelian E, Hussein AA, Hussain MB, Tripathi YC, Khan MU, Shariati MA. Phytochemical and pharmacological attributes of piperine: A bioactive ingredient of black pepper. European journal of medicinal chemistry. 2019 Aug 15;176:149-61.

31. Imenshahidi M, Hosseinzadeh H. Berberis vulgaris and berberine: an update review. Phytotherapy research. 2016 Nov;30(11):1745-64.
32. Viuda-Martos M, Fernández-López J, Pérez-Álvarez JA. Pomegranate and its many functional components as related to human health: a review. Comprehensive reviews in food science and food safety. 2010 Nov;9(6):635-54.
33. Gaur PK, Mishra S, Purohit S, Dave K. Isolation and evaluation of piperine from black pepper and white pepper. Int J Res Ayurveda Pharm. 2013;4(2):210–3.
34. Fischer U, Benskin CMH, Nordby HE, Zeller WJ. Influence of pH on the color stability of pomegranate anthocyanins. Eur Food Res Technol. 2015 May;240(5):1059–68.
35. Srinivasan K. Black pepper (Piper nigrum) and its bioactive compound, piperine. InMolecular targets and therapeutic uses of spices: Modern uses for ancient medicine 2009 (pp. 25-64).
36. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. International journal of chemical studies. 2020 Mar 1;8(2):603-8.
37. For TLC: Bele AA, Khale A. An overview on thin layer chromatography. International Journal of Pharmaceutical Sciences and Research. 2011 Feb 1;2(2):256.
38. Migas P, Heyka M, Pobłocka-Olech L, Krauze-Baranowska M. BMD-TLC—the useful technique for quantitative analysis of chelidonine, chelerithrine and berberine in herbal drugs. JPC–Journal of Planar Chromatography–Modern TLC. 2012 Oct;25:439-44.
39. Basera IA, Girme A, Bhatt VP, Shah MB. A validated high-performance thin-layer chromatography method for the simultaneous estimation of berberine, berbamine, palmatine, magnoflorine and

jatrorrhizine from Berberis aristata. JPC–Journal of Planar Chromatography–Modern TLC. 2021 Apr;34(2):147-55.

40. Gadkari P, Daharwal SJ. Quantification of punicalagin in pomegranate peels from high-performance thin-layer chromatography. Biomedical and Biotechnology Research Journal (BBRJ). 2022 Oct 1;6(4):586-90.
41. Kolhe SR, Borole P, Patel U. Extraction and evaluation of piperine from Piper nigrum Linn. International Journal of Applied Biology and Pharmaceutical Technology. 2011;2(2):144-9.
42. Shah RB, Tawakkul MA, Khan MA. Comparative evaluation of flow for pharmaceutical powders and granules. Aaps Pharmscitech. 2008 Mar;9:250-8.
43. Shah DS, Moravkar KK, Jha DK, Lonkar V, Amin PD, Chalikwar SS. A concise summary of powder processing methodologies for flow enhancement. Heliyon. 2023 Jun 1;9(6).
44. Agrawal R, Naveen Y. Pharmaceutical processing–A review on wet granulation technology. International journal of pharmaceutical frontier research. 2011 Apr;1(1):65-83.
45. Hussan SD, Santanu R, Verma P, Bhandari V. A review on recent advances of enteric coating. IOSR J Pharm. 2012 Nov;2(6):05-11.
46. Koli M, Nogai L, Bhandari M, Mishra R, Pathak R, Sharma H. Formulation And Evaluation Of Berberine Hydrochloride Film Coated Tablet. Journal of Pharmaceutical Negative Results. 2023 Apr 1;14(2).
47. Kristensen HG, Schaefer T. Granulation: a review on pharmaceutical wet-granulation. Drug development and industrial pharmacy. 1987 Jan 1;13(4-5):803-72.
48. Patra CN, Priya R, Swain S, Jena GK, Panigrahi KC, Ghose D. Pharmaceutical significance of Eudragit: A review. Future Journal of Pharmaceutical Sciences. 2017 Jun 1;3(1):33-45.
49. Seitz JA, Flessland GM. Evaluation of the physical properties of compressed tablets I: Tablet hardness and friability. Journal of Pharmaceutical Sciences. 1965 Sep 1;54(9):1353-7.
50. Nair AB, Gupta R, Kumria R, Jacob S, Attimarad M. Formulation and evaluation of enteric coated tablets of proton pump inhibitor. Journal of basic and clinical pharmacy. 2010 Nov 15;1(4):215.

51. Rathore SB, Sharma A, Garg A, Sisodiya DS. Formulation and evaluation of enteric coated tablet of Ilaprazole. International current pharmaceutical journal. 2013 Jun 1;2(7):126-30.
52. Tavade S, Patil K, Kurangi B, Suryawanshi S. Development and validation of UV-spectrophotometric method for estimation of berberine hydrochloride in marketed formulation and poly lactic co-glycolic acid nanoparticles. Indian J. Pharm. Educ. Res. 2022 Jul 1;56(3):873-0.
53. Bala I, Bhardwaj V, Hariharan S, Kumar MR. Analytical methods for assay of ellagic acid and its solubility studies. Journal of pharmaceutical and biomedical analysis. 2006 Jan 23;40(1):206-10.
54. Murti YB, Hartini YS, Hinrichs WL, Frijlink HW, Setyaningsih D. UV-Vis spectroscopy to enable determination of the dissolution behavior of solid dispersions containing curcumin and piperine. Journal of Young Pharmacists. 2019;11(1):26.
55. Shingate PN, Dongre PP, Kannur DM. New method development for extraction and isolation of piperine from black pepper. International Journal of Pharmaceutical Sciences and Research. 2013 Aug 1;4(8):3165.