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FORMULATION, EVALUATION, MOLECULAR DOCKING AND ANTIBACTERIAL ACTIVITY OF *COLEUS AMBOINICUS* EXTRACT-BASED HERBAL SYRUP AGAINST DIARRHEA-CAUSING BACTERIAL PATHOGENS

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ABSTRACT

Diarrheal diseases remain a major global health concern, particularly in developing countries, due to bacterial pathogens such as *Escherichia coli*, *Salmonella*, *Shigella*, and *Vibrio cholerae*. The increasing incidence of antimicrobial resistance has created an urgent need for safer and more effective herbal alternatives. The present study aimed to formulate and evaluate a herbal antibacterial syrup containing the ethanolic extract of *Coleus amboinicus* leaves and to investigate its antibacterial potential through in vitro and in silico approaches. Fresh leaves of *Coleus amboinicus* were collected, authenticated, and subjected to extraction using a Clevenger apparatus with ethanol. Four syrup formulations (F1–F4) were prepared using different bases, namely distilled water, simple syrup, sorbitol, and honey, while maintaining a constant concentration of the plant extract. The formulations were evaluated for organoleptic properties, pH, viscosity, drug content, UV-visible spectroscopy, accelerated stability, and antibacterial activity using the agar well diffusion method against *Escherichia coli*. Molecular docking studies were performed using the major phytoconstituents, thymol and carvacrol, against selected diarrhea-causing pathogens to predict their binding affinity and possible antibacterial mechanism. All formulations exhibited acceptable physicochemical characteristics, including satisfactory appearance, pH, viscosity, stability, and drug content. The antibacterial study demonstrated that the herbal formulations produced measurable zones

of inhibition against *E. coli*, indicating promising antibacterial efficacy, with activity comparable to the standard drug in selected formulations. UV-visible spectral analysis confirmed the presence and stability of phytoconstituents within the formulations. Molecular docking studies revealed favorable binding interactions of thymol and carvacrol with bacterial target proteins, supporting their potential inhibitory activity and validating the observed antibacterial effects. Overall, the findings suggest that *Coleus amboinicus*-based herbal syrup represents a promising natural therapeutic candidate for the management of bacterial diarrhea. Further pharmacological, toxicological, and clinical investigations are warranted to establish its efficacy, safety, and therapeutic applicability.

KEYWORDS: *Coleus amboinicus*, Herbal syrup, Antibacterial activity, Diarrhea, Molecular docking, Thymol, Carvacrol.

1. INTRODUCTION

Anti-bacterial agents are substances that work against bacteria by either killing them (bactericidal) or stopping their growth (bacteriostatic). In the context of diarrhea, antibacterial agents are important because many diarrheal diseases are caused by harmful bacteria such as Escherichia coli, Salmonella, Shigella, and Vibrio cholerae. These bacteria infect the intestinal tract, produce toxins, or disturb the normal gut flora, leading to watery stools, abdominal cramps, and dehydration.

Bacteria are microscopic, single-celled organisms that lack a true nucleus. They are among the oldest life forms on Earth. Bacteria are found everywhere, including air, water, soil, and inside plants and animals. They reproduce quickly by a process called binary fission. Bacteria have different shapes such as cocci, bacilli, and spirilla. Some bacteria are useful in food production, digestion, and making medicines. They also help in nutrient recycling and maintaining environmental balance. However, some bacteria are harmful and cause diseases like diarrhea, cholera, tuberculosis, and typhoid. Pathogenic bacteria enter the body through contaminated food or water. Proper hygiene and sanitation help prevent bacterial infections. Antibiotics are used to treat bacterial diseases. Thus, bacteria play both beneficial and harmful roles in India.

Types of Bacteria

Based On Shape

- Cocci (round or spherical)

- Bacilli (rod shape)
- Spirilla(spiral shape)
- Vibrios (coma shape).

Based On Gram Staining:

- Gram positive bacteria
- Gram negative bacteria.

1.1 Liquid Dosage Form – Syrups

Definition

Syrup is a concentrated aqueous liquid dosage form containing one or more medicinal substances dissolved in a sweetened vehicle, usually sucrose, or a substitute such as sorbitol. Syrups are intended for oral administration and are used to mask unpleasant taste, improve patient compliance, and provide rapid drug action. Syrups may be medicated (containing active pharmaceutical ingredients) or non-medicated (used mainly as vehicles or flavouring agents).

Ideal Characteristics of Syrups

- Clear, transparent, and homogeneous preparation.
- Pleasant taste and acceptable sweetness.
- Free from particulate matter and crystallization.
- Physically, chemically, and microbiologically stable.
- Suitable viscosity for easy pouring and accurate dosing.
- Non-irritant and safe for oral use.

2. PLANT PROFILE

Synonyms: *Plectranthus amboinicus, plectranthus aromaticus, coleus aromaticus, ocimum amboinicum.*

Other Names: Indian borage, cuban oregano, Mexican mint, spanish thyme, country borage, Broadleaf thyme, soup mint, thick leaf thyme, False oregano.

Vernacular Names

Table: 1 Vernacular Names.

Languages	Vernacular names
English	Spanish thyme, Indian borage, cuban oregano
Tamil	Karpuravalli

Hindi	Patta ajwain
Telugu	Vamu aakulu
Spanish	Oregano frances

Taxonomy

- Kingdom: Plantae
- Clade: Tracheophytes
- Clade: Angiosperms
- Clade: Eudicots
- Clade: Asterids
- Order: Lamiales
- Family: Lamiaceae
- Genus: Coleus
- Species: *Coleus amboinicus*

Morphology: *Coleus amboinicus* is a succulent, aromatic herb or small shrub with thick, soft stems that are square in cross-section and covered with fine hairs. The leaves are fleshy, broadly ovate, velvety to the touch, and arranged oppositely on the stem. They have coarsely crenate (scalloped) margins and emit a strong, oregano-like aroma when crushed. The plant occasionally produces small, tubular, bluish-purple flowers arranged in terminal spikes. Its overall growth is bushy and spreading, making it a common ornamental and medicinal plant in tropical regions.

Botanical Description: *Coleus amboi*nicus (syn. *Plectranthus amboinicus*), commonly known as Indian borage or Mexican mint, is a succulent, aromatic, perennial herb or subshrub belonging to the family Lamiaceae. The plant has a thick, fleshy, and quadrangular stem, which is usually green or purplish and covered with fine hairs.

Composition Of Phytochemicals: *Coleus amboinicus* contains several important phytochemicals, mainly found in its aromatic leaves. The essential oils include carvacrol, thymol, and eugenol, which have strong antimicrobial and anti-inflammatory properties. It also contains flavonoids like quercetin and apigenin, known for antioxidant activity. Phenolic compounds such as Rosmarinus acid and caffeic acid further enhance its medicinal value. Additionally, the plant has terpenoids, tannins, saponins, and alkaloids that support its traditional therapeutic uses.

Fig: 1 Coleus amboinicus.

3. MATERIALS AND METHODS

3.1 Collection of Plant Material: The plant leaves are collected from the student's home by selecting healthy, disease-free leaves early in the morning to ensure freshness and quality.

3.2 Authentication: Plant was authenticated by Dr.KN Sunil Kumar, Research Officer and HOD, Department of Pharmacognosy and Dr.A.Kanagarajan Asst. Director (Siddha), Sci-IV and HOD clinical for Assistant Director In/C, (FORM NO. PCOG002-ACF DATED ON 23.06.2025) From Siddha Central Research Institute, Arumbakkam, Chennai-600106.

3.3 Preparation of Ethanolic Extraction: The process of *Coleus amboinicus* extraction carried out by distillation method by using Clevenger apparatus**.**

- **Preparation:** leaves of *Coleus amboinicus* are freshly collected and washed. Then washed leaves are finely chopped.
- **Distillation:** The Clevenger apparatus used for extraction process. The essential oil from the fresh of *Coleusamboinicus* was extracted using a Clevenger apparatus by distillation, where sufficient amount of finely chopped leaves were placed in a round bottom flask with sufficient quantity of ethanol as solvent and heated for 3 to 4 hours, the condensed ethanolic extract was collected.

3.4 Preparation of Syrup

The prepared extract was mixed with the syrup base and certain excipients were added. These were mix together using a laboratory stirrer.

General procedure for formulation F1 – F4

- **Preparation of Extract Solution:** The required quantity of *Coleus amboinicus* extract was dissolved in a measured volume of ethanol with gentle stirring to obtain a uniform extract solution.
- **Preparation of Preservative Solution:** Sodium benzoate was dissolved separately in a small quantity of warm distilled water and kept aside.
- **Preparation of Base**

F1 (Honey base): Honey was diluted with distilled water to obtain a uniform syrup base.

F2 (Simple syrup base): Simple syrup was prepared by dissolving sucrose in distilled water and filtered if required.

F3 (Distilled water base): Distilled water was used as the base.

F4 (Sorbitol base): Sorbitol solution was diluted with distilled water to achieve the desired consistency.

- **Mixing of Ingredients:** To the prepared base, glycerine was added with continuous stirring. The preservative solution (sodium benzoate) was then incorporated, followed by the ethanolic extract solution of *Coleus amboinicus.*
- **Addition of Colouring Agent:** A permitted colouring agent was added in minimal quantity to obtain a uniform and acceptable appearance.
- **Make Up the Final Volume:** The final volume was adjusted using certain base for each formulation and the formulation was mixed thoroughly to ensure homogeneity.

Table: 2 Formulation Table.

S. No.	INGREDIENTS	F1	F2	F3	F4
1.	Extract	5ml	5ml	5ml	5ml
2.	Colouring agent	Q.S.	Q.S.	Q.S.	Q.S.
3.	Sodium benzoate	1gm	1gm	1gm	1gm
4.	Glycerine	7ml	7ml	7ml	7ml
5.	Base	Honey	Simple syrup	Distilled water	Sorbitol
6.	Ethanol	1ml	1ml	1ml	1ml

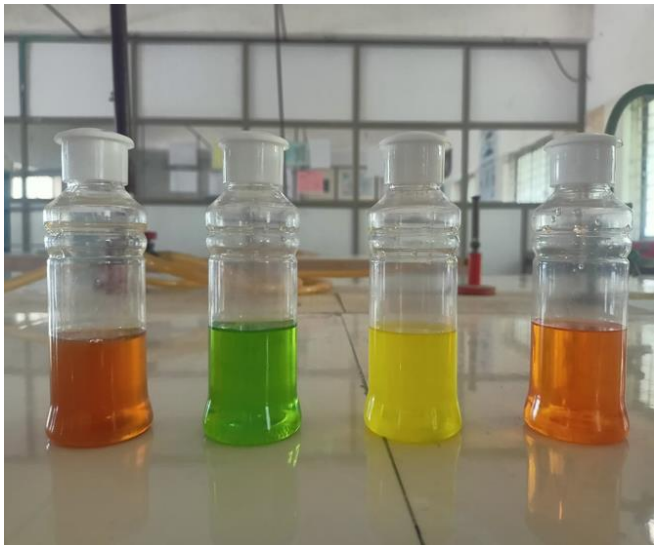


Fig: 2 Syrup.

3.5 Preliminary Phytochemical Screening Of Ethanolic Extract Of "*Coleus Amboinicus*" Leaves:

Test For Essential Oil

- **Ferric Chloride Test:** To a small amount of the hexane fraction (evaporate and redissolve in small ethanol), add a drop of 5% $FeCl_3$.positive green, blue ,purple suggests phenolic constituents present thymol and carvacrol.
- **Sodium Bicarbonate Test:** To add small amount of ethanolic extract with 5% NaHCO3(sodium bicarbonate) and shake well. Result will be no effervescence obtained.
- **Bromine Water Test:** Take a extract in test tube and add bromine water dropwise in the test tube and observe the tube decolorization of bromine water with white precipitate till to presence of activated phenolic ring, typical of thymol and carvacrol.
- **Libermann's Test:** Dissolve the extract in chloroform and add concentrated H2SO4 along the side of the test tube and observe reddish, violet or green coloration. Indicate phenolic monoterpenes like thymol and carvacrol.

3.6 Evaluation of Herbal Syrup

- **Organoleptic Evaluation:** Organoleptic evaluation refers to the assessment of the syrup using sensory organs such as sight, smell, and taste. It helps to determine patient acceptability and product quality.
- **PH Determination:** The pH of the syrup was measured using a calibrated digital pH meter and found to be within the acceptable range (4.5–6.5), indicating good stability and suitability for oral administration**.**

- **Viscosity:** The viscosity of the syrup was determined using a Brookfield viscometer at 25°C and was found to be within acceptable limits, ensuring good pourability and patient compliance.
- **Drug Content:** Drug content of the syrup was determined by UV–Visible spectrophotometry and was found to be within the acceptable range, indicating uniform distribution of the active ingredient.
- **UV Analysis:** The UV–visible spectroscopic analysis demonstrated distinct absorption characteristics for the *Coleus amboinicus* plant extract and its formulated syrups when compared with the reference drug ciprofloxacin. Ciprofloxacin showed maximum absorption in the range of 274–278 nm, whereas the plant extract (Formulation 0) exhibited a predominant peak at 237 nm with minor peaks at higher wavelengths, confirming that the extract possesses a different spectral profile and does not overlap with the reference drug. This indicates the presence of unique phytoconstituents in the extract. The formulated preparations (F1–F4) showed noticeable shifts of absorption into the visible region, depending on the base used (honey, simple syrup, distilled water, or sorbitol). These shifts suggest interactions between the plant extract and the respective formulation bases, leading to the presence and stabilization of bioactive secondary metabolites such as essential oil and flavonoids. Among the formulations, the honey- and distilled water–based preparations exhibited relatively prominent absorbance in the visible range, indicating better retention or expression of phytochemical components. Overall, the UV–visible analysis confirms that *Coleus amboinicus* extract and its formulations contain characteristic phytochemicals with absorbance in both UV and visible regions, supporting their potential pharmaceutical relevance. The variation in absorption patterns across formulations highlights the influence of different bases on the spectral behavior and possible bioactivity of the plant extract.
- **Accelerated stability studies:** The formulated herbal syrup remained stable under accelerated storage conditions, indicating good physical and chemical stability.

3.7 Molecular Docking Study

Molecular docking was performed as an insilico approach to evaluate the binding interactions of carvacrol and thymol with selected target proteins of diarrhea-causing bacteria. The docking analysis aimed to predict the binding affinity, interaction patterns, and possible inhibitory potential of these phytoconstituents at the molecular level. The results provide mechanistic insight into the antibacterial activity of *Coleus amboinicus* and support its traditional use in the management of Anti bacterial.

3.8 Anti-Bacterial Activity

Agar Diffusion Method Measured By Zone Of Inhibition: formulation was evaluated by the agar well diffusion method against *Escherichia coli.*The antibacterial activity of *Coleus amboinicus* extract based diarrheal syrup coli. The zone of inhibition(mm) produced by different formulation (F1,F2,F3,F4), *Coleus amboinicus* extract, standard drug (ciprofloxacin) was measured and compared**.**

4. RESULTS AND DISCUSSION

4.1 Organoleptic Evaluation

Table: 3 Organoleptic Evaluation.

Formulation	Colour	Odour	Taste	Appearance
Distilled water F1	Colourless	Aromatic	Pleasant	Clear
Simple syrup F2	Green	Aromatic	Sweet	Clear
Sorbital F3	Orange	Aromatic	Pleasant	Clear
Honey F4	Brown	Aromatic	Sweet	Clear

4.2 Physio-Chemical Evaluation

Table: 4 Physio-Chemical Evaluation.

Formulation	PH	Viscosity	Percentage
Distilled water F1	7	1.0 centipoise at 20°C	85%
Simple syrup F2	5.5	100 centipoise at 20°C	92%
Sorbital F3	4	110 centipoise at 25°C	95%
Honey F4	6.5	10000 centipoise	90%

4.3 Accelerated Stability Studies

Table: 5 Accelerated Stability Studies.

Formulation	Colour	Odour	Taste	PH	Viscosity	Drug content
Distilled water F1	Nil	Nil	Nil	Nil	Nil	Nil
Simple syrup F2	Nil	Nil	Nil	Nil	Nil	Nil
Sorbital F3	Nil	Nil	Nil	Nil	Nil	Nil
Honey F4	Nil	Nil	Nil	Nil	Nil	Nil

4.4 Antibacterial Assay

Table: 6 Antibacterial Assay.

S.No.	Microorganisms	Control	CE	1	2	3	4	Ciprofloxacin
		Zone of inhibition in mm						
1.	*Escherichia coli*	-	11	28	13	09	-	35

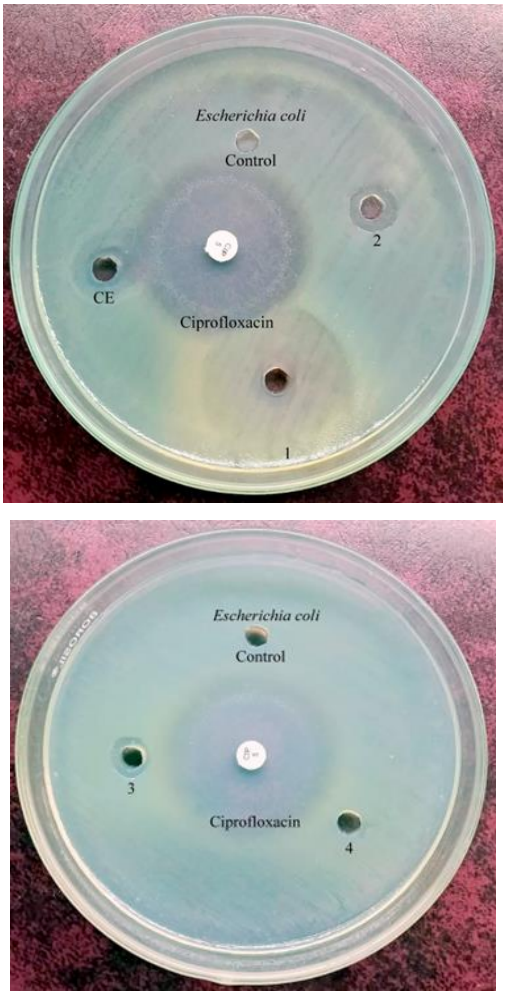


Fig: 3 Antibacterial Assay.

4.5 UV SPECTROSCOPY ANALYSIS

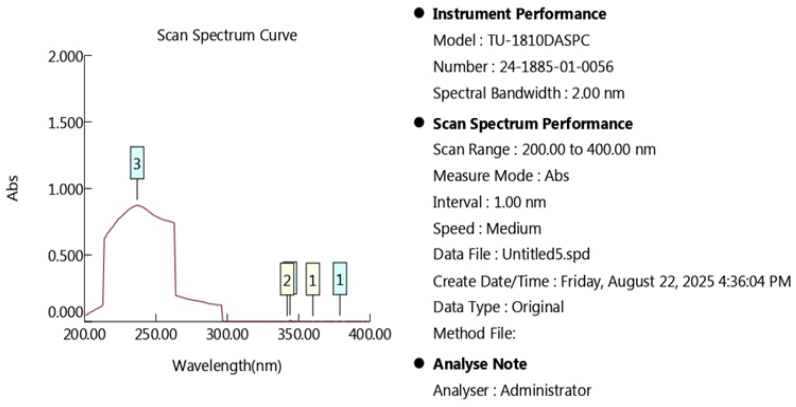


Fig: 4 Plant Extract.

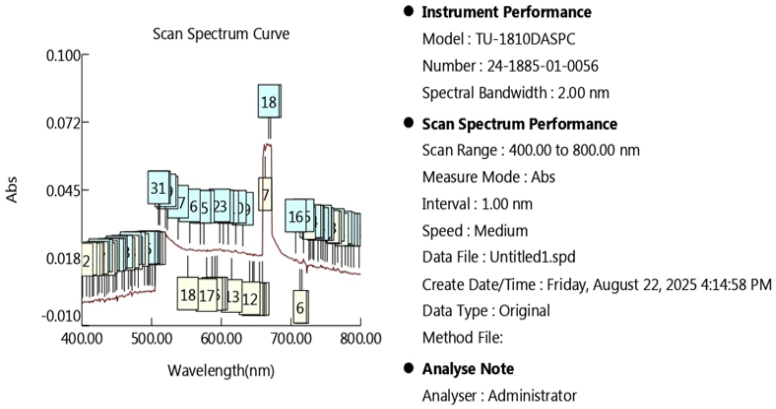


Fig: 5 Formulation F1.

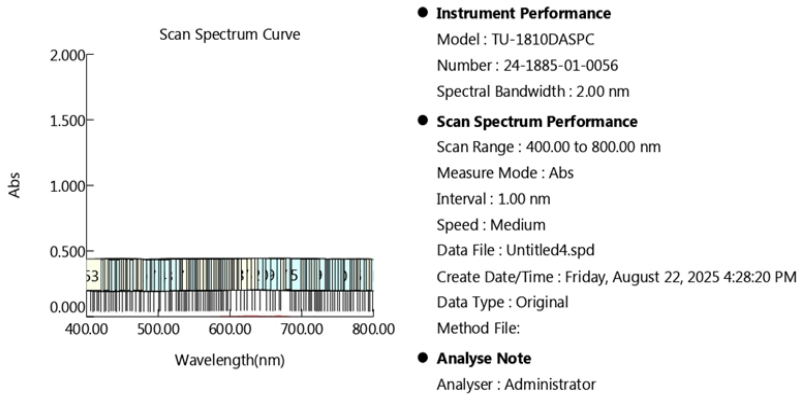


Fig: 6 Formulation F2.

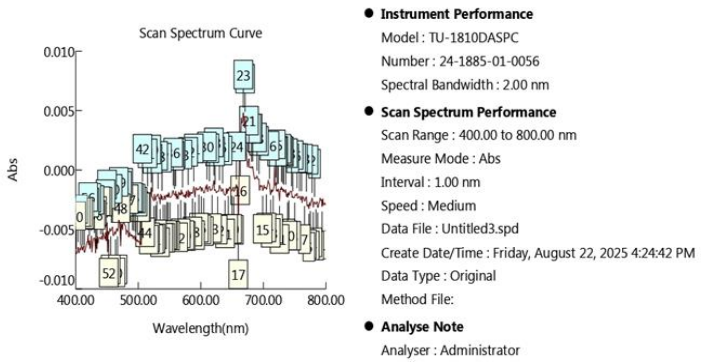


Fig: 7 Formulation F3.

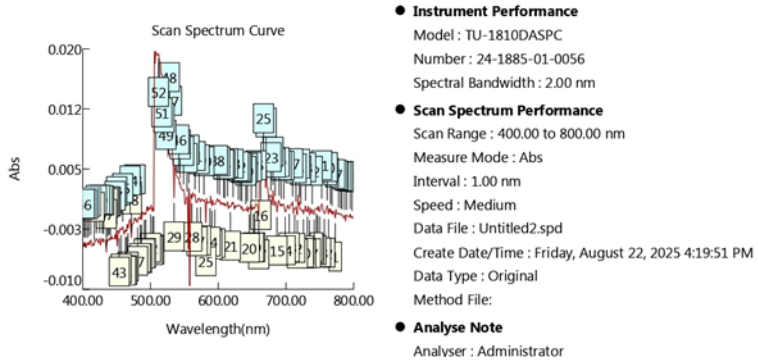


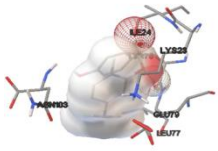
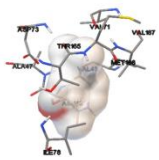
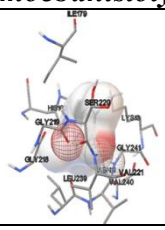
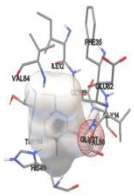
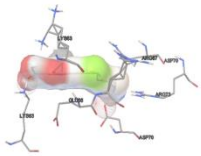
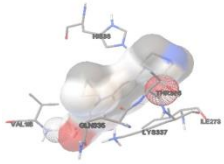
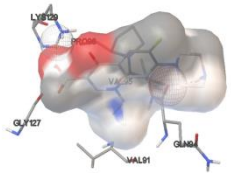
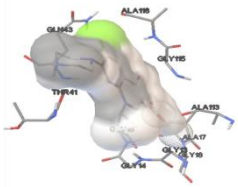
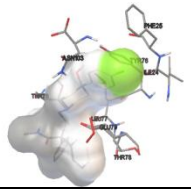
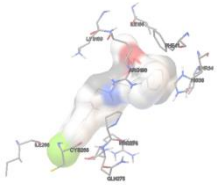
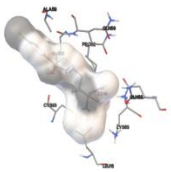
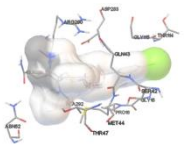
Fig: 8 Formulation F4.

4.6 Molecular Docking Study

Table: 7 Molecular Docking Study.

Drug	*Vibrio cholerae*	*Escherichia coli*	*Entamoebahistolytica*	*Giardia lamblia*
Thymol	**-6.61**	**-5.25**	**-5.90**	**-6.22**
Carvacrol	**-7.26**	**-7.60**	**-6.53**	**-9.29**
Ciprofloxacin (Reference drug)	**-7.71**	**-8.20**	**-7.06**	**-9.51**

Table: 8 Molecular Docking Study. (Protein & Ligands)

Drug	*Vibrio cholerae*	*Escherichia coli*	*Entamoebahistolytica*	*Giardia lamblia*
Thymol				
Carvacrol				
Ciprofloxacin (Reference drug)				

DISCUSSION

The present study successfully formulated and evaluated an antibacterial herbal syrup containing the ethanolic extract of *Coleus amboinicus* leaves for the management of bacterial

diarrhea. Four formulations (F1–F4) were prepared using different syrup bases, namely distilled water, simple syrup, sorbitol, and honey, to investigate the influence of the vehicle on the physicochemical properties, stability, and antibacterial efficacy of the herbal preparation. The overall findings demonstrated that the formulated syrups possessed acceptable pharmaceutical characteristics and exhibited promising antibacterial activity against *Escherichia coli*.

The organoleptic evaluation revealed that all formulations exhibited acceptable colour, aromatic odour, pleasant taste, and clear appearance, indicating good patient acceptability. Herbal syrups intended for oral administration should possess desirable sensory characteristics to improve compliance, particularly among pediatric and geriatric patients. The presence of honey and simple syrup not only enhanced sweetness but also improved the overall appearance and palatability of the formulations. These findings suggest that the selected excipients were compatible with the plant extract and contributed positively to the formulation quality.

The physicochemical evaluation demonstrated that all formulations complied with acceptable quality parameters. The pH values ranged between 4.0 and 7.0, which is considered suitable for oral liquid preparations and minimizes the risk of microbial growth while maintaining chemical stability. Variations in viscosity were observed depending on the type of syrup base employed. The honey-based formulation exhibited the highest viscosity, whereas the distilled water formulation showed the lowest viscosity. Increased viscosity contributes to improved mouthfeel, better stability of suspended phytoconstituents, and prolonged contact time within the gastrointestinal tract, thereby potentially enhancing therapeutic efficacy. Drug content analysis confirmed uniform distribution of the extract throughout the formulations, indicating efficient mixing and formulation homogeneity.

Accelerated stability studies indicated that none of the formulations exhibited noticeable changes in colour, odour, taste, pH, viscosity, or drug content during storage under accelerated conditions. The absence of significant physicochemical alterations demonstrates the stability of both the herbal extract and formulation excipients. Stability is a critical quality attribute for herbal dosage forms because phytoconstituents are often susceptible to degradation due to oxidation, hydrolysis, and environmental factors. The present findings suggest that the selected preservatives and formulation bases effectively maintained product integrity throughout the storage period.

The antibacterial evaluation using the agar well diffusion method demonstrated appreciable antibacterial activity of the *Coleus amboinicus* formulations against *Escherichia coli*. The

herbal extract itself produced a measurable zone of inhibition, while one formulation demonstrated a notably larger inhibition zone compared with the others, although ciprofloxacin remained the most active reference drug. The observed antibacterial activity can be attributed to the presence of essential oil constituents such as thymol and carvacrol, together with flavonoids and phenolic compounds naturally present in *Coleus amboinicus*. These phytochemicals are known to disrupt bacterial cell membranes, increase membrane permeability, interfere with enzyme systems, and inhibit bacterial growth. The findings support the traditional medicinal use of *Coleus amboinicus* in gastrointestinal infections and suggest that it may serve as a promising herbal alternative for managing bacterial diarrhea.

UV-visible spectroscopic analysis confirmed the presence of bioactive phytoconstituents in both the crude extract and formulated syrups. Differences in absorption patterns among formulations indicated that the type of syrup base influenced the spectral behaviour of the phytochemicals. Honey-based and distilled water-based formulations showed relatively better expression of characteristic absorption peaks, suggesting improved retention or stabilization of active constituents. These findings indicate that formulation excipients may influence phytochemical stability and bioavailability without altering the fundamental chemical characteristics of the extract.

Molecular docking studies further supported the antibacterial potential of the major phytoconstituents present in *Coleus amboinicus*. Carvacrol demonstrated stronger binding affinities than thymol against several diarrhea-causing pathogens, including *Vibrio cholerae*, *Escherichia coli*, *Entamoeba histolytica*, and *Giardia lamblia*. Although the docking scores were slightly lower than those obtained with ciprofloxacin, the interactions indicate that these naturally occurring compounds possess considerable affinity toward microbial target proteins. The docking analysis provides mechanistic evidence supporting the observed in vitro antibacterial activity and suggests that thymol and carvacrol may interfere with essential microbial metabolic pathways. The integration of in vitro microbiological studies with in silico molecular docking strengthens the scientific validity of the herbal formulation and supports its potential development as a phytopharmaceutical product.

Overall, the present investigation demonstrates that *Coleus amboinicus* possesses significant pharmaceutical and antibacterial potential. The herbal syrup formulation exhibited desirable physicochemical properties, acceptable stability, and promising antibacterial efficacy against *Escherichia coli*. The synergistic contribution of essential oils, flavonoids, and phenolic compounds, together with favourable molecular docking interactions, supports the therapeutic potential of this medicinal plant. Nevertheless, further studies involving

quantitative phytochemical estimation, toxicity evaluation, pharmacokinetic investigation, animal models, and randomized clinical trials are required before the formulation can be recommended for routine clinical use. Such investigations will provide comprehensive evidence regarding its efficacy, safety, dosage optimization, and long-term therapeutic benefits.

5. CONCLUSION

The present study successfully formulated and evaluated an antibacterial herbal syrup containing the ethanolic extract of *Coleus amboinicus* leaves using different formulation bases. All formulations exhibited satisfactory organoleptic characteristics, acceptable physicochemical properties, uniform drug content, and excellent stability under accelerated storage conditions. The antibacterial investigation demonstrated that the formulated syrup possessed significant inhibitory activity against *Escherichia coli*, indicating its potential usefulness in the management of bacterial diarrhea. UV-visible spectroscopic analysis confirmed the presence and stability of the plant phytoconstituents within the formulations, while molecular docking studies demonstrated favorable binding interactions of the major bioactive compounds, particularly carvacrol and thymol, with selected microbial targets, providing mechanistic support for the observed antibacterial activity. Overall, the findings suggest that *Coleus amboinicus*-based herbal syrup is a promising natural oral formulation with potential therapeutic application against bacterial gastrointestinal infections. Further preclinical toxicity studies, pharmacological investigations, and well-designed clinical trials are necessary to establish its safety, efficacy, dosage regimen, and suitability for large-scale pharmaceutical development.

6. REFERENCES

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