STUDY OF BIOENHANCERS FOR EFFICACY IMPROVEMENT OF A MODEL ANTIBIOTIC DRUG

By

PRAMOD DHAKAL B.Pharm.,
Reg. No. 13PU309

A Dissertation Submitted to the

Rajiv Gandhi University of Health Sciences, **Karnataka, Bangalore**

In partial fulfillment of the requirements for the

MASTER OF PHARMACY
In
PHARMACEUTICS
Under the guidance of,

Mr. VEDAMURTHY JOSHI
M.Pharm.,

DEPARTMENT OF PHARMACEUTICS
SAC COLLEGE OF PHARMACY
B.G. NAGARA, KARNATAKA -571448
2015.

Rajiv Gandhi University of Health Sciences, Karnataka.

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"STUDY OF BIOENHANCERS FOR EFFICACY IMPROVEMENT OF A MODEL ANTIBIOTIC DRUG"** is a bonafide and genuine research work carried out by me under the guidance of **Mr. VEDAMURTHY JOSHI.,** Department of Pharmaceutics, SAC College of Pharmacy. B.G. Nagara.

Date:

Place: B.G.Nagara

Mr. PRAMOD DHAKAL
B. Pharm.,

Dept. of Pharmaceutics,
SAC College of Pharmacy,
B.G.Nagara- 571448,
Karnataka.

SAC COLLEGE OF PHARMACY

B.G.NAGARA-571448

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"STUDY OF BIOENHANCERS FOR EFFICACY IMPROVEMENT OF A MODEL ANTIBIOTIC DRUG"** was carried out by him in the laboratory of SAC College of Pharmacy, B.G.Nagara, under my direct supervision and guidance.

Date:

Place: B.G.Nagara.

Mr. VEDAMURTHY JOSHI
M.Pharm.,

Department of Pharmaceutics,
SAC College of Pharmacy,
B.G.Nagara- 571448.

SAC COLLEGE OF PHARMACY

B.G.NAGARA- 571448

ENDORSEMENT BY THE HEAD OF THE DEPARTMENT

This is to certify that the dissertation entitled **"STUDY OF BIOENHANCERS FOR EFFICACY IMPROVEMENT OF A MODEL ANTIBIOTIC DRUG"** is a bonafide research work carried out by **Mr. PRAMOD DHAKAL** under the guidance of **Mr. VEDAMURTHY JOSHI,** Department of Pharmaceutics, SAC College of Pharmacy, B.G.Nagara.

Date:

Place:B.G.Nagara

Dr. MOHAMMAD GULZAR AHEMED

M.Pharm., PHD

Professor and Head,
Department of Pharmaceutics,
SAC College of pharmacy,
B.G.Nagara- 571448,
Karnataka

SAC COLLEGE OF PHARMACY

B.G.NAGARA- 571448

ENDORSEMENT BY THE PRINCIPAL

This is to certify that the dissertation entitled **"STUDY OF BIOENHANCERS FOR EFFICACY IMPROVEMENT OF A MODEL ANTIBIOTIC DRUG"** is a bonafide research work carried out by **Mr. PRAMOD DHAKAL** under the guidance of Mr.**VEDAMURTHY JOSHI,** Department of Pharmaceutics, SAC College of Pharmacy, B.G.Nagara.

Date:

Dr. B. RAMESH
M Pharm., Ph.D

Place: BG Nagara

Principal,
Dept. of Pharmaceutical Chemistry
SAC College of pharmacy,
B.G.Nagara – 571448,
Karnataka

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the **Rajiv Gandhi University of Health Sciences, Karnataka** shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic / research purpose.

Date:

Place: B.G.Nagara

Mr. PRAMOD DHAKAL
B. Pharm.,
Depertment. of Pharmaceutics,
SAC College of Pharmacy,
B.G.Nagara- 571448,
Karnataka.


DEDICATED TO MY BELOVED
PARENTS

ACKNOWLEDGEMENT

I offer my adoration to my Family specially my father and mother who gave me the strength and courage to complete my dissertation and gave me the opportunity.

I express my generous thanks to my project guide Mr. VEDAMURTHY JOSHI Assistant Professor Department of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, BG Nagara, not only for his excellent guidance, unabated inspiration but also for his timely attention and kind interest whose suggestions and constant guidance has contributed much towards the completion of my dissertation work successfully.

It's my privilege to thank to Dr. B. Ramesh Principal, Sri Adichunchanagiri College of Pharmacy for his great support in bringing out this Formulary.

I extend my whole hearted gratitude to Dr.Mohammed Gulzar Ahmed, professor and head, Department of Pharmaceutics, for his valuable help and suggestion throughout my academic period here.

I express my deep and heart-felt thanks to Dr. Janakiram K Asso. Professor, Department of Microbiology, Dr. Annya Gowda, Asso. Professer, Department of Pharmacognosy. Mr G.B. Kiran kumar, Asso. Professor, Department of Pharmaceutics, Mr.Senthilkumar, Asso professor, Department of Pharmaceutics, Mr. Abdul Nasir Kurnool, Asst. professor, Department of Pharmaceutics, Dr. NK Satish, professor and Head, Department of Pharmaceutical Analysis, and other faculty members of Department of pharmaceutics, S.A.C. College of Pharmacy, B.G.Nagara for their invaluable support and encouragement in completing my project successfully.

"There is nothing better than the encouragement of a good friend". I am really grateful to my friends Ankit Acharya, Suyash Adhikari, Ravi Chaudhary, Amrit Timilsina, Pramod Kheral, Jasti Harika, Gummadi Ashwani, Pabithra, Chetana SR, Akshatha RS, Schin A B, Guru, Hansiya Sultana, Braj kishor Yadav, Manoj Lamichhane, Narendra who have been with me through these years during crucial juncture at B.G.Nagara.

I shall forever remember my friends, *Laxman Wagle* for their inspiration, moral support, encouragement that helped me to grow and expand my thinking.

I thank my seniors Puru, Diwakar, Rakesh, Madhu, Vinaya for their professional help during my research work.

I am great full to laboratory technician Prasad for providing me microorganism the project time.

I thank .....for their professional help during my research work.

Thanks to all my teachers, friends, relatives and acquaintances who don't find a mention here, but to whom I remain indented.

Date:

Place: B.G. Nagara

Pramod Dhakal

LIST OF ABBREVIATIONS

%	=	**Percentage**
°C	=	**Degree Centigrade**
abs	=	**Absorbance**
IP	=	**Indian Pharmacopoeia**
conc	=	**Concentration**
cm	=	**Centimeter**
cfu	=	**Colony factor unite**
FT-IR	=	**Fourier transform infrared spectroscopy**
CUD	=	**Cow Urine Distillate**
HBS	=	**Hydrodynamic balanced system**
Hrs	=	**Hours**
ICH	=	**International council for harmonization**
USP	=	**United State Pharmacopeia**
GGI	=	**gamma glutamyl trenspeptidase**
µg	=	**Microgram**
mg	=	**Milligram**
min	=	**Minute**
ml	=	**Milliliter**
mm	=	**Millimeter**
nm	=	**Nanometer**
µm	=	**micrometer**

DMSO	**= Dimethyl sulfoxide**
pH	**= Negative logarithm of hydrogen ion concentration**
RH	**= Relative humidity**
RPM	**= Revolution per minute**
ATCC	**= American Type Culture Collection**
UV	**= Ultraviolet**
w/w	**= Weight by weight**
w/v	**= Weight by volume**
CYP	**=**
DNA	**= Deoxyribonucleic acid**
P-GP	**= Permeability glycoprotein**

ABSTRACT

The objective of the study is to screen the selected herbal extracts for bio-enhancing property when given along with a model antibiotic Ciprofloxacin (CF) on selected strains of organisms. Ethanolic herbal extracts of powdered seeds of pepper (P), powdered roots of turmeric (T) and zinger (Z), dried powdered leaves of drumstick (D) and Cow urine distillate (CUD). The extracts were tested for antimicrobial activity alone and in combination with CF against *Staphylococcus aureus*, *Klebsiella pneumonia* A, *Pseudomonas aeruginosa* and *E-coli* by cup plate diffusion method. 10% DMSO solution and ciprofloxacin 10µg/ml acts as negative and positive control. Results depicted that test samples and negative control did not show any antimicrobial activity alone. However in combination with drug showed significant activity. Amongst the test samples combination of with CF, P, CUD and Z showed 5 to 50% increase activity when compared to the positive control. Bioenhancer activity was concentration dependent and the order follows; Pepper > CUD > Turmeric > Zinger. Further, tablets were prepared using the herbal extracts with CF by wet granulation method. Pre compression and post compression parameters were found be in limits. FTIR studies confirmed that no significant interaction between drug and excipient . Accelerated stability studies at 40 $\pm$ 2^0C / 75$\pm$5 % RH for 60 days showed no significant variation in physical and chemical properties. It can be concluded that use of bio-enhancers along with antibiotics potentiates antimicrobial activity thus drug related and dose related side effects can be minimized.

Key words: Bioenhancer, Cup Plate Method, Ciprofloxacin, Pepper, Cow Urine Distillate, Turmeric, Zinger.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Antibiotics are molecules that kill, or stop the growth of microorganisms, including both bacteria and fungi (1). Antibiotics revolutionized medicine in the 20th century, and have together with vaccination lead to the near eradication of diseases such as tuberculosis in the developed world. Their effectiveness and easy access led to overuse, especially in live-stock raising, prompting bacteria to develop resistance. This has led to widespread problems antibiotic resistance, so much as to prompt the World Health Organization to classify antimicrobial resistance as a "serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country"(2)(3).

Now day's modern pharmaceutical research is concerned with all aspects of identifying new chemical substances with new mode of action. Scientists is doing to modified conventional dosage form for the propose of increasing potency of drug, reducing the cost and amount of drug, increasing bioavailability decrease the side effect and improving the patient compliance. They are followed the novel drug delivery technique, modified dosage form.(4)

It is evident that microbes including bacterium, virus or parasite are responsible causing diseases like typhoid, tuberculosis, malaria, pneumonia, diarrhea, dysentery etc.(5) An antibiotics are the agents that inhibits the growth or kills bacteria in low concentration.(6). These antibiotics acts on the microbes in various mechanisms; inhibition of cell wall synthesis, protein synthesis, DNA gyrase, interference with DNA synthesis, DNA functioning, cause misreading of m-RNA code and affect permeability, cause leakage from cell membrane and interfere with intermediary metabolism.(7) Recently few studies revealed that the potency and efficacy of most of antibiotics in humans are decreasing and antibiotic resistant strains are evolving.(8)

Strain of many highly pathogenic species resistance to all widely available antibiotics have emerged and proliferated at rates that can never be envisaged. It commonly assumed that infection caused by multiple resistant strain occur throughout the developing world. In other sense, a generalized sense of alarm regarding the problem of multidrug resistant microorganism is arising simultaneously in a number of countries in the world. This suggests that we are on the threshold of uncertain future in which the conditions that existed before 1940, in the preantibiotic era may recur. In the word of Alexandar Thomasz "The specter of a pathogen resistant to all antimicrobial agent is closer to science fact than science fiction". Resistant pathogens are increasing in prevalence, for example: multidrug resistant *Acinobactor baumanni,* methicillin resistant *staphylococcous aureus*, vancomycin resistant beta-lactamase producing Enterococcus species has been observed in hospital acquired Pathogen. The For instance, *P.falciparum* developed resistance to Atovaquone by changing DNA sequence in mitochondria.(9) Some microbes developed resistance to Fluroquinolones by altering DNA gyrase enzyme. In case of B-lactum antibiotic, Penicillin, gram negative bacteria develops Penicillinase enzyme in the outer membrane. *Enterobactor* is largely resistant to Cephalosporin by the producing B-lactamase. Tetracycline was effective against gynecologic infection due to becteroides, but now these organisms are resistance due to the presence of plasmid mediated protein that promotes efflux of the drug.(10) This phenomenon is termed as **antibiotic resistance.**

The phenomenon of antibiotic resistance is the ability of the microorganism to survive and reproduce in the presence of antibiotic doses that were previously thought effective against them(11). The antimicrobial drug resistance is caused by selective pressure, mutation, gene transfer, societal pressures, inappropriate drug use, inadequate diagnostics, hospital use agricultural use and the inappropriate antibacterial treatment and the overuse of antibiotics.(12)

Many outcomes can be expected because of antibiotic resistance; primarily the treatment is inadequate or failure, secondly cost of the treatment increases as existing antibiotics became obsolete and new one has to be explored, finally the treatment is time consuming involving huge capital (8).

The development of bacterial resistance to presently available antibiotic has necessitated the need to search for few antimicrobial agents. Gram positive bacteria such as *Staphylococcus aureus* is mainly responsible for post-operative wound infection, toxic shock syndrome, endocarditis, osteomylitis and food poisoning(13). Gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicemia(14)(15). Different antibiotics exercise their inhibitory activity on different pathogenic organisms(16). Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. Drug resistant microbes such as Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections. MRSA infections are very difficult to cure because MRSA strains are resistance against almost all clinically available antibiotics. For most MRSA strains, glycopeptides-type drugs such as vancomycin are the only effective antimicrobial agents. However, vancomycin-resistant *S. aureus* (VRSA) has been reported. *Pseudomonas aeruginosa* also causes nosocomial infections as a result of its ubiquitous nature, ability to survive in moist environments and resistance to many antibiotics and antiseptics. A main problem is the emergence of multidrug-resistant *P. aeruginosa* strains resistant to different antimicrobial agent classes. Perhaps, this

high degree of multidrug resistance related to the presence of antibiotic efflux systems which provide resistance to multiple antimicrobial agents(19).

Multidrug-resistant *Enterobacteriaceae*, mostly Escherichia coli, produces extended-spectrum β lactamases (ESBLs) such as the CTX-M enzymes. These enzymes were named for their greater activity against cefotaxime than other oxyimino-beta-lactam substrates such as ceftazidime, ceftriaxone, or cefepime have emerged within the community setting as an important cause of urinary tract infections (UTIs). Recent reports have also described ESBL-producing *E. coli* as a cause of bloodstream infections associated with these community-onsets of UTI (20)(11). This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs(17).

Some plants exhibit significant potency against human bacterial pathogens. However, at present, plant extracts are rarely used as antimicrobials or as a systemic antibiotics and this may be due to their low level of activity, especially against gram-negative bacteria(21). The concept of bienhancer is new to the modern science. It was first time reported by bose in 1929, who described the increase in the asthmatic effects of vasaka (*Adhatoda vasica*) leaves by the addition of long piper to it. A bioenhancer is an agent capable of enhancing the bioavaibility and the efficacy of a drug with which it is co-administered, without any pharmacological activity of its own at the therapeutic dose used. They tend to decrease the dose activity drug of drug required for the optimal end point of the treatment strategy, bypass the need to use injectable route of administration to a large extent, might help in overcome the resistance to antimicrobials and saving the precious raw materials for the manufacturing of medicines(22).

Table 1: Evolution of Resistance of Antibiotic

Antibiotic	Year developed	Resistance observed
Sulfonamide	1030	1040s
Penicillin	1943	1946s
Streptomycin	1943	1959
Chloramphenicol	1947	1959
Tetracycline	1948	1953
Erythromycin	1953	1988
Vancomycin	1956	1988
Methicillin	1960	1961
Ampicillin	1961	1973
Cephalosporin	1960s	Late 1960s

Table 2: Major antibiotics: structural classes, and resistance mechanisms

Antibiotic	Target	Resistance mechanism
Cell well		
β-Lactums	Transpeptidase/transglycosylase	β-lactimase, PBP mutants
vancomycins	D-ala-D-ala termini of	Reprogramming of D- Ala-
teicoplanin	peptidoglycon and of lipid II	D-Ala to D- Ala D-lac
Protein synthesis		
Erythromycin	Peptidyltransferase/ribosome	rRNA methylation/efflux
Tetracycline	Peptidyltransferase	Drug efflux
Aminoglycoside	Peptidyltransferase	Drug modification
DNA replication/repair		
Fluroquinolones	DNA gyrase	Gyrase mutation

These antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as

'forgotten plants'. Taking into the account, increasing demand for natural ingredients that might be used as food additives, components of functional foods, preventing plant diseases and nutraceuticals as well as for other applications. It is reasonable to revise the 'forgotten plants' by assessing their applicability and benefits using modern scientific analysis methods. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents(18).

Basic information of Bacteria

Bacterial structure

The majority of bacteria fall within the general dimensions of 0.75-4 *(Xm.* They are unicellular structures which may occur as cylindrical (rod-shaped) or spherical (coccoid) forms. In one or two genera, the cylindrical form may be modified in that a single twist (vibrios) or many twists like a corkscrew (spirochaetes) may occur(23).

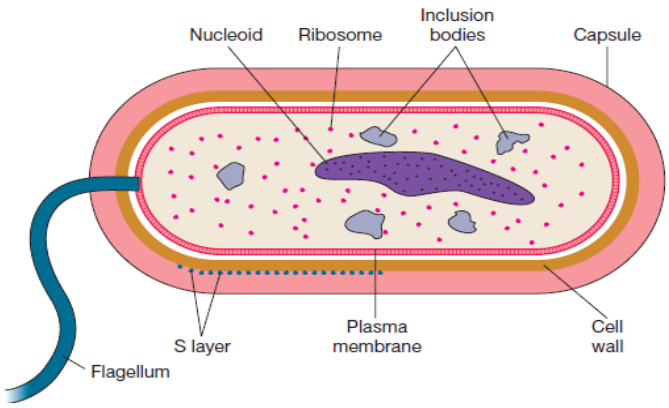


Figure 1: Morphology of a Gram-Positive Bacterium

The majority of the structures shown here are found in all gram-positive cells. Only a small stretch of surface proteins in the S-layer has been included to simplify the drawing; when present, these proteins cover the surface.

Component of the bacterial cell envelope(24)

A. The cytoplasmic membrane

The cytoplasmic membrane of Gram- positive and Gram- negative bacteria are indistinguishable. Each is compose of the protein, lipids, phospholipids and a small amount of carbohydrate.

It has five principal functions:

1. To act as an osmotic barrier.
2. To serve as the site of selective permeability and carrier-mediated transport.
3. To serve as the site of cytochrome activity and generation of proton motive force (PMF).
4. To synthesize the cell wall.
5. To provide a site to implant the chromosome.

B. The periplasma

The periplasma is the space between the inner and outer membrane of the Gram-negative bacterium, and the cell wall lies within it. The periplasma contains enzymes that hydrolyze large molecules, contain enzyme that hydrolyze antibiotics, and binding protein that facilitate transport.

C. The cell wall

The cell wall is a web-like structure that is sometimes called the murien saccules. It is compose of the peptidoglycan. The cell wall provides the cell with its shape and osmotic stability. The cell wall constituents are peptidoglycan, teichoic acid and lipoteichoic acids.

D. The outer membrane

Only Gram- negative bacteria have an outer membrane. Porins and porin-like proteins in the outer membrane allow the membrane to act as a molecular sieve, restricting the access of the some molecules to the cell wall and periplasm. The most clinically significant component of the outer membrane is a phospolipid like molecule called lipopolysaccharide (LPS) that shown in figure no. 2

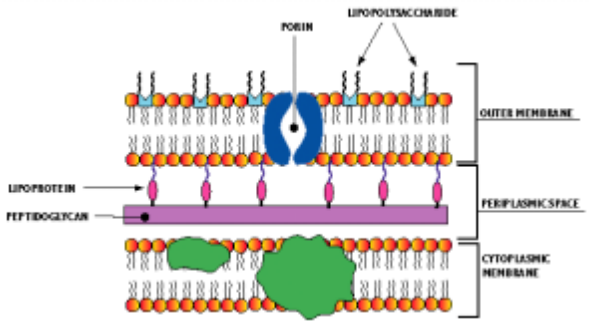


Figure 2: The cell wall of gram-negative bacteria

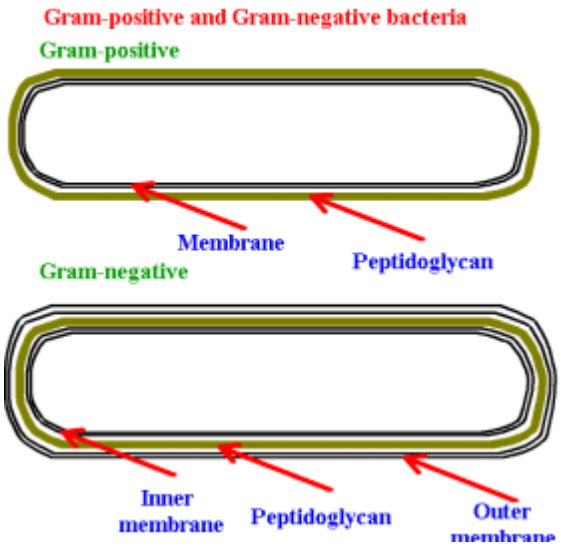


Figure 3: Comparison of the structure of cell wall between gram-positive and gram-negative bacteria.

Mechanism action of bioenhancer

There are several mechanisms of action by which herbal bioenhancers act. Different herbal bioenhancers may have same or different mechanism of action. Nutritional bioenhancers enhance absorption by acting on gastrointestinal tract. Antimicrobial bioenhancers mostly act on drug metabolism process. Among the various mechanisms of action postulated for herbal bioenhancers some are as follows:

(a) Reduction in hydrochloric acid secretion and increase in gastrointestinal blood supply.

(b) Inhibition of gastrointestinal transit, gastric emptying time and intestinal motility.

(c) Modifications in GIT epithelial cell membrane permeability.

(d) Cholagogous effect.

(e) Bioenergetics and thermogenic properties.

(f) Suppression of first pass metabolism and inhibition of drug metabolizing enzymes. Stimulation of gamma glutamyl transpeptidase (GGT) activity which enhances uptake of amino acids(25)(26).

Bioavailability-enhancing activity of natural compounds from the medicinal plants may be attributed to various mechanisms, such as P-gp inhibition activity by flavone, quercetin, and genistein(27). inhibition of efflux transporters, such as P-gp and breast cancer resistance protein (BCRP),(28)(29) by naringin and sinomenine thus preventing drug resistance; DNA receptor binding, modulation of cell signaling transduction, and inhibition of drug efflux pumps(30)(31) by stimulating leucine amino peptidase and glycyl-glycine dipeptidase activity, thus modulating the cell membrane dynamics related to passive transport mechanism as seen with piperine(32); nonspecific mechanisms, such as increased blood supply to the gastrointestinal tract, decreased hydrochloric acid secretion, preventing breakdown of some drugs(33); and inhibition of

metabolic enzymes participating in the biotransformation of drugs, thus preventing inactivation and elimination of drugs and thereby, increasing their bioavailability(34).

Modern drug development processes achieve oral bioavailability enhancement by a number of approaches:

(a) Increasing the polarity of the drug through chemical modification.

(b) Salt preparation or complexation.

(c) Prodrug formation.

(d) Micronization and nanonization.

(e) Specific polymorphic form selection.

(f) Targeted delivery of the drug to the site of action.

(g) Controlled drug delivery through film coating.

(h) Sustained drug release through polymorphic matrices formation.

(i) Liposomal microencapsulation and so forth.

(j) Application of P-glycoprotein inhibitors(35)(36).

Classification of bioenhancer

The uses of bioenhancers are familiar concept in Ayurveda as 'Yagavahi' which was used to enhance bioavailability, tissue distribution and efficacy of drug especially those with poor bioavailability. It can be classified based on origin and mechanism of action

1. Classification of Bioenhancers Based on Origin

Plant origin

Moringa oleifera (Niaziridin)

Caumarin cyminum (cumin)

Carum carvi (Caraway)

Curcuma lunga (Curcumin)

Zingiber officinale (Ginger)

Rivea corymbosa (Lysergol)

Glycyrrhiza glabra (Glycyrrhizin)

Animal origin

Cow urine distillate (kamdhenu ark)

2. Classification of Bioenhancers Based on Mechanism of Action

- **Inhibition of P-gp efflux pump and other efflux pumps:**

Examples: Carum carvi (Caraway), Genistein, Sinomenine, cuminum cyminum cyminum (Black cumin), Naringin, Quercetin

- **Suppressors of CYP-450 enzyme and its isozymes**

Examples: Naringin, Garlic acid and its esters, Quercetin

- **Regulators of GIT function to facilitate better absorption:**

Example: *Aloe vere* (aloe), Niaziridin (Drumstick pods), Zingiber officinale (Ginger), Glycyrrhizin (Liquorice)(37).

Oral dosage form:

Oral dosage is most popular dosage form. Where, it is regarded as the safe, convenient and economical method of drug delivery with highest patient compliance and convenience. It has wide acceptance up to 50-60 % of total dosage form.

Solid dosage forms are popular because of ease of administration, accurate dosage, self medication, pain avoidance and most importantly the patient compliance. The most popular solid dosage forms are being tablets and capsules. During the past four decades, the pharmaceutical industry has invested vast amounts of time and money in the study of tablet compaction.

The expenditure is quite reasonable when one considers how valuable tablets, as a dosage form, are to the industry. Because oral dosage forms can be self-administered by the patient, they are obviously more profitable to manufacture than parental and other dosage forms that must be administered, in most cases, by trained personnel.

Tablet:

Tablet is a most popular solid dosage form then capsule. It is usually obtained by single or multiple compressions of powders or granules. In certain cases tablets may be obtained by molding or extrusion techniques and can be uncoated or coated according to nature of drug substance. Tablets are normally right circular solid cylinders, the end surfaces of which are flat or convex and the edges of which may be beveled. They may have lines or break-marks, symbols or other markings. Tablets can contain one or more active ingredients. They may contain excipient such as diluents, binders, disintegrating agents, colouring agents, flavouring agents, glidants, lubricants, substances capable of modifying the behaviour of the dosage forms and the active ingredient in the gastrointestinal tract. When such excipient are used necessary to ensure that they do not adversely affect the stability, dissolution rate, bioavailability, safety or efficacy of the active ingredient there must be compatibility between any of the components of the dosage form(38).

CHAPTER 2

OBJECTIVES

2. OBJECTIVES

The objective of the present study was Study of Bioenhancer property of selected herbs by using a model antibiotic.

The specific objectives of the research include:

- To chose a model antibiotic and different strain of microbes for antimicrobial activity.
- To identify suitable plant and for the proposed activity.
- To obtain extract by suitable extraction procedure.
- To perform antimicrobial activity of identified above and in combination of model antibiotic.
- To identify the suitable extract and optimize its dose to formulate into tablet dosage form in combination with antibiotic.
- To characterize the formulations for drug-excipient interaction and carryout for short term stability studies as per standard guidelines.
- To compile the data and report

Plan of the work:

1. To carry out the antimicrobial assay of ciprofloxacin and combination with selected natural bioenhancer.
2. To carry out the compatibility studies for possible drug and extract interactions by FT-IR studies.
3. To carry out the Preformulation studies by various parameters.
4. To formulate Ciprofloxacin tablets by using natural polymers like polyvinyl pyrrolidone and Hydroxyl propyl methyl cellulose.

5. To evaluate the pre-compression and post-compression parameters such as angle of repose, bulk density, tapped bulk density and compressibility index, hardness, friability and weight variation, drug content respectively.
6. To carry out the *in-vitro* release studies.
7. Stability studies were conducted for the optimized formulations as per ICH guidelines.

Expected outcomes:

- Primarily, we are expecting that plant extracts when used along with antibiotic well potentiate antimicrobial activity of antibiotic thus dose reduction can be thought off. Secondly, antibiotic resistance by the organisms can be minimized and dose related side effects can also be minimized.
- If this research is fruitful, opens a new arena of the combination of Herbal drug along with the allopathic medicine. Where a scientist can explore all the possibilities to cure the disease with minimum synthetic drug dose in combination approved herbs creating a environmental friendly drugs.
- This research attracts public and creates general awareness towards the proper use of herbs as medicine. Further, this research may encourage medicinal plant cultivation.
- Furthermore, this study may help to add new ayurvedic and allopathic formulation in pharmaceutical industry.

CHAPTER 3

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

Literature survey was carried out on the proposed topic by referring various scientific journals, book website an internet. The survey reveals that no such articles were reported on the proposed work and some related articles are mentioned below.

- **Ghanshyam B *et al.,*** have comprehensively reviewed on pharmacotherapeutics of Piperine as a bioenhancer. They concluded that piperin is shown enhance bioavailability and bioefficacy of different classes of drugs such as antibiotics, antituberculosis, antiviral, antifungal, and anticancerous drugs at low doses. They also revealed that piperin improved oral absorption of nutraceuticals like vitamins, minerals, amino acids, and certain herbal compounds mainly through absorption process, drug metabolism, and action on drug target.(1)
- **Singh R *et al.,*** discussed about Indian Herbal bioenhances.which when used in small concentration improves the general health and also enhances the efficacy of many classes of drugs and nutrients. They discussed the various works reported on plants like *Piper nigrum, Piper longum,zinger officinalis, Aloe vera, Black cummin, Moringa oleifera, Glycyrrhiza glabra, carum carvi, Allum sativum.* (2)
- **Rakshitha MN *et al.,*** studied the bioenhancing role a Cow urine distillate (CUD) as antibacterial activity of methanol extract of *Capsicum fructeress* fruit. They found that CUD did not have any antibacterial activity as such but when present in combination with methanolic extract of *Capsicum fructeress* enhanced the antibacterial activity (3).
- **Randhawa KG *et al.,*** studied on Cow urine distillate as bioenhancer. They found the potential role of cow urine in treatment of bacterial infections and cancer, and demonstrated that cow urine can enhance the efficacy and potency of other drugs.(4)
- **Chutima J *et al.,*** studied on bioavailability enhancement techniques of curcumin. They prepared various Curcumin derivatives in form of polycurcumin, PEGylated Curcumin,

Curcumin-amino acid conjugates and Curcumin conjugated hyaluronic acid. They proved that these derivatives increased stability, solubility, and/or permeability of Curcumin leading to bioavailability enhancement of Curcumin.(5)

- **Pattanaik S *et al.,*** evaluated the effect of simultaneous administration of Piperine and Carbamazepine in epileptic patients undergoing Carbamazepine monotherapy. They observed that Piperine significantly enhanced the oral bioavailability of carbamazepine possibly by decreasing the elimination or by increasing its absorption of carbamazepine. They concluded that piperine significantly increased the mean plasma concentrations of carbamazepine in experimental groups.(6)

- **Kasibhatta R *et al.,*** studied the influence of Piperine on the pharmacokinetics of Nevirapine under fasting conditions by randomized, crossover and placebo controlled study. They administered Piperine or placebo to healthy adult males for 6 day. On seventh day Piperine or placebo was administered with Nevirapine. Blood samples were collected post-dose. The results of the study showed that there was an enhanced bioavailability of Nevirapine when administered with Piperine.(7)

- **Mekala P *et al.,*** reviewed on bioenhancer of Curcumin, Ginger, Cumin, Grape fruit, Citric fruit. They were found Ginger and Black cumin increases the bioavailabity of Amoxicillin, Cloxacilline, Cefalaxin, Cefadroxil, Erythromycin and Azithromycin. They suggested that the bioenhancer reduces the toxicity associated with over dosage due to poor bioavailability and minimize the development of drug resistance by microbes which are not only a problem to animals but also to human beings.(8)

- **Sushma D *et al.,*** studied on use of herbal bioenhancers to increase the bioavailabity of drug. They use Piperine, Ginger, Drumstick Pods, Liquorice, Black cumin, Garlic,

Quercetin. They found Drumstick Pod enhance the activity of Rifampicine, Ampicilline, Nalidixic acid by 1.2 -12 fold against the garm positive strain.(9)

- **Deepthi V *et al.,*** studied on the natural biaenhancers. They found pipermint oil improves the oral bioavailabity of cyclosporine and Capsaicin enhances the bioavailability of Thiophylline. (10)

- **Emad B. B. *et al.,*** studied on self-nanoemulsifying drug delivery system (SNEDDS). They developed and optimized SNEDDS formulations containing surfactants reported improvement of dissolution and oral absorption of lacidipine (LCDP) concluding that the surfactant itself act as bioenhancer.(11)
- **Thanou M**. ***et al.,*** studied on oral drug absorption enhancement by chitosan and its derivatives. They used Chitosan in solution which was able to interact with the tight junctions and to provoke their opening allowing for paracellular permeation of hydrophilic macro- molecular drugs and integrity of the epithelium or the cell membranes are minimal.(12)
- **Singh A *et al.,*** reviewed on Piperine as a bioenhancer. They found bioenhancer activity of Piperine certain drugs Ciprofloxacin, Rifampicin, Dapson, phnenobarbitone and Piperine enhances Cmax of different drugs significantly.(13)
- **Sibanda t *et al.,*** studies on the challenges of overcoming antibiotic resistance used plant extracts as potential sources of antimicrobial and resistance modifying agents. They were searching such a compounds which can be combined with antibiotics in the treatment of drug resistant infections may be an alternative to overcoming the problem of resistance in bacteria. Crude extracts of medicinal plants stand out as veritable sources of potential resistance modifying agents and the African biosphere promises to be a potential source of such compounds owing to its rich plant species diversity.(14)

- **Naidu M. U. R** was studied on Influence of Piperine on the Pharmacokinetics of Nevirapine under Fasting Conditions. This pilot study provided evidence for enhanced bioavailability of Nevirapine when administered with Piperine. Further in-depth studies in a large number of patients receiving different dosage regimens are required to confirm these results and further our understanding of a possible clinical advantage arising from the bioenhancement capabilities of Piperine in the treatment of HIV infection.(15)
- **Kesarwani K *et al.,*** reviewed on Bioavailability enhancers of herbal origin. They were explain many herbal compounds including quercetin, genistein, naringin, sinomenine, piperine, glycyrrhizin and nitrile glycoside have demonstrated capability to enhance the bioavailability. They are summarizing various available novel drug delivery technologies which have been developed for delivery of drugs (herbal), and to achieve better therapeutic response. An attempt has also been made to compile a profile on bioavailability enhancers of herbal origin with the mechanism of action (wherever reported) and studies on improvement in drug bioavailability, exhibited particularly by natural compounds.(16)
- **Karan *et al*.**, studied the effect of trikatu on the pharmacokinetic profile of indomethacin in rabbits. The results showed that TRIKATU enhanced the absorption of indomethacin which was supposed to be the result of an increase in the gastrointestinal blood flow and an increased rate of transport across gastrointestinal mucosa**.**(17)
- **Singh M *et al.,*** studied the alteration of pharmacokinetics of oxytetracycline following oral administration of *Piper longum* in hens. Their studies revealed that the prior administration of *P.longum* increases total duration of antimicrobial action and enhances the therapeutic efficacy of oxytetracycline in poultry birds. There was reduction in loading and maintenance dose and thus the subsequent side effects (18)

- **Atal *et al.,*** worked on biochemical basis of enhanced drug bioavailability by piperine. The study was aimed at understanding the interaction of piperine with enzymatic drug biotransforming reactions in hepatic tissue. They found that piperine shows little discrimination between different cytochrome P-450 forms and is a non-specific inhibitor of drug metabolism. Piperine strongly inhibited the hepatic AHH and UDP-glucuronyltransferase activities when orally administered to rats. The results of the experiment demonstrated that piperine is a potent inhibitor of drug metabolism**.**(19)
- **Surabhi KS *et al.,*** studied on Cow urine distillate as a Bioenhancer of antibacterial activity of *Polyalthia longifolia* Thw fruit pericarp. They were found the antibacterial effect of extract and CUD combination was higher than the inhibition caused by extract alone. Moreover, inhibition of test bacteria was observed with less extract concentration of extract on combining with CUD.(20)
- **Santos Dos I *et al.,*** studied on Improvement of Norfloxacin oral bioavailability by EDTA and sodium caprate. They were found that absorption kinetic of Norfloxacin was markedly accelerated when mixed with EDTA or Na caprate in a ratio of 1:1. When mixed with the absorption enhancers in a ratio of 1:5, only Na caprate improved Norfloxacin bioavailability significantly. In vitro dissolution tests demonstrated that EDTA and Na caprate increased Norfloxacin dissolution kinetic. However, the correlation between bioavailability and in vitro dissolution improvement was not clearly established. So, we can conclude that the solubilizing property of EDTA and Na caprate did not take a prominent part in Norfloxacin absorption.(21)
- **Yi-Dong Yan *et al.,*** studied on Enhanced oral bioavailability of docetaxel in rats by four consecutive days of pre-treatment with curcumin. They were found, curcumin, in comparison to the currently marketed P-glycoprotein inhibitors CYP3A and CYP3A, is

safe and possesses inherent anti-cancer properties, making it an ideal candidate for improving the oral bioavailability of docetaxel.(22)

- **Junginger H.E. *et al.,*** studied on Trimethylated chitosan as polymeric absorption enhancer for improved peroral delivery of peptide drugs. Various in vivo studies in different animal models confirmed the ability of N-trimethyl chitosan chloride (TMC) to increase the absorption of the peptide drugs buserelin and octreotide after intraduodenal or jejunal administration. However, TMC has always been administered as a solution in these studies. The impracticality of administering a solution, as well as the fact that most peptides are unstable in the presence of water, have led to the need for a solid oral dosage form with which TMC can be administered together with peptide drugs. Recent studies have focused on the development and in vivo evaluation of solid oral dosage forms.(23)
- **Acharya SG. *et al,*** studied on Piperine as a Bio-enhancer. They were found at site of absorption Molecular structure of piperine is suitable for enzyme inhibition and it inhibits various metabolizing enzymes like cytochrome bs, NADPH cytochrome, CYP3A4, UDP-glucose dehydrogenase (UDP-GDH), aryl hydrocarbon hydroxylase (AAH) and UDP-glucuronyl transferase. Structural modification of piperine provides selective inhibitors of various cytochrome p450 enzymes. Inhibition of these enzymes by piperine results in enhanced bioavailability of drugs and nutrients like oxytetracyclin, metronidazole, ampicillin, norfloxacin, ciprofloxacin, acefotaxime, amoxicillin trihydrate, curcumin, beta-carotene, carbamazepine, gallic acid, nimesulide, tiferron, nevirapine, pentobarbitone, phenytoin, resveratrol, vasicine and sparteine by different mechanisms. Thus piperine is an absorption enhancer and a potent inhibitor of drug metabolism.(24)
- **Patil U *et al.,*** has studied on Role of Piperine as a Bioavailability Enhancer. They were shown to possess bioavailability enhancing activity with various structurally and therapeutically diverse drugs. It has been found that piperine's bioavailability-enhancing

property may be attributed to increased absorption, which may be due to alteration in membrane lipid dynamics and change in the conformation of enzymes in the intestine. Piperine has been demonstrated to increase the serum levels and lengthen the serum half lives of some nutritional substances, such as coenzyme Q10 and beta-carotene. The mechanism of this action is unknown. It is speculated that piperine may act as a so called thermonutrient and increase the absorption of certain nutritional substances from the gastrointestinal tract by producing a local thermogenic action. The present review is an attempt to highlight the bioenhancing ability of piperine when it is given along with various drugs and nutrients.(25)

3.1 DRUG PROFILE

CIPROFLOXACIN HYDROCHLORIDE

Ciprofloxacin Hydrochloride contains not less than 98.0percent and not more than 102.0 percent of $C_{17}H_{18}FN_3O_3 \cdot HCl$.

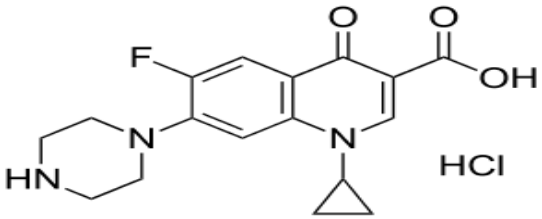


Fig 4: Structure of ciprofloxacin

Empirical formula: $C_{17}H_{18}FN_3O_3$

Chemical name: 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.

Molecular mass: 367.8

Solubility: Soluble in water, slightly soluble in methanol, vary slightly soluble in ethanol and practically insoluble in acetone, ethyacetate.

Description: It is a faintly yellowish to light yellow crystalline substance

Physical State: Solid

pK: pK_a: 6.43 (Predicted), pK_b: 8.68 (Predicted**)**

Melting point: 311-320°C

Bioavailability: 69%

Peak Serum concentration: 2.4µg/ml

Oral Dose: 250-500 mg

Half life: 4 hours

Pharmacokinetics(26)(27)(28)(29)**:**

Absorption:

Ciprofloxacin given as an oral tablet is rapidly and well absorbed from the gastrointestinal tract after oral administration. The absolute bioavailability is approximately 70% with no substantial loss by first pass metabolism. Ciprofloxacin maximum serum concentrations and area under the curve are shown in the chart for the 250 mg to 1000 mg dose range.

Distribution:

The binding of ciprofloxacin to serum proteins is 20 to 40% which is not likely to be high enough to cause significant protein binding interactions with other drugs. Oral administration of ciprofloxacin is widely distributed throughout the body. Ciprofloxacin is present in active form in saliva, nasal and bronchial secretions, mucosa of the sinuses, sputum, skin blister fluid, lymph, peritoneal fluid, bile, and prostatic secretions. Ciprofloxacin has also been detected in lung, skin, fat, muscle, cartilage, and bone. The drug diffuses into the cerebrospinal fluid (CSF); however, CSF concentrations are generally less than 10% of peak serum concentrations.

Metabolism:

Metabolism occur through liver including CYP1A2 and also Four metabolites have been identified in human urine which together account for approximately 15% of an oral dose. The metabolites have antimicrobial activity, but are less active than unchanged ciprofloxacin.

Excretion:

The serum elimination half-life is approximately 4 hours. Approximately 40 to 50% of an orally administered dose is excreted in the urine as unchanged drug. After a 250 mg oral dose, urine concentrations of ciprofloxacin usually exceed 200 μg/ml during the first two hours and are approximately 30 μg/ml at 8 to 12 hours after dosing. The urinary excretion of ciprofloxacin is virtually complete within 24 hours after dosing. The renal clearance of

ciprofloxacin, which is approximately 300 ml/minute, exceeds the normal glomerular filtration rate of 120 ml/minute.

Mechanism of action:

Ciprofloxacin is a broad-spectrum antibiotic active against both Gram positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell division.

Indication(30)(31)**:**

1. Urinary tract infection
2. Lower respiratory tract infection
3. Acute sinusitis
4. Skin and skin structure infection
5. Bond and joint infection
6. Complicated intra-abdominal infection
7. Infectious diarrhoea
8. Typhoid fever
9. Sexual transmitted disease
10. Pheonephritis in children

Side effects:

Common adverse reactions involve the GI tract, with 3% to 17% of patients reporting mostly mild nausea, vomiting, and/or abdominal discomfort. Diarrhea and antibiotic-associated colitis have been unusual. CNS side effects involve mild headache and dizziness; have been seen in 0.9% to 11% of patients. Rarely, hallucinations, delirium, and seizures

Interactions(32)

1. Ciprofloxacin + Antacid (Aluminum hydroxide, magnesium hydroxide): To reduce the absorption.
2. Ciprofloxacin + Calcium salt: To reduce the absorption.
3. Ciprofloxacin + Cyclosporine : Increase the risk of nephrotoxicity
4. Ciprofloxacin + Ferrous salt: To reduce the absorption
5. Ciprofloxacin + Ibuprofen: Increase the risk of convulsion
6. Ciprofloxacin + warfarin: To enhance the anticoagulant effect
7. Ciprofloxacin + Zinc sulphate: To reduce the absorption

Contraindications:

Concomitant administration with Tizanidine is contraindicated. Anyone with a history of hypersensitivity to any member of the quinolone class of antimicrobial agents, including ciprofloxacin, or any of the product components is contraindicated.

3.2 EXCIPIENTS PROFILE

DIMETHYL SULPHOXIDE (DMSO) (33)(34)

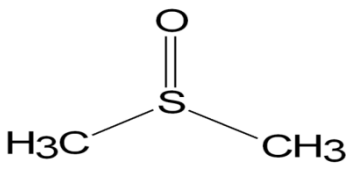


Fig11: Structure of Dimethyl sulfoxide

Empirical formula: C_2H_6OS

Molecular mass: 78.13 g/mol

Functional Category: Penetration enhancer, solvent

Melting point: 19°C

Boiling point: 189°C

Density: 1.10 g/ cm^3

Dipole moment: 4.3 at 20°C

Dissociation constant (pKa):31.3

Enthalpy of fusion: 3.43cal/mol

Solubility:

DMSO is miscible with water with evolution of heat, also miscible with ethanol, ether and most organic solvents; immiscible with paraffin, hydrocarbon sand practically insoluble in acetone, chloroform, ethanol and ether.

Incompatibility

Dimethyl sulphoxide can react with oxidizing materials.

Description:

Dimethyl sulphoxide occurs as a colourless, viscous liquid, or as colourless crystal that are miscible with water, alcohol, and ether. The material has a slightly bitter taste with a sweet aftertaste and is ordourless, or has a slight odour characteristic of dimethyl sulphoxide. Dimethyl sulphoxide is extremely hygroscopic, absorbing up to 70% of its own weight in water with evolution of heat.

Stability and Storage Condition

Dimethyl sulfoxide is reasonably stable to heat but upon prolonged reflux it decomposes slightly to methyl mercaptan and bismethylthiomethane. This decomposition is aided by acids, and is retarded by many bases, when heated to decomposition, toxic fumes are emitted. At temperature between 40-60°C, it has been reported that dimethyl sulfoxide suffers a partial breakdown, which is indicated by changes in physical properties such as refractive index, density, and viscosity. It should be stored in airtight, light resistant containers.

POLYVINYL PYRROLIDONE(35)

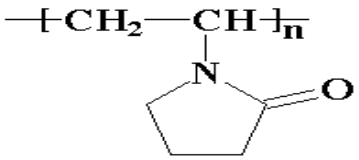


Fig 6: Chemical structure of Polyvinylpyrrolidone

Synonym: plasdone k-30, luviskol k-30, plasdone, povidone, pvp p, pvp k-30; pvp; polyvinylpyrrolidone; poly(1-(2-oxo-1-pyrrolidinyl)ethylene); povidone k-30; poly(n-vinylbutyrolactam); poly(1-vinylpyrrolidinone)

Chemical name: poly (1-vinyl-2-pyrrolidinone)

Chemical formula: $(C_6H_9NO)_n$

Functional category: suspending agent; tablet binder

Molar mass: 2.500-2.5000.000g.mol-1

Density: 1.2g/cm^3

Melting point: 150-1800C

Boiling point: 1930C

Description: it is a fine, white to creamy-white colored, odorless, hygroscopic, amorphous powder.

Incompatibility: reactive with oxidizing agents.

Solubility: soluble in cold water, soluble in chloroform, alcohol, chlorinated hydrocarbons, amines, nitroparaffin's, lower weight fatty acids.

Application: PVP k series can be used as film forming agent, viscosity enhancement agent, lubricator and adhesive. In tableting, PVP solutions are used as binders in wet granulation process. PVP is also added to powder blends in the dry forms and granulated *in-situ* by addition of water, alcohol or hydroalcoholic solutions. PVP solutions may also be used as

coating. It is also used as a suspending, stabilizing or viscosity- increasing agents in topical and oral suspensions and solutions.

Stability and storage conditions: PVP darkens to some extent on heating at 150oC, with a reduction in aqueous solubility. PVP may be stored under ordinary conditions without undergoing decomposition or degradation. It stored in an airtight container in a cool place, dry place.

Safety: when consumed orally, PVP may be regarded as essentially nontoxic since it is not absorbed from the gastrointestinal tract or mucous membranes. PVP has no irritant effect on the skin and causes no sensitization.

MAGNESIUM STEARATE(36)

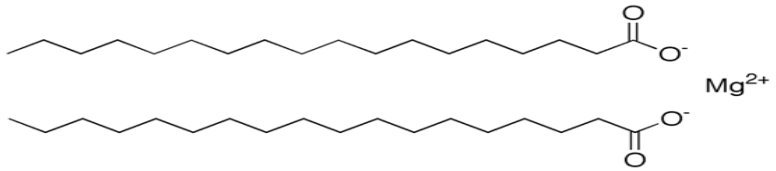


Fig 7: Chemical structure of magnesium stearate

Synonym: Magnesium octadeconate, Octadecanoic acid magnesium salt, stearic acid magnesium salt.

Chemical name: Octadecanoic acid magnesium salt

Functional category: Tablets and capsules lubricant

Description: It is a fine, white, precipitated or milled, impalpable powder with low bulk density. Insoluble in water, powder shows a faint odour of stearic acid, tasteless. The powder is greasy to touch and readily adhere to skin.

Applications in pharmaceutical formulations and technology:

It was extensively used in cosmetic formulations, food and pharmaceutical formulations. It is primarily used as a lubricant in tablets and capsule fabricating processes at a concentration of 0.25-5.0% also used to prepare barrier creams

Stability and storage: It is a stable chemical substance. It shall be stored in a well closed, air tight container in a cool and dry place

Incompatibilities: It is incompatible with strong acids, iron salts and should be avoiding mixing with strong oxidizing agents. It should not be included in the formulations containing aspirin, some vitamins, and most of the alkaloidal salts.

Safety: It is one of the mostly used pharmaceutical excipient as it is an non-toxic when ingested through oral route. Upon consumption of large amount produces laxative effect and can irritates mucosal layer of G.I.T.

TALC(37)

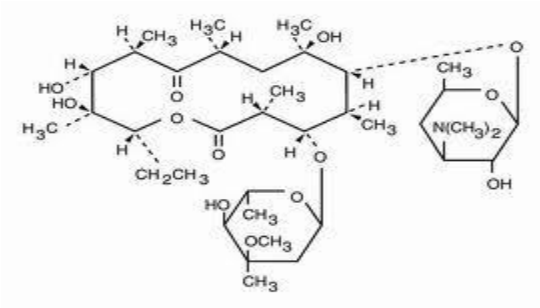


Fig 8: Chemical structure of talc

Synonyms: Magsil star, powdered talc, Purified French chalk, Purtalc, steatite, Soapstone

Empirical Formula: $Mg_6 (Si_2O_5)_4(OH)_4$

Functional Category: Anti-caking agent, Glidant, tablet and capsule diluents, tablet and capsule lubricant.

Description: Talc is a very fine, white to grayish-white colored, odorless, impalpable, unctuous, crystalline powder. It adheres readily to the skin, it soft to the touch, and free from grittiness.

Applications in Pharmaceutical Formulation or Technology:

Talc was once widely used in oral solid dosage formulations as a lubricant and diluents, although today it is less commonly used. However, it is widely used as a dissolution retardant in the development of controlled-release products. Talc is also used as a lubricant in tablet formulations) in a novel powder coating for extended release pellets and as an adsorbent.

Stability and Storage Conditions: Talc is a stable material and may be sterilized by heating at 1600 C for not less than 1 hour. It may also be sterilized by exposure to ethylene to ethylene oxide or gamma irradiation. Talc should be stored in a well-closed container in a cool, dry place

Safety: Talc is mainly used in tablet and capsule formulation. Oral ingestion talc is not absorbed systemically and regarded as a nontoxic material.

LACTOSE(38)

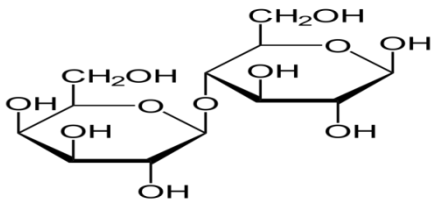


Fig 9: Chemical Structural of lactose

Formula weight: $C_{12}H_{22}O_{11}$

Molecular weight: 342.30

Description: Lactose occurs as white to off-white crystalline particles or powder. Several different brands of anhydrous lactose are commercially available which contain anhydrous β-lactose and anhydrous α-lactose. Anhydrous lactose typically contains 70–80% anhydrous β-lactose and 20–30% anhydrous α-lactose.

Functional Use: Binding agent, directly compressible tablet excipient, lyophilization aid, tablet and capsule filler.

Solubility: Soluble in water; sparingly soluble in ethanol (95%) and ether.

Applications in Pharmaceutical Formulation or Technology

Anhydrous lactose is widely used in direct compression tablet applications and as a tablet and capsule filler and binder. Anhydrous lactose can be used with moisture-sensitive drugs due to its low moisture content.

Safety:

Lactose is diluent and filler-binder in oral capsule and tablet formulations. It may also be used in intravenous injections. Adverse reactions to lactose are largely due to lactose intolerance, which occurs in individuals with a deficiency of the intestinal enzyme lactase, and is associated with oral ingestion of amounts well over those in solid dosage forms.

3.4 BACTERIA USED

Staphylococcus aureus

Domain: Bacteria

Kingdom: Eubacteria

Phylum: Firmicutes

Class: Coccus

Order: bacilaes

Family**:** Staphylococceae

Genus: *Staphylococcus*

Species: ***S. aureus***

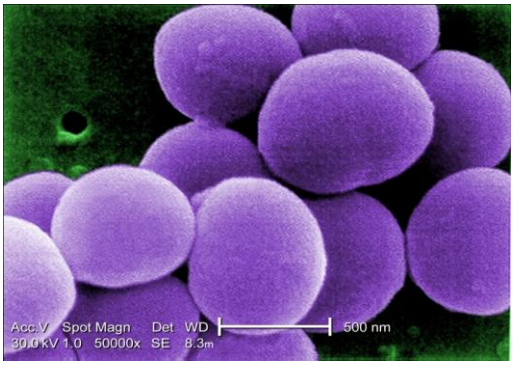


fig 10: Structure of *Staphylococcus aureus*

General characteristics

The *S. aureus* is Gram-positive cocci. It is spherical cell (0.5 to 1.5µm) that appears singly, in pairs, and in irregular clusters that has been described as looking like "branches of grape". This organism is non-motile, non-spore forming, facultative anaerobe and chemoorganograph (both respiratory and fermentative metabolism). Colonies appear creamy, white or light gold and sometimes yellow to orange. The optimum temperature is 30-37°C.(39)

Clinical significance

S. aureus is responsible for a wide variety of infections and disease due to toxins.

Table 3: Disease associated with *S. aereus*

Disease associated with *S. aereus*
1. Localized infection • Skin infection (folliculitis, impetigo, furuncles, carbuncles) and wound infections • Infection caused by S. aereus are supparative and pyogenic. Some of the common skin infections are boils, carbuncles, folicullitis and bullous impetigo. These opportunistic infections occur usually as a result as a result of previous skin injuries.
2. **Systemic infections** • Bacteremia, septicaemia
3. Toxin production • Food poisoning. • *S. aereus* produces enteroxins that have been identified and associated with gastrointestinal upset.

Antibiotic susceptibility characteristics

Penicillin became more widely available and used. By the 1950s, isolated strain of *S. aureus* was resistant to penicillin by producing an enzyme that cleaves its β- lactum ring. The penicillinase-resistant penicillin, which was nafcillin, methicillin and oxacillin, were used to treat the more resistant isolates. The 1970s, resistance developed to these compounds. The MRSA have become a costly problem in hospitals. The rest anti-infective therapy against MRSA is vancomycin(40).

E-coli

Domain: Bacteria

Phylum: Proteobateria

Class: Gammaproteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: *Escherichia*

Species: ***E-coli***

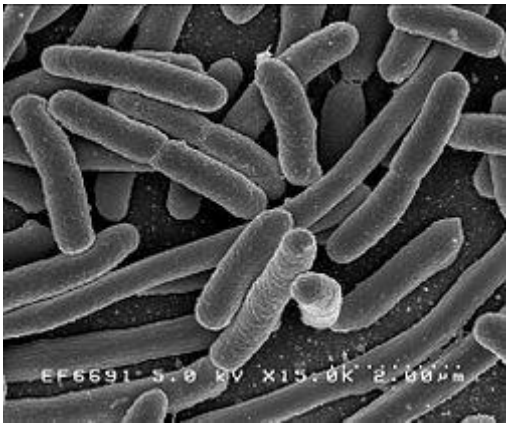


fig 4: Structure of *E-coli*

General characteristics

E. *coli* is a Gram negative, straight rod (1.1-1.5 μm x 2.0-6.0) that occurs singly or in pairs. It is facultatively anaerobes and chemoorgranotrophs. The optimal temperature is 37°C. It occurs as normal flora in the lower part of the intestine of warm-blooded animal.

Clinical significance

Table 4: Infections associated with E. coli

Group	**Type of infection**	**Comments**
Nephropathogenic E- coli	Urinary tract infection (UTI) (pyelonephritis)	Common in women, young children in diapers and catheterized patients.
Enteropathogenic E-coli (EPEC)	Watery diarrhea	Common in infants, outbreaks in nurseries
Enteropathogenic E-coli (EPEC)	Traveler's diarrhea	Common in travelers to endemic area
Enteropathogenic E-coli (EPEC)	Haemorragic colitis, hemolytic uremic syndrome	Associated with undercooked ground beef, raw milk, other foods, acute renal failure, may be fatal.

Enteroinfasive E- coli (EIEC)	Bloody diarrhea	Dysentery-like diseases, most common in young children in developing countries.
Enteroaggregative E. coli (EAEC)	Watery diarrhea	Most common in young children in developing countries, diarrhea may be acute or chronic.

***Pseudomonas aeruginosa*:**

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gamma Proteobacteria

Order: Pseudomonadales

Family: Pseudomonadaceae

Genus: *pseudomonas*

Species: ***p. aeruginosa***

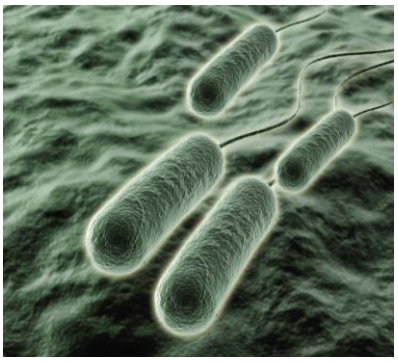


Fig 12: Structure of *p. aeruginosa*

General characteristic

P. aeruginosa is a Gram negative, straight or slightly curved rod (0.5-1.0) μm x 1.5-5.0 μm). It wildly distributed in nature. It is extremely hardy organism, surviving under condition that would kill most other bacteria.

Clinical significance

This organism can occasionally cause disease in healthy individuals. The infections in debilitated or immunocompromised host are significantly more common and more serious. This species is a very important opportunistic pathogen in hospitalized patients.

Table 5: Infections associated with *P. aeruginosa*.

Infections	**Comments**
Burns and wounds	Wounds may be due to accidental or surgical trauma, infection is often accompanies with blue-green pus due to pigment production.
Bactereia/septicemia	Result of progressive infection seen in immunocompromised individuals.
Ecthyma gangrenosum	Syndrome with painful and occur in association with bacteremia.
Ostiomyelitis	Inflammation of bone, associated with deep wounds and compound fractures, may be local or spreading.
Otitis externa	"Swimmer's ear" in children who spend prolonged time in swimming pools.
Pneumonia and lungs abscess	Associated with neutropenia, immunosuppression and cytotoxic drugs.
Meningitis	Seen mostly in the immunocompromised.
UTI	Associated with catheters and medical producers.
Endocarditis	Seen mostly in drug addicts, occasionally seen in patients with prosthetic heart valves.

Antibiotic susceptibility characteristics

P. aeruginosa is one of the most highly resistant organisms encountered in clinical laboratories. Usually penicillin is used together with a aminoglycoside. The newer quinolones (Ciprofloxacin), aztreonam, imipenem, and other third generation Cephalosporine are also active against the organisms.

Klebsela pneumonia

Kingdom: Bacteria

Phylum: Proteobaceria

Class: Enterobacteriales

Family: Enterobacteriacae

Genus: *Klebsiella*

Species: ***K. Pneumoniae***

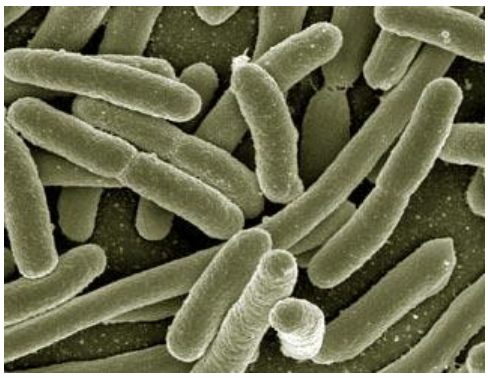


fig 13: structure of *K. Pneumoniae*

Klebsiella pneumoniae is a Gram-negative, non motile, encapsulated, lactose fermenting, facultative anaerobic, rod-shaped bacterium. It naturally occurs in the soil, and about 30% of strains can fix nitrogen in anaerobic conditions. It is rod-shaped and measures 2 μm by 0.5 μm. In 1882, Friedlander C. Uber first discovered *Klebsiella* to be a pathogen that caused pneumonia.

Table 6: Clinical significance *K. Pneumoniae* (41)

Infection	**Comment**
Urinary tract infection	Frequency, urgency, dysuria, hesitancy, low back pain, and suprapubic discomfort. Systemic symptoms such as fever and chills are usually indicative of a concomitant pyelonephritis or prostatitis.
Nosocomial infection	Bacteremia, wound infection, cholecystitis, and catheter-associated bacteriuria, cholangitis, meningitis, endocarditis, and bacterial endophthalmitis. The latter occurs especially in patients with liver abscesses and diabetes. These infectious presentations are relatively uncommon.
Chronic genital ulcerative disease	An ulcerogranulomatous presentation is most common and is characterized as a beefy red ulcer. A hypertrophic or verrucous

	presentation may mimic condylomata acuminate. A necrotic presentation is characterized by a deep ulcer. Sclerotic and cicatricial presentations are rare.
Respiratory tract infection	Pneumonia, bronchopneumonia bronchitis, lung abscess, cavitation, empyema, and pleural adhesions

Antibiotic susceptibility characteristics

Klebsiella spp. were naturally sensitive or intermediate to several Penicillins, all tested Cephalosporins, Aminoglycosides, Quinolones (Ciprofloxacin), Tetracyclines, Trimethoprim, Cotrimoxazole, Chloramphenicol and Nitrofurantoin**.** It is naturally resistant or intermediate to amoxicillin, ticarcillin and to antibiotics to which other Enterobacteriaceae are also intrinsically resistant.(42)

3.5 AGAR MEDIA

Muller Hinton agar(43)(44)

Muller-Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing. **Composition**(45)

Table 7: Composition of Muller Hinton agar

Ingredients	**Gram/liter**
Beef, infusion form	300.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.00

Final PH (at 25°C) 7.3±0.1

Method of preparation:

1. Suspend 38 g of the medium in one liter of purified water

2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Pour cooled Mueller Hinton Agar into sterile Petri dished on a level, horizontal surface to give uniform depth. Allow to cool to room temperature
5. Check prepared mauler hinton agar to ensure the final pH is 7.3±0.1 at 25°C

Quality Control(46)

Appearance

Cream to yellow homogeneous free flowing powder

Color and Clarity of prepared medium

Light amber colored clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.8%w/v aqueous solution at 25°C, pH 7.3±0.1

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

3.6 NATURAL BIOENHANCERS

3.6.1 ANIMAL PRODUCT

Cow Urine Distillate(47)**:**

Cow Urine distillate is used an ancient therapy, part of Ayurveda, which has been re-established by study, research and work of over several years. Cow urine is the most important ingredient of cow therapy. Cow urine contains various elements which are present and are required in our body. It tries to balance those elements, hence, re-establishing the equilibrium for a healthy body. The holy texts like Athva Veda, Charak Samhita, Rajni

Ghuntu, Vridhabhagabhat, Amritsagar, Bhavprakash, Sushrut samhita and more contain beautiful description about these things. To remove such poisons from our body cow urine is a very effective medicine. In fact, many poisonous ayurvedic herbs (like *dhatura*) are kept in cow urine to remove the poison.

Cow urine has the potency to cure diseases like diabetes and thyroid. It is also been successfully tested on cancer. Many cancer patients have got cured by regular consumption of cow urine. Cow urine is not only a good medicine but also a good tonic. It balances *kapha*, *vaata* and *pitta*. It increases the immunity of the body and purifies the blood.

Cow urine used as following ailments:(48)

Cow Urine Distillate can be used as a flu, arthritis, tridosa's, snake poison, small pox, chicken pox ,indigestion, edema, hepatitis, obesity, gastric, ulcers, stress, tensions, nervous disorder, cardiovascular disease, asthma, tetanus, Parkinson's disease, morning sickness, fever, diabetes, severe skin disease, blood purification, leucorrhea, irregular menstrual cycle, urinary disorders, enhances memory power and DNA Protection.

Chemical composition of distilled cow urine and uses(49):

1. Nitrogen (N_2, NH_2): Removes blood abnormalities and toxins, Natural stimulant of urinary track. It activates kidneys and help diuretic.
2. Sulphur (S): Supports motion in large intestines. Cleanses blood.
3. Ammonia (NH_3): Stabilize bile, mucous and air of body. Stabilizes blood formation.
4. Copper (Cu): Controls built up of unwanted fats.
5. Iron (Fe): Maintains balance and helps in production of red blood cells & hemoglobin. Stabilizes working power.
6. Urea CO $(NH_2)_2$: Affects urine formation and removal. Germicidal.
7. Uric Acid ($C_5H_4N_4O_3$): Removes heart swelling or inflammation. It is diuretic therefore destroys toxins.

8. Phosphate (P): Helps in removing stones from urinary track.
9. Sodium (Na): Purifies blood. Antacid.
10. Potassium (K): Cures hereditary rheumatism. Increases appetite. Removes muscular weakness and laziness.
11. Manganese (Mn): Germicidal, stops growth of germs, protects against decay due to gangrene.
12. Carbolic acid (HCOOH): Germicidal, stops growth of germs and decay due to gangrene.
13. Calcium (Ca): Blood purifier, bone strengthener, germicidal.
14. Salt (NaCl): Decreases acidic contents of blood, germicidal. Vitamins A, B, C, D, and E: Vitamin B is active ingredient for energetic life and saves from nervousness and thirst, strengthens bones and reproductive ingredient for energetic life and saves from nervousness and thirst, strengthens bones and reproductive power.
15. Other Minerals: Increase immunity.
16. Lactose ($C_6H1_2O_6$): Gives satisfaction, strengths heart, removes thirst and nervousness.
17. Enzymes: Make healthy digestive juices, increase immunity.
18. Water (H_2O): It is a life giver. Maintains fluidity of blood, maintains body temperature.
19. Hipuric acid (CgNgNox): Removes toxins through urine.
20. Creatinin ($C_4HgN_2O_2$): Germicide.
21. Aurum Hydroxide (AuOH): It is germicidal and increases immunity power. AuOH is highly antibiotic and anti-toxic.

3.6.2 HARBAL PRODUCT

Turmeric

Scientific classification

Kingdom: Plantae

Order: Zingiberales

Family: Zingiberaceae

Genus: *Curcuma*

Species: ***C. longa***

fig 14: Turmeric powder

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger. It is native in southeast India, and needs temperatures between 20 and 30°C and a considerable amount of annual rainfall to thrive. Plants are gathered annually for their rhizomes, and propagated from some of those rhizomes in the following season. The wide range of turmeric health benefits come mainly from its main ingredient, curcumin. This widely researched component of turmeric is highly therapeutic and is used in various drugs and pharmaceutics mainly because of its immunity boosting and anti-oxidant properties.

Uses:

- Digestive Disorders

Turmeric used as a digestive bitter and a carminative. It is a cholagoue, stimulating bile production in the liver and help excretion of bile via the gallbladder. This improves the body's ability to digest fats.

- Liver Diseases:

Turmeric is a liver protecting compounds that milk thistle and artichoke leaves contains.

- Cancer:

Turmeric can cure host of disease also restrain the growth of various types of cancer. It is also use for treatment of skin cancerous pre cancerous skin conditions.

- Atherosclerosis:

Turmeric helps in preventing the blockage of arteries that can cause a heart attack or strock. Turmeric lowers the cholesterol level and inhibits the oxidation of LDL (bad cholesterol).

- Osteoarthritis:

It helps relieve the symptom of osteoarthritis.

- Menstrual problem of women:

Turmeric is an antispasmodic to smooth muscles so it reduces the digestive and menstrual cramping.

- Eye Disorder:

Curcumin prove to be as effective as corticosteroids in the uvitis.

Black Piper

Scientific classification

Kingdom: plantae

Order: Piperales

Family: Piperaceae

Genus: *Piper*

Species: ***P. nigrum***

fig 15: Piper nigrum

It is a perennial vine and climber that requires supporting tree or pole to grow in height; thus it has similar growth characteristics that of beetle leaf plant. The pepper plant start producing small round berries after about three to four years of plantation. Technically, the pepper berry is a drupe, measuring about 5 mm in diameter, containing a single large seed at its center**.**

Application of Piperrine:(50)

- It is increase the absorption of selenium, vitamin B and β- carotene as well as other nutrients.
- It can be stimulate pancreatic and intestinal digestive enzymes, also increases biliary bile acid secretion when orally administrated.
- It prevents and minimizes diarrhea produced by oil and also reduces the intestinal fluid accumulating in mouse intestine.
- Its involvement in increasing the absorption of nutrients in the body and also novel applications like helping to fight against colon cancer.

It has also anti-inflammatory, thermogenic, growth stimulatory, anti-thyroid and chemo preventive activities.

- It is inhibition of hepatic drug metabolism; enhancing pentobarbitone induced hypnosis, bioavailability of oxyphenyl butazone, hepatoprotective activity, inhibition of lipid peroxidation during experimental inflammation, antifertility and radio protective effects.
- It has been found to inhibition of human CYP3A4, P-glycoprotein and enzymes important for metabolism and transport of xenobiotics and metabolites.

Drumstick

Scientific classification

Kingdom: Plantae

Order: Brassicales

Family: Moringaceae

Genus: *Moringa*

Species: ***M. oleifera***

fig 16: Drumstick

Moringa oleifera is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. English common names include: moringa,

drumstick tree (from the appearance of the long, slender, triangular seed-pods), horseradish tree (from the taste of the roots, which resembles horseradish), Ben tree, or benzoil tree (from the oil which is derived from the seeds). It is a fast-growing, drought-resistant tree, native to the southern foothills of the Himalayas in northwestern India, and widely cultivated in tropical and subtropical areas where its young seed pods and leaves are used as vegetables. It can also be used for water purification and hand washing, and is sometimes used in herbal medicine.

Medicinal properties:(51)

- Antihypertensive, diuretic and cholesterol lowering activities
- Antispasmodic, antiulcer and hepatoprotective activities
- Antibacterial and antifungal activities
- Antitumor and anticancer activities
- Aqueous leaf extracts regulate thyroid hormone and can be used to treat hyperthyroidism and exhibit an antioxidant effect
- *M. oleifera* leaf may be applicable as a prophylactic or therapeutic anti-HSV (Herpes simplex virus type 1) medicine.

Zinger

Scientific classification

Kingdom: Plantae

Clade: Angiosperms

Clade: Monocots

Clade: Commelinids

Family: Zingiberaceae

Genus: *Zingiber*

Species: ***Z. officinale***

Fig 17: Zinger

Zinger rhizome is knobby and fleshy that is covered in rings. This part is used in food and medicine. Rhizomes grow underground but they are not roots but stem. Laterally compressed, ovate, flattish, oblique irregularly branched. Pieces about 7-12 cm long and 1-2 cm thick and longitudinal wrinkled.

Medicinal Uses

- Ginger is good for the respiratory system
- Ginger offers substantial protection from stroke and heart attack because of its ability to prevent blood clotting.
- Ginger juice is good for upset stomach and indigestion.
- It is very effective as a cleansing agent through the bowels and kidneys and also through the skin.
- Helps to clear chest and sore throats when massaged with butter.
- It is crucial in the battle against cardiovascular disease.
- Relieves headaches and pains.
- Decoction of rhizome along with Artemisia dubia is taken as antipyretic.

CHAPTER 4

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1. MATERIALS

4.1.1 List of material used

Table 8: List of chemical used in formulation

Sl No	Ingredients	Company Name
1.	Muller Hinton agar	Hi Media Laboratories Pvt. Ltd, Mumbai, India
2.	Ciprofloxacin	Biocon Lab, Bangalore
3.	Turmeric (*Curcuma longa*)	Local market
4.	Black Piper (*Piper nigrum)*	Local market
5.	Drumstick (*Moringa oleifera*)	Local market
6.	Zinger (*Zingiber officinale*)	Local market
7.	Cow Urine Distillate	Maa Gou Product Pvt. Ltd. Banguluru
8.	Lactose	S.D. Fine Chem. Ltd, Mumbai, India
9.	Magnesium stearate	Hi Media Laboratories Pvt. Ltd, Mumbai, India
10.	Talc	S.D. Fine Chem. Ltd, Mumbai, India

4.1.2 LIST OF INSTRUMENTS

Table 9. List of Equipments

Sl No	Equipments	Model/Company
1	Electronic Balance	Citizen, India
2	Tablet compression machine	Lab press Multi punch machine
3	Tablet hardness tester	Monsanto hardness tester
4	Dissolution test apparatus	Lab India, Mumbai, India
5	Disintegration test apparatus	Campbell Electronics
6	Friability test apparatus	Roche friabilator
7	UV-Visible Spectrophotometer	Shimadzu UV"1800, Japan
8	Flourio Transformer Infrared Spectrophotometer	Nicolet thermo 380, India
9	pH meter	Consolidated Electrical Industries, Bangalore
10	U FLC	Shimadzu, Japan
12	Hot air oven	Kadavil electromechanical ind., Kerala
13	Autoclave	Thermo scientific, Bangalore
14	Water bath	Thermo scientific, Bangalore

4.2 ANALYTICAL METHOD USED IN THE DETERMINATION OF CIPROFLOXACIN.

The UV Spectrophotometric method was developed for the analysis of the drug using Shimadzu 1800 spectrophotometer.

4.2.1 Determination of λ max

Ciprofloxacin is freely soluble in water. An absorption maxima of Ciprofloxacin was determined by scanning 200-400nm using 10µg/ml concentration after correcting the baseline. The λmax of the drug was found to be 278 nm.

4.2.2 Standard Curve for Ciprofloxacin

100 mg of Ciprofloxacin was accurately weighed and dissolved in 100 ml of water to prepare first stock solution (1mg/ml). 10ml of first stock solution was taken and diluted to 100 ml with the same solvent to prepare II stock solution (100mcg/ml). The aliquots of stock solution II was further diluted with water to get 1, 2, 3, 4, 5 and 6µg/ml of the final solution. The absorbance was measured in a UV spectrophotometer at 278nm against water as blank.

4.3 Extraction of plant:

4.3.1 Preliminary preparation

Powdered roots of *Curcuma longa*, dried roots of *Zinger officinalae* and dried seeds of *Piper nigrum* were purchased from the local market of Bellur and dried at 40^0C for 4 hours in hot air oven, finely powdered and packed in self sealing plastic cover until further use. Fresh leaves of *Moringa oleifera* were collected from BG Nagara in Mandya district. Leaves were separated, dried in cool place of laboratory for 5 days, finely powdered and packed in self sealing plastic cover until further use.

4.3.2Extraction

Dried powders were extracted with 99% ethnol using a Soxhlet extractor apparatus. The 100g power was put in soxhlet thimble and put into a Soxhlet thimble tube. 1000 ml of ethanol was added to a Soxhlet flask, and then extracted at 60°C until the extract was not clear or about 8 hour. The ethanol was removed under pressure using a rotator evaporator. Then dried residue crude extract were stored in dark bottle a 4°C.

4.4 Preparation of media plate:

- Suspend 38 g muller hinton agar (Hi-media) powder in one liter of distilled or deionized water in a conical flask and sterilize at 121^{o} C (15 lbs. of pressure) for 15 minutes.
- Cool to 40-45^{o} C and pour the molten agar to a depth of ¾ of the sterilized plate in a laminar flow.
- Keep the Petri dishes horizontally until the medium completely solidifies. Turn dishes upside-down and stack them up for storage.

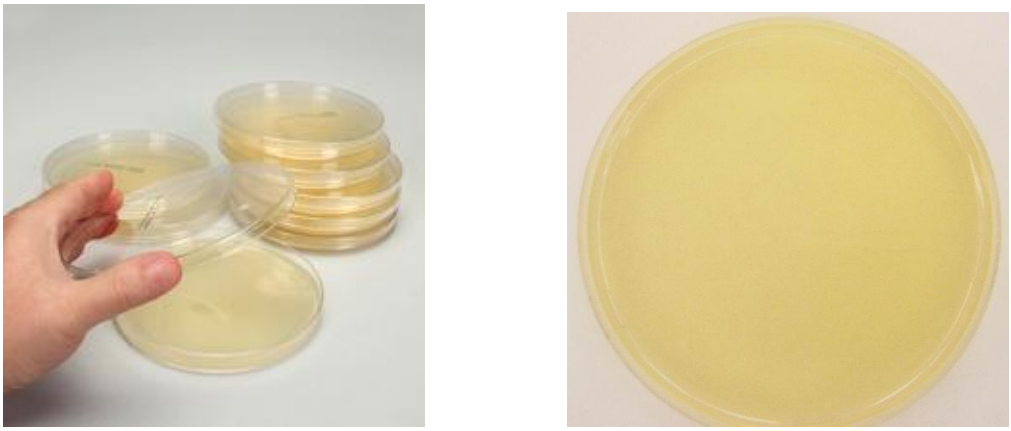


Fig18: Mueller Hinton media plate

4.5 Bacterial Strains

Strain of *E-coli*, *K. pneumonae*, *Staphylococcus aureus*, *P.aureoginosa* were used as tested organisms in all antibacterial assays. These ATCC grade organism stains were taken from

Department of Microbiology, Sri Adichunchanagiri Institute of Medical Sciences. These organisms were selected because they are among many pathogens often implicated in food borne outbreak in the world. Different strains of bacteria were streaked onto Tryptone Soy agar (TSA) to obtain pure isolated colonies, following a standard aseptic technique and the four-way streak plate inoculation .Once the isolated colonies were obtained, the bacterial strains were enumerated with Mueller Hinton Broth (MHB) for the next step of the experiment, the antimicrobial assay.

4.5.1 Antimicrobial Assay:

Prepared bacterial concentrations 1.5 x 10^6 cfu/ml of were spread on to the surface of the Mueller Hinton using sterile swabs. Sample was dissolved in 10% DMSO to make different concentration 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml. The 9mm diameter bored in sterile Mueller Hinton agar plate and added different concentration of sample in to bored accurate 100µl. Plates were incubated at 37°C for 24 hours in upright position and mean value of zone of inhibition was recorded. Measured inhibition zone were recorded as mean diameter in mm.

4.5.3 Determination of Minimum inhibitory concentration (MIC)(21)

The minimum inhibitory concentration is defined as the lowest concentration of the antimicrobial agent that results in inhibition of visible growth after incubation at 30°C for 24 hours. Bacterial concentration 1 x 10^6cfu/ml was spread by cotton swap on Mueller Hinton agar plate. This assay was performed in a 96 plate. MICs were determined for each crude extract and combination with drug in agar plate bye cup plate method. Ciprofloxacin was used as a positive control as a concentration 10 µg/ml and 10% DMSO was used as a negative control. The test was carried out in quadrate. Zone of inhibition were measured in mm after 24 h of growth.

4.6 COMPATIBILITY STUDY USING FT-IR

FT-IR Nicolet thermo 380 spectra of the prepared formulations were taken and compared with the spectrum of pure drug. The characteristic peaks of drug were checked in the formulation spectra.

4.7 FORMULATION DEVELOPMENT CIPROFLOXACIN TABLETS

Table 10. Selected excipient for prototype formulation

Sl NO	EXCIPIENT	FUNCTION
1	Lactose	Diluent
2	PVK_{30}+ CUD	Binding agent
3	Talc	Flow enhancer
4	Magnesium stearate	Lubricant

Table 11. Formulations containing & various concentrations of excipients

INGREDIENTS	WEIGHT OF ONE TABLET (MG/TAB)
Ciprofloxacin	300
Pepper	75
Turmeric	75
Zinger	75
Lactose	125
PVK_{30} pest	q .**s**
Talc	30
Mg. stearate	20
Total	700

4.8 PREPARATION OF CIPROFLOXACIN TABLETS BY WET GRANULATION METHOD

All the ingredients were passed through sieves separately and weighed. Weighed ingredients were transferred into mortar and mixed for 15 minutes. After mixing thoroughly the granules are pass through the sieve and subjected for drying. The granules were evaluated for various pre-compression parameters like bulk volume, tapped volume, bulk density, tapped density and angle of repose.

After compression they were evaluated for appearance, diameter, tablet weight, thickness, hardness, and friability, uniformity of dispersion, weight variation, content uniformity and dissolution profile. Stability studies were also carried out.

4.9 EVALUATION OF BLENDED CHARACTERISTICS OF CIPROFLOXACIN

4.9.1 Evaluation of Granules(22)(23)(24)

Angle of Repose:

The angle of repose of granules was determined by the funnel method. The accurately weighed granules were taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the granules. The granules were allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation

$$\mathbf{Tan\ \theta = \boldsymbol{h}/\boldsymbol{r}}$$

Where, ***h*** and ***r*** are the height and radius of the powder cone respectively.

Bulk Density (D_b): It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed powder into a measuring cylinder and the volume was noted. It is expressed in gm/ml and is given by

D_b= Mass powder/Volume

Tapped density (D_t): It is the ratio of total mass of powder to the tapped volume of powder. The tapped volume was measured by tapping the powder to constant volume. It is expressed in gram/ml and is given by

$$D_t = M/V_t$$

Where, **M** - Mass of the powder

V_t – Tapped volume of the powder.

Compressibility index (I) and Hausner's ratio: Carr's index and Hausner's ratio measure the propensity of granule to be compressed and the flow ability of granule. Carr's index and Hausner's ratio were calculated using following formula.

$$C.I = (D_t - D_b)100/D_t$$

Where, D_t – Tapped density of the powder

D_b – Bulk density of the powder

4.10 EVALUATION OF CIPROFLOXACIN TABLETS

The matrix tablets prepared were evaluated for the following parameters:

1. Weight variation
2. Hardness
3. Friability
4. Drug content
5. *In-vitro* Dissolution Studies
6. Stability Studies

Weight Variation Test

To study weight variation, 20 tablets of each formulation were weighed using an electronic balance and the test was performed according to the official method.

Table12 : IP standards of Uniformity of weight

Sl. No	Avg Wt of Tablet	% of Deviation
1	≤ 80 mg 10	10
2	>80mg- 250mg	7.5
3	≥250	5

Hardness and Friability

For each formulation, the hardness and friability of 6 tablets were determined using the Monsanto hardness tester (Cadmach, Ahmedabad, India) and the Roche friabilator (Campbell Electronics, Mumbai, India) respectively.

The percent friability calculated as follows

$$\%F = \frac{W_1 - W_2}{W_1} \times 100$$

Drug Content:

Five tablets were weighed and triturate, from that transfer an accurately weighed portion of the powder equivalent to about 100mg of Ciprofloxacin in a 100ml volumetric flask containing buffer solution and then concentration is measured at λ max 278 nm.

4.10 *IN-VITRO* DISSOLUTION STUDIES

The *in-vitro* dissolution studies were performed using the USP-II (Paddle) dissolution apparatus at 50 rpm. The dissolution medium consisted of 900ml of phosphate buffer pH 6.8, maintained at 37±0.50C. An aliquot (5ml) was withdrawn at specific time intervals and drug content was determined by UV-visible spectrometer at 278nm. The study was performed in triplicate.

4.10.1 KINETIC ANALYSIS OF *IN-VITRO* RELEASE RATES OF ORAL RELEASE TABLETS OF CIPROFLOXACIN

The results of *in-vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows:-

1. Zero- order Kinetic model – Cumulative % drug released versus Time.
2. First- order Kinetic model – Log cumulative % drug remaining versus Time.
3. Higuchi's model- Cumulative percent drug released versus square root of time.
4. Korsmeyer equation / Peppa's model- Log cumulative percent drug released versus log time.

Zero order kinetics:

Zero order release would be predicted by the following equation:-

$$\mathbf{A_t = A_0 - K_0 t}$$

Where,

A_t= Drug release at time't'.

A_0= Initial drug concentration

K_0=Zero-order rate constant (hr^{-1}).

When the data is plotted as cumulative % drug release versus time, if the plot is linear then the data obeys zero- order release Kinetics, with a slope equal to K^0.

First order Kinetics:

First order release would be predicted by the following equation:-

$$\textbf{Log C = log } C_0 - K_t / 2.303$$

Where,

C = Amount of drug remained at time't'.

C_o = Initial amount of drug.

K = First – order rate constant (hr^{-1}).

When the data is plotted as log cumulative % drug remaining versus time yields a straight line, indicating that the release follows first order kinetics. The constant 'K' can be obtained by multiplying 2.303 with the slope values.

Higuchi's model:

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation.

$$Q = A\,(D(2C - Cs)\,Cs.\,t\,)^{1/2}$$

Where Q is the amount of drug released in time t per unit area A, *C* is the drug initial concentration, *Cs* is the drug solubility in the matrix media and *D* is the diffusivity of the drug molecules (diffusion coefficient) in the matrix substance.

Korsmeyer equation/ Peppa's model:

To study the mechanism of drug release from the sustained – release matrix tablets of losartan potassium, the release data were also fitted to the well – known exponential equation (Korsmeyer

equation/ peppa's law equation), which is often used to describe the drug release behavior from polymeric systems.

$$M_t / M_a = Kt^n$$

Where,

M_t / M_a = the fraction of drug released at time 't', K = Constant incorporating the structural and geometrical characteristics of the drug/ polymers system, N = Diffusion exponent related to the mechanism of the release.

Above equation can be simplified by applying log on both sides,

$$\text{Log } (M_t / M_a) = \text{Log } K + n \log t$$

When the data is plotted as log of drug released versus time, yields a straight line with a slope equal to 'n' and the 'K' can be obtained from y- intercept. For Fickian release 'n' = 0.5 while for anomalous (non- Fickian) transport 'n' ranges between 0.5 and 1.0

Table 13 . Mechanism of Drug Release as per Korsmeyer Equation/ Peppa's Model

S. No	'n' value	Drug release
1.	0.45	Fickian release
2.	0.45 <n<0.89	Non- Fickian release
3.	n>0.89	Class II transport

4.11 STABILITY STUDIES

Stability of a pharmaceutical product may be defined as the capability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications throughout its shelf life.

ICH specifies the length of study and storage conditions.

Long Term testing: $25^0C \pm 2^0C$ / 60% RH ± 5% for 12 months

Accelerated Testing: $40^0C \pm 2^0C$ / 75% RH ± 5% for 6 months

4.11.1 Method

The optimized formulation was subjected for two month stability study according to ICH guidelines**.** The selected formulations were packed in aluminum foils, which were in wide mouth bottles closed tightly. They were then stored at 40ºC / 75% RH for 2 months. Then samples were evaluated for their content and *in vitro* dissolution studies.

CHAPTER 5

RESULTS

5. RESULTS

5.1 STANDARD CURVE OF CIPROFLOXACIN:

The absorbance was measured in a UV spectrophotometer at 278nm against in deionized water. The absorbances obtained table and Calibration curve was plotted.

Table 14: Spectrophotometric Data for the Estimation of Ciprofloxacin in deionozed water.

Sl No.	CONCENTRATION (µg/ml)	ABSORBANCE (278nm)
1	0	0.000
2	1	0.085
3	2	0.242
4	3	0.312
5	4	0.397
6	5	0.489

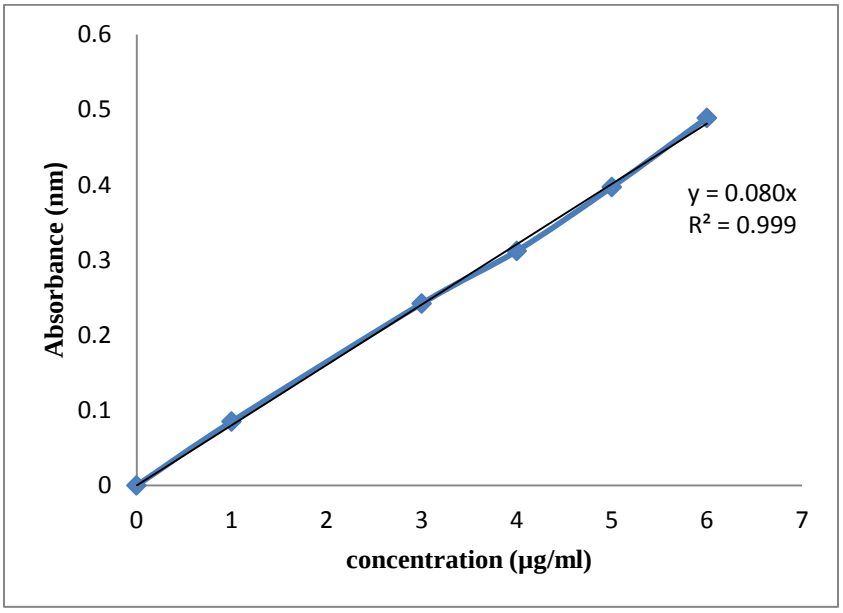


Figure 19: Standard Curve of ciprofloxacin in water

5.2 Antibacterial activity drug and sample:

Antibacterial activity was shown in different concentration and combination in Ciprofloxacin and sample. Where following symbol indicate sample and concentration:

C= Cow urine distillate

P= Pepper extract

T= Turmeric extract

Z= Zinger

1, 2, 3, 4 indicate the concentration of ciprofloxacin in 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml respectively. And **a, b, c, d** indicate the combination concentration of ciprofloxacin and sample in 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml respectively.

5.2.1 Ciprofloxacin and Cow Urine Distillate

Table15: Antibacterial activity of Ciprofloxacin and Cow Urine Distillate

	Staphyl. aureus		***E. coli***		***P. auregenosa***		***K. pneumonae***	
Sample	**ZOI (cm)**	***%* increase**	**ZOI (cm)**	***%* increase**	**ZOI (cm)**	***%* increase**	**ZOI (cm)**	***%* increase**
DMSO	0	-	0	-	0	-	0	-
Cipro	3.2	-	3	-	3.5	-	2.05	-
C1	0	-	0	-	0	-	0	-
C2	0	-	0	-	0	-	0	-
C3	0	-	0	-	0	-	0	-
C4	0	-	0	-	0	-	0	-
Ca	3.4	6.25	3.3	10	3.6	2.85	2.15	4.86
Cb	3.6	12.5	3.4	13.33	3.75	7.14	2.41	17.56
Cc	3.8	18.75	3.5	16.66	3.9	11.42	2.65	29.26
Cd	4	25	3.8	26.66	4.2	20	2.95	43.9

1, 2, 3, 4 indicate the concentration of ciprofloxacin in 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml respectively. And **a, b, c, d** indicate the combination concentration of ciprofloxacin and CUD in 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml respectively.

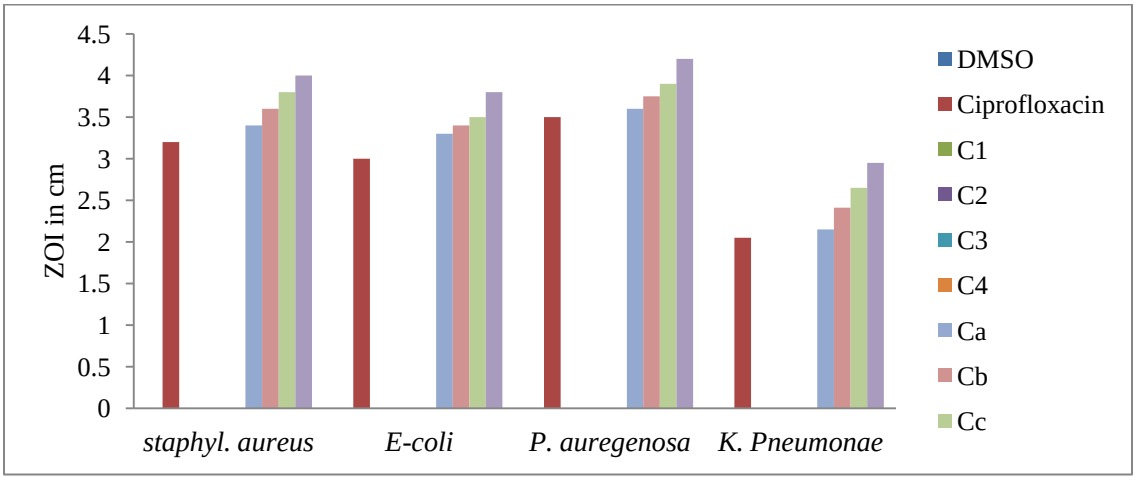


Fig 20: Antibacterial activity combination of Ciprofloxacin and CUD

5.2.2 Ciprofloxacin and Pepper

Table16: Antibacterial activity of Ciprofloxacin and pepper

Sample	*staphyl. aures*		*E-coli*		*P. auregenosa*		*K. Pneumonae*	
	ZOI (cm)	**% increase**	**ZOI (cm)**	**% increase**	**ZOI (cm)**	**% increase**	**ZOI (cm)**	**% increase**
DMSO	0	-	0	-	0	-	0	-
Cipro	3.2	-	3	-	3.5	-	2.05	-
P1	0	-	0	-	0	-	0	-
P2	0	-	0	-	0	-	0	-
P3	0	-	0	-	0	-	0	-
P4	0	-	0	-	0	-	0	-
Pa	3.5	9.37	3.4	13.33	3.9	11.42	2.3	12.2
Pb	3.7	15.62	3.6	20	4.1	17.14	2.68	30.73
Pc	3.9	21.89	3.9	30	4.43	26.57	2.98	45.37
Pd	4.4	37.5	4.3	43.33	4.52	29.14	3.2	56.1

1, 2, 3, 4 indicate the concentration of ciprofloxacin in 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml respectively. And **a, b, c, d** indicate the combination concentration of ciprofloxacin and Pepper in 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml respectively.

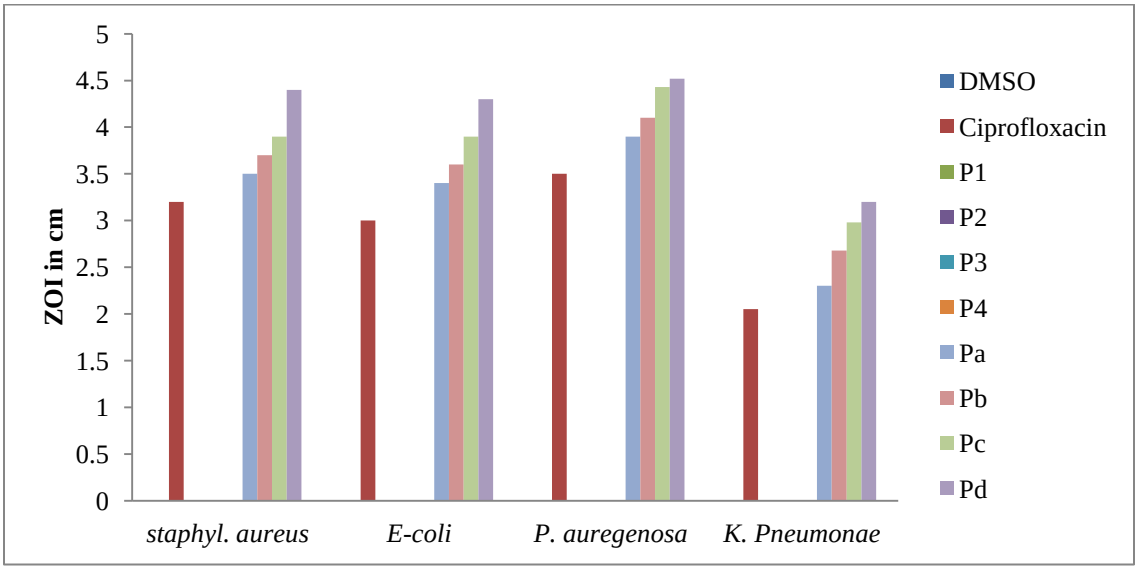


Fig 21: Antibacterial activity combination of Ciprofloxacin and pepper

Ciprofloxacin and Zinger

Table 17: Antibacterial activity of Ciprofloxacin and Zinger

Sample	*staphyl. aureus*		*E-coli*		*P. auregenosa*		*K. Pneumonae*	
	ZOI (cm)	**% increase**	**ZOI (cm)**	**% increase**	**ZOI (cm)**	**% increase**	**ZOI (cm)**	**% increase**
DMSO	0	-	0	-	0	-	0	-
Cipro	3.2	-	3	-	3.5	-	2.05	-
Z1	0	-	0	-	0	-	0	-
Z2	0	-	0	-	0	-	0	-
Z3	0	-	0	-	0	-	0	-
Z4	0	-	0	-	0	-	0	-
Za	3.3	3.13	3	0	3.62	3.42	2.22	8.3
Zb	3.4	6.25	3.1	3.33	3.75	7.14	2.35	14.63
Zc	3.5	9.58	3.2	6.67	3.89	11.14	3.64	28.78
Zd	3.7	15.63	3.4	13.33	3.92	12	3.76	34.64

1, 2, 3, 4 indicate the concentration of ciprofloxacin in 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml respectively. And **a, b, c, d** indicate the combination concentration of ciprofloxacin and Pepper in 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml respectively.

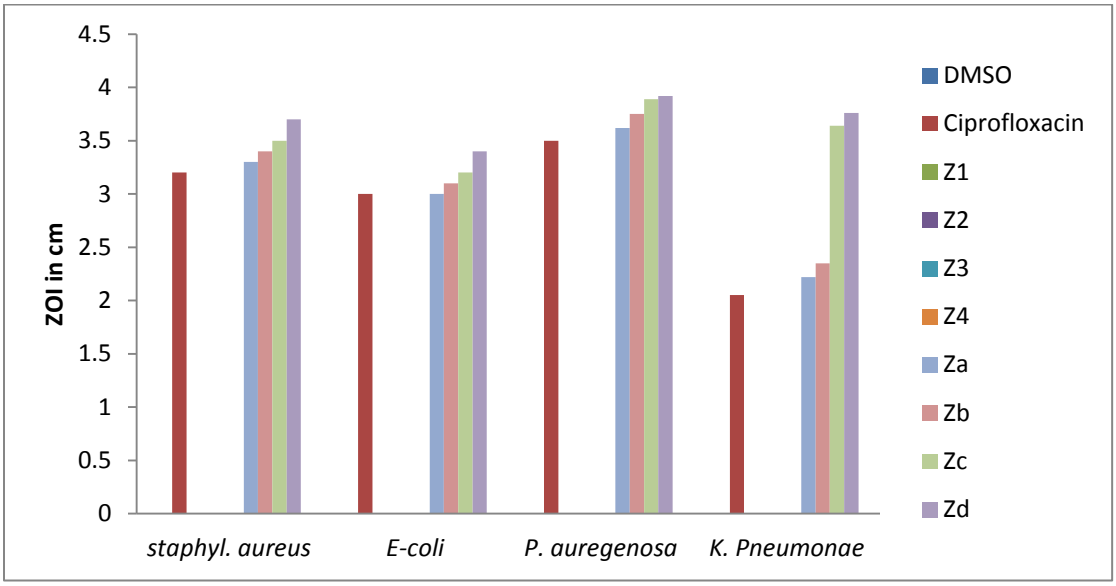


Fig 22: Antibacterial activity combination of Ciprofloxacin and Zinger

Ciprofloxacin and Turmeric

Table18: Antibacterial activity of Ciprofloxacin and Turmeric

Sample	*staphyl. aureus*		*E-coli*		*P. auregenosa*		*K. Pneumonae*	
	ZOI (cm)	**% increase**	**ZOI (cm)**	**% increase**	**ZOI (cm)**	**% increase**	**ZOI (cm)**	**% increase**
DMSO	0	-	0		0		0	
Cipro	3.2	-	3		3.5		2.05	
T1	0	-	0		0		0	
T2	0	-	0		0		0	
T3	0	-	0		0		0	
T4	0	-	0		0		0	
Ta	3.3	3.13	3.1	3.13	3.63	3.13	2.36	3.13
Tb	3.4	6.25	3.2	6.25	3.75	6.25	2.58	6.25
Tc	3.6	12.5	3.4	12.5	3.97	12.5	2.74	12.5
Td	3.8	18.75	3.6	18.75	4.1	18.75	2.92	18.75

1, 2, 3, 4 indicate the concentration of ciprofloxacin in 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml respectively. And **a, b, c, d** indicate the combination concentration of ciprofloxacin and Pepper in 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml respectively.

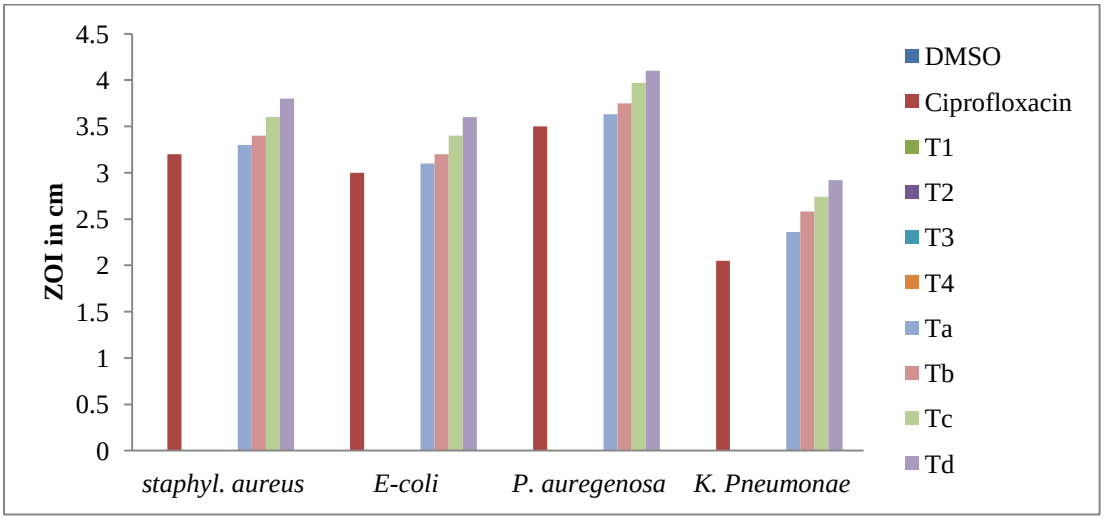
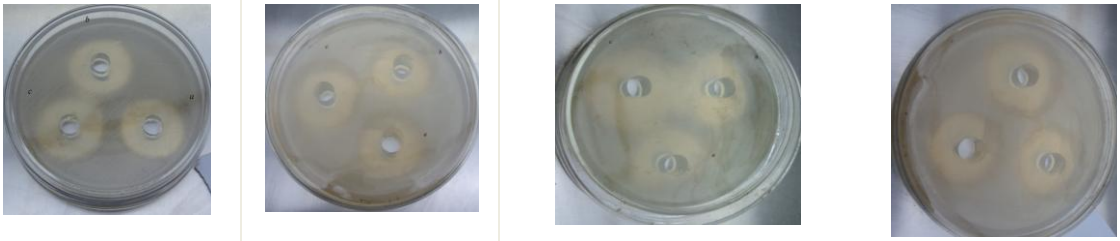


Fig 23: Antibacterial activity combination of Ciprofloxacin and Turmeric



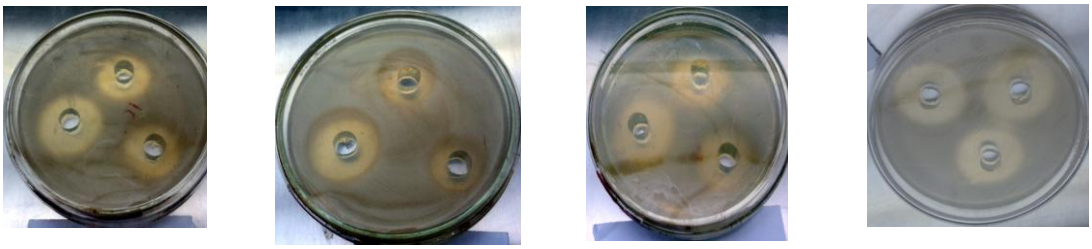
Cipro+pepper in *k.pneumonae*

Cipro+pepper in *P.auregenosa*

Cipro+pepper *in E-coli*

Cipro+pepper *in* s.aureus

Fig 24: Best antibacterial activity of combination of ciprofloxacin and pepper extract in different microorganism



Cipro+CUD in *K. pneumonae*

Cipro+ CUD in *P. auregenosa*

Cipro+CUD in *E.coli*

Cipro+CUD in *S.aureus*

Fig 25: Antibacterial activity of combination of ciprofloxacin and CUD in different microorganism

Compatibility study

Table 19: Compatibility study of drug and excipient using FTIR

Peaks (cm^{-1})	Groups	Peak assignment
3500-3450	Hydroxyl group	O-H stretching vibration, intermolecular H-bonded
3000-2950	Aromatic, cyclic enes	υ=CH and Ar-H
1750-1700	CO group of acid	C=O stretching vibration
1650-1600	Quinolines	δN-H bending vibration
1450-1400	Carbonyl group	υC-O
1300-1250	Hydroxyl group	δO-H bending vibration
1050-1000	Fluorine group	C-F stretching

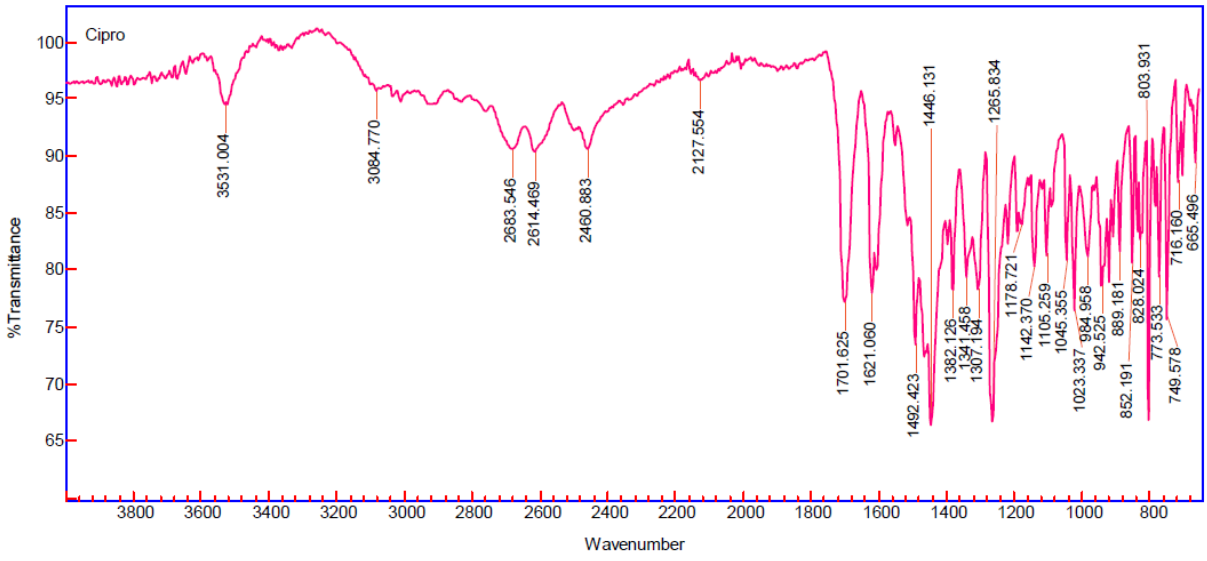


Fig 26: FT-IR spectra of Ciprofloxacin

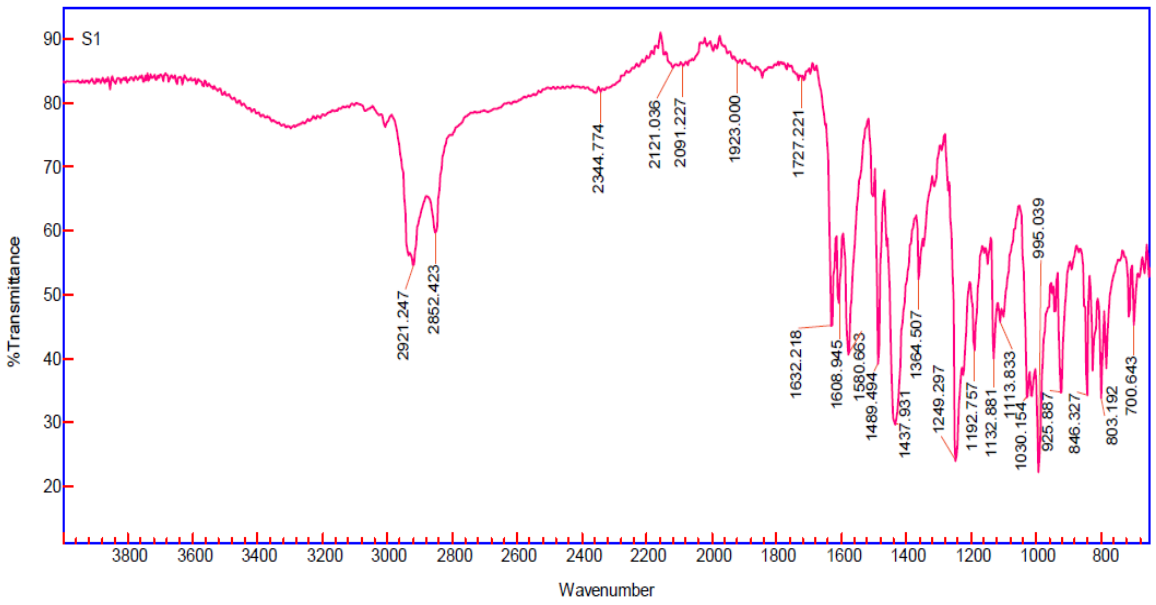


Fig 27: FT-IR spectra of pepper

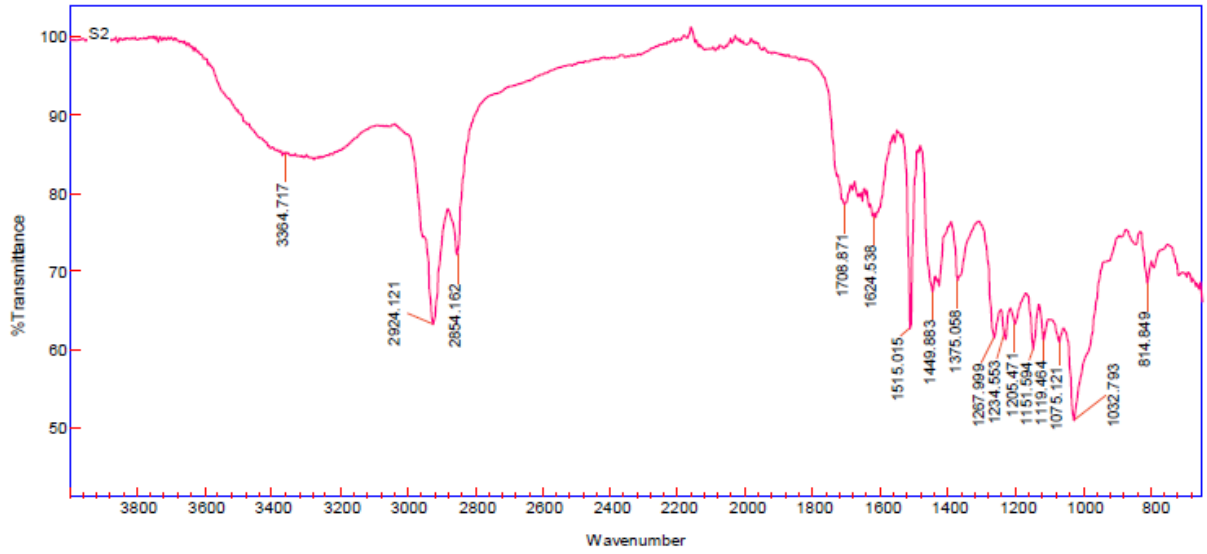


Fig 28: FT-IR spectra of zinger

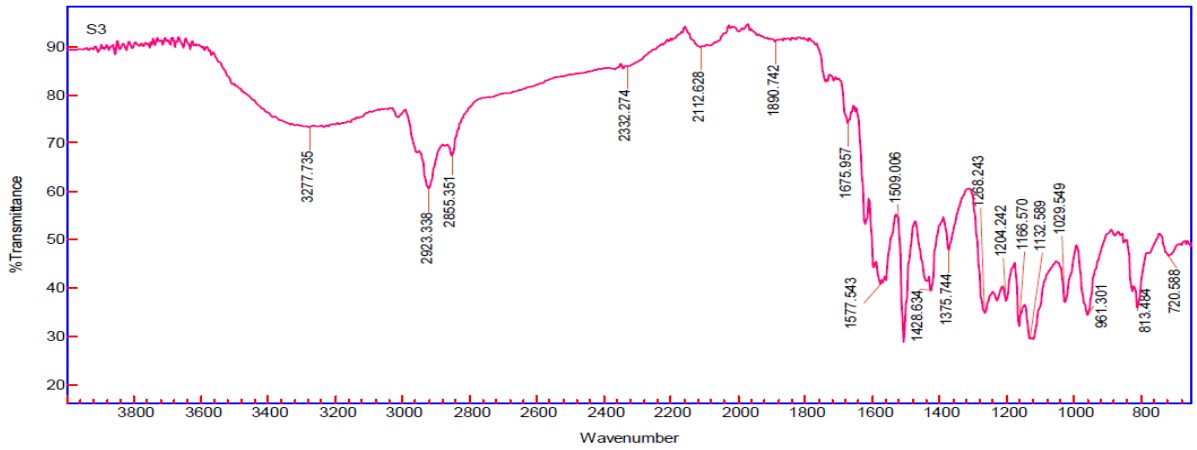


Figure 29: FT-IR spectra of turmeric

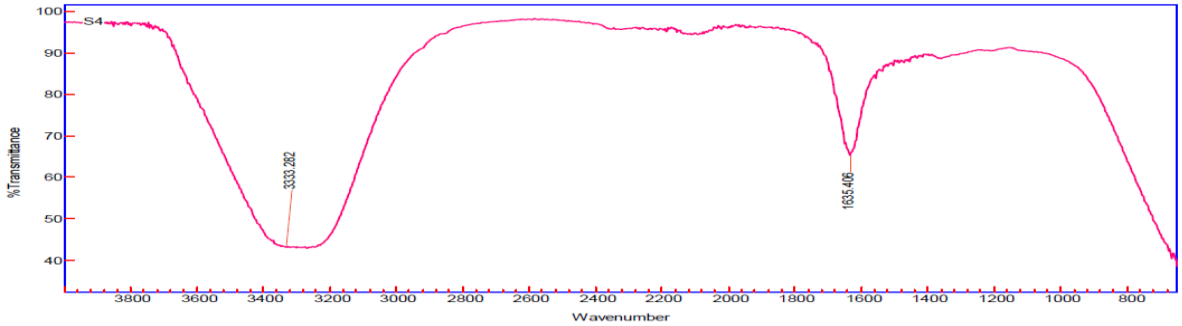


Fig 30: FT-IR spectra of cow urine distillate

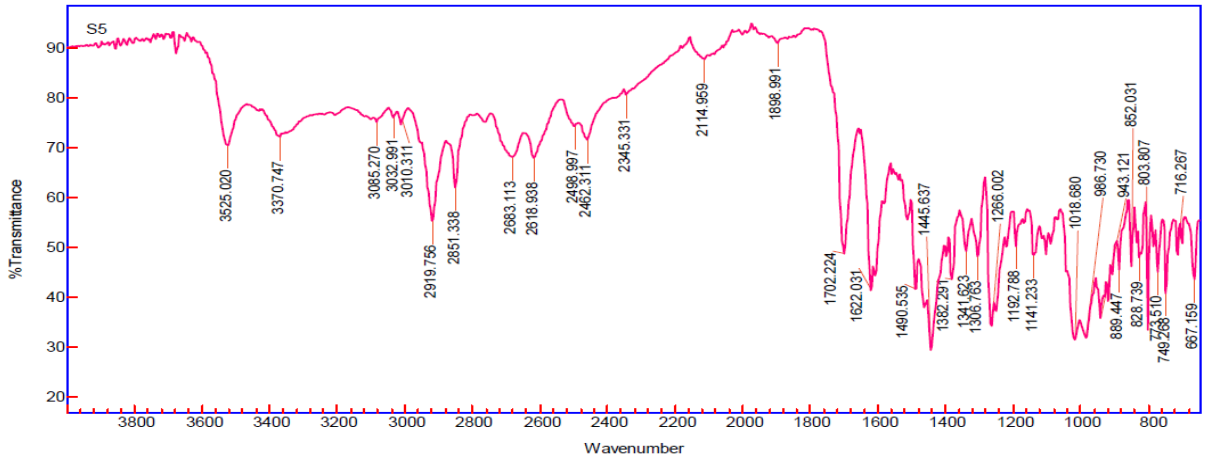


Figure 31: FT-IR spectra of combination of ciprofloxacin, pepper, zinger, turmeric and CUD

Table 20: FT-IR Characteristic peak of Pure drug (Ciprofloxacin), sample and Drug with Sample

SL. No.	Functional Group	IR Range (cm^{-1})	IR Observed Peaks					
			Pure drug	Pepper	Turmeric	Zinger	CUD	Mixture
1	O-H	3500-3400	3531	-	3277.73	3364.71	3333.29	3525.02
2	Ar-H ,υ=CH	3000-2950	2930	2921.24	2923.33	2924.12	-	2919.75
3	C=O	1750-1700	1701.62	1727.22	-	1708.87	-	1702.22
4	δN-H	1650-1600	1621.06	1632.21	1675.95	1624.53	35.4016	1622.03
5	δC-O	1450-1400	1442.42	1437.93	1428.63	1449.88	-	1445.63
6	δO-H	1300-1200	1307.19	1249.29	1268.24	1267.95	-	1266.02
7	C-F	1050-1000	1045.35	1030.15	1029.54	1075.12	-	1018.68

5.3 EVALUATION OF BLENDED CHARACTERISTICS OF CIPROFLOXACIN FORMULATION

Table 21: Pre-compression parameter results

Bulk density gm/ml (mean± sd)	0.538±0.006
Tapped density gm/ml (mean± sd)	0.622±0.011
Carr's index (mean± sd)	15.78±1.70
Haunser's ratio (mean± sd)	1.18±0.020
Angle of repose (°)	22.1±0.59

5.4. POST- COMPRESSION EVALUATION PARAMETERS

Table 22: Post- compression parameter results

Weight Variation mean± sd (g)	0.698±0.003
Hardness mean± sd (kg/cm^2)	4.20±16
Friability mean± sd (%)	0.16±0.010
Drug contain mean± sd (%)	99.37±0.70
Thickness mean± sd (mm)	2.96± 0.02
Disintegration time mean± sd (sec)	13± 0.97

5.5 *IN-VITRO* DRUG RELEASE STUDIES:

Table 23: Cumulative % of drug release

Time in minutes	% of cumulative Release
0	0
15	31.23
30	64.34
45	77.67
60	85.56
75	91.66
90	98.12

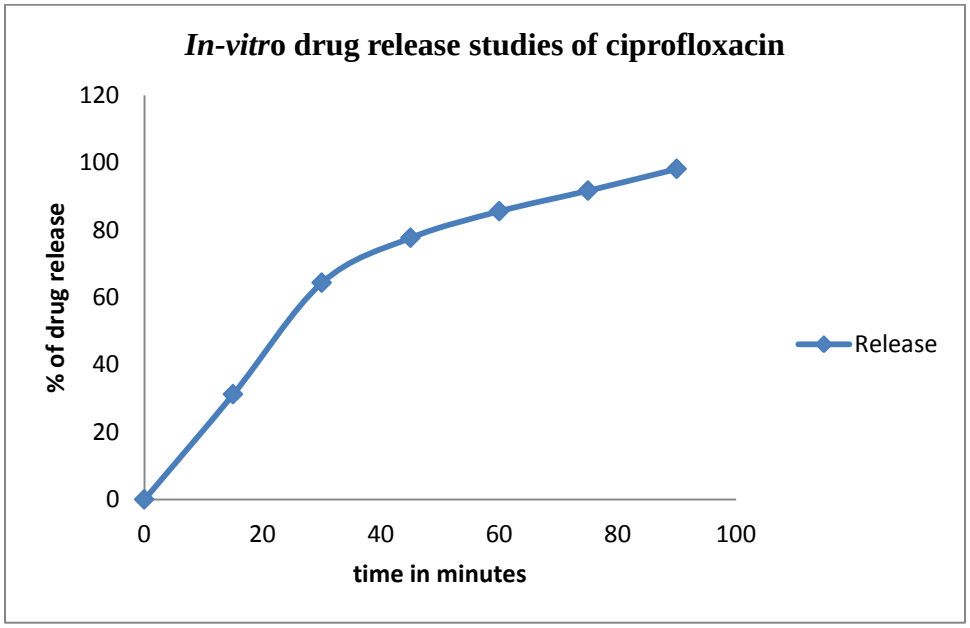


Figure 32: Cumlative % drug release of formulations

Table 24: Mathematical modelling and drug release kinetics of Ciprofloxacin

Drug release kinetics (R^2)				Release exponential (n)
Zero order	First order	Higuchi	Korsmeyer	
0.877	0.949	0.975	0.967	0.928

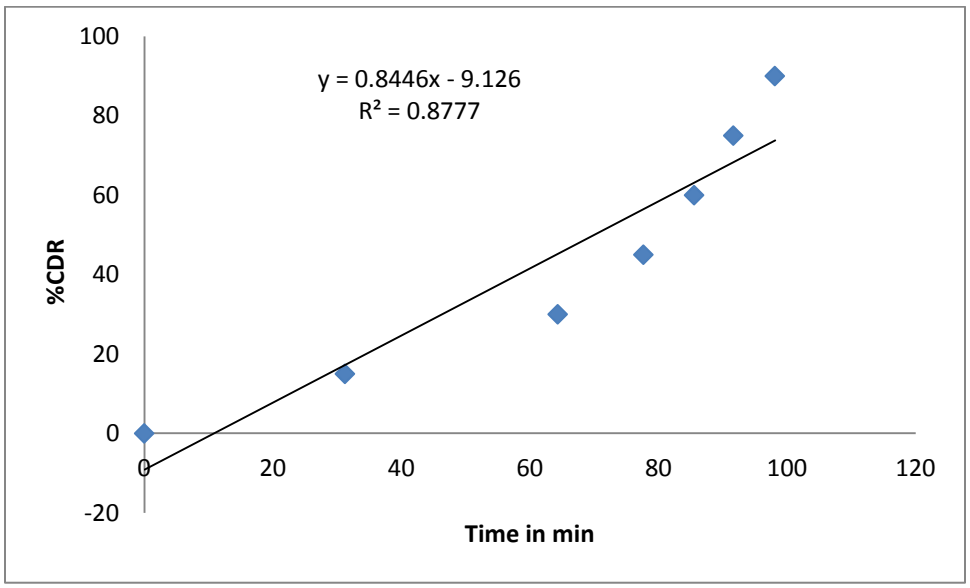


Figure 33: Zero Order release profile of formulations

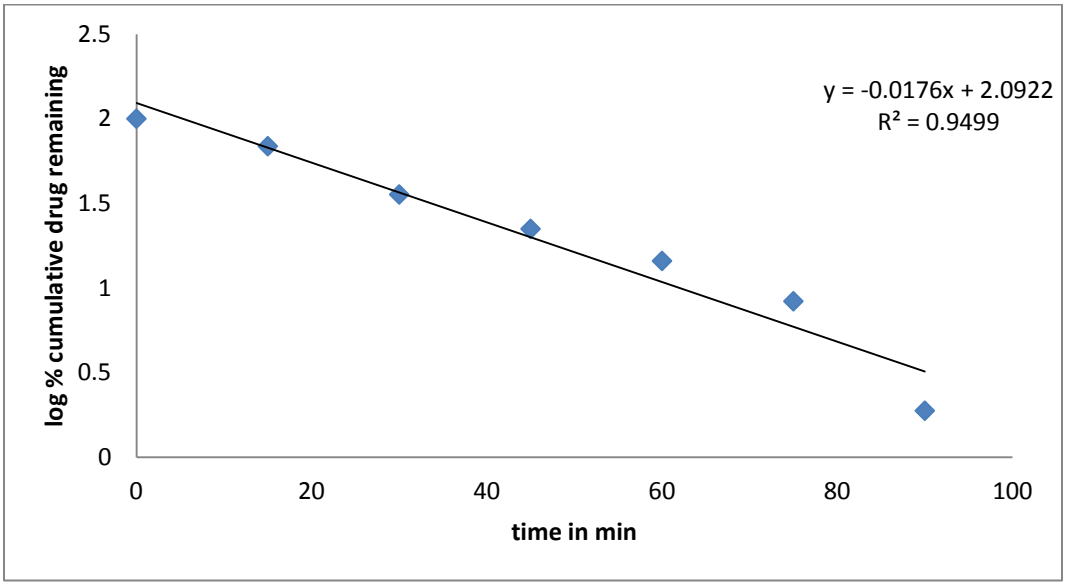


Figure 34: First Order release profile of formulations

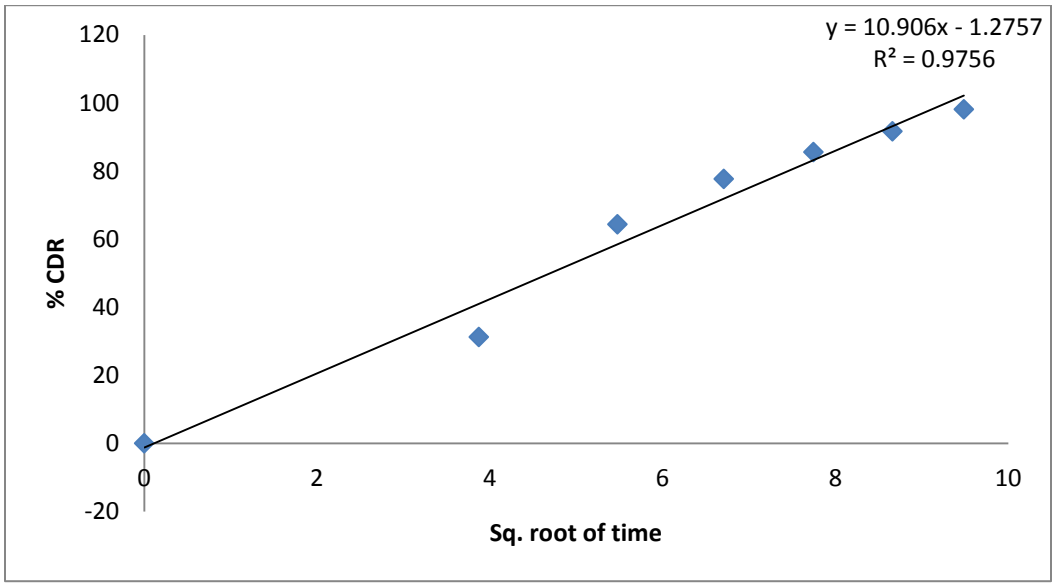


Figure 35: Higuchi release profile of formulations

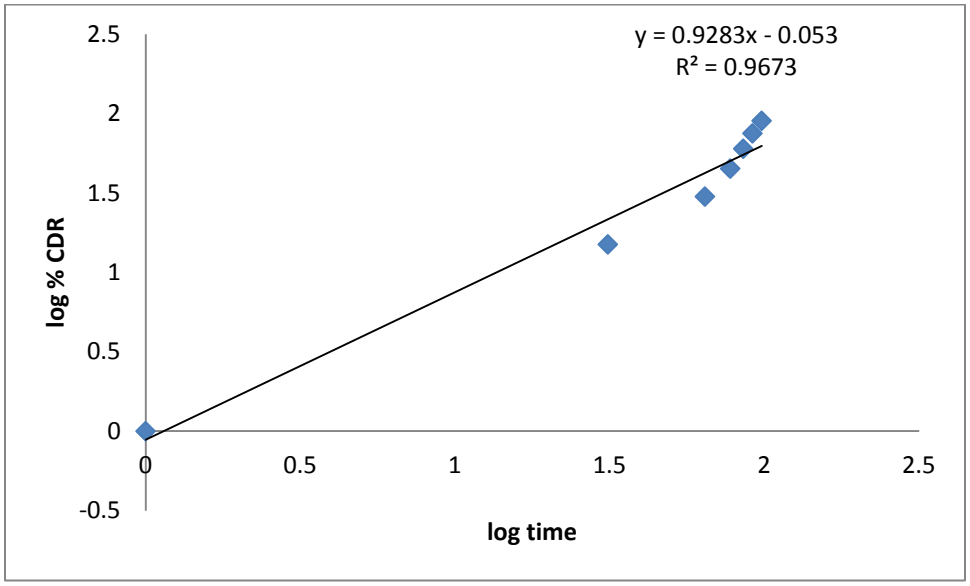


Figure 36: Korsmeyer release profile of formulations

5.7 STABILITY STUDIES:

After the stability studies the formulations were subjected to content estimation and the results shown in table

Table 25: Drug content data of stability study

S NO.	TIME IN DAY	FORMULATIONS (Content estimation in %)
1	15	99.37
2	30	98.79
3	45	98.63
4	60	98.23

After the stability studies the formulations were subjected to *in vitro* dissolution and the results shown in table

Table 26. *In-vitro* drug release of tablets stability study of formulation

Time in min	Before stability studies	After stability studies
0	0	0
15	31.23	32.01
30	64.34	64.57
45	77.67	76.74
60	85.56	85.88
75	91.66	91.89
90	98.12	98.93

CHAPTER 6

DISCUSSION

6. DISCUSSION

This project is designed with an objective to enhance the antibacterial activity of Ciprofloxacin. In this project, activity of ciprofloxacin was determined alone and in combination with selected herbal extracts. Further, dose of selected herbal extracts are optimized and formulated into tablets followed by its evaluation of properties along with stability and compatibility studies.

Ciprofloxacin was found to be water soluble and showed the absorption maxima at 278nm in water. Calibrations curve the ciprofloxacin in water showed good linearity with the regression co-efficient of 0.998. ATCC strains of *K. pneumonae, Staphylococcus aereus, Escherichia coli,* and *Pseudomonas aeruginosa* were obtained from AIMS institute. Clinical isolates of *Escherichia coli,* and *Pseudomonas aeruginosa* were also obtained as ATCC strains found resistant to Ciprofloxacin drug. Powdered roots of *Curuma longa* and *Zingibar officinale*, dried powdered seeds of *Piper nigrum,* and dried powdered leaves *of Moringa olifera,* are extracted using 99% ethanol and concentrated using rotator evaporator. Cow urine distillate is also used as a test sample. Antibacterial activity of Ciprofloxacin observed alone and in combination with four different concentrations such as 50,100,150 and 200µg/ml of extracts in 10% DMSO solution. 10% DMSO solution and ciprofloxacin 10µg/ml acts as negative and positive control. Test samples in all concentrations and negative control showed no activity however in combination with drug showed almost increased activity compared to positive control in all four organisms. The activity was found depended on the concentration of the extract. These results clearly indicated the bioenchancing activity of samples. Bioenhancing property was in the following order pepper> CUD> Turmeric> Zinger extracts. Drum stick extract did not show any bioenhacing property.

Zone of inhibition in K. *Pneumonae* was increased more than 40 % in case of Pc, Pd, Cd, and Td. Similarly zone of inhibition of *E. coli, S. aereus & P. aurigenosa* was more than 30 % in case of Pd. Above results indicated that the all the herbal extracts and CUD was quite active at higher concentration. During experimentation with ATCC strains of *E. coli* and *S. aereus* was found to be resistant to Ciprofloxacin hence Clinical isolates were obtained from Microbiology department, AIMS and continued experiment. Based on the above input, herbal extracts of pepper, turmeric, zinger and CUD were included in the tablet formulation containing 300mg of ciprofloxacin. An FTIR study was performed to find out the suitable interaction between drug – excipient in a tablet formulation. Ciprofloxacin showed characteristic peaks at 3531.004m^{-1} due to hydroxyl group O-H stretching vibration, intermolecular H-bonded, 2930.54cm^{-1} due to Aromatic, cyclic enes, 1701.62 cm^{-1} due to CO group of acid peak assignment C=O stretching vibration, at 1621.06 indicate quinolines because δN-H bending vibration, at 1492.42 indicate carbonyl group because υC-O, at 1307 .19 peak assignment by δO-H bending vibration and at 1023.33 indicate fluorine group due to C-F stretching. Moreover, same peaks were observed for the mixture of drug with different herbal extract Pepper, Turmeric, Zinger, and animal product Cow urine distillate respectively. Hence, it was found that all the herbal extract and animal product used in formulations were compatible with Ciprofloxacin.

Ciprofloxacin tablets were prepared by wet granulation method. In this method, CUD is used in place of water; Extracts of Pepper, turmeric and zinger were 75mg each; other expients include PVP-K30 (binder) lactose (diluents) magnesium stearate and talc (flow enhancers). Granules were prepared and evaluated. Flow property of the granules was found to good. The bulk density, tapped density and cars index was found to be 0.538 gm/ml, 0.622 mg/ml and 15.78 respectively.

The Hunsers ratio and angle of repose was 1.18 and 22.1^{o} respectively. Tablets were punched and post compression parameters were evaluated. The hardness of the tablet was about 4 Kg and friability was below 0.18 %. The drug content was above 98% well within the specified limit. Disintegration time was found to below 15min. *In vitro* release studies showed 90 % release in 75 min. This delay may be due to binding nature of herbal extracts.

The release kinetics of tablet was fitted into zero, first, Higuchi and Korsmeyer models where 'r' was for first order release, zero order and Higuchi's was 0.949, 0.877 and 0.975. It was understood to be predominant Higuchi's release pattern. Further, to understand the drug release mechanism, the data were fitted into Peppas exponential model $M_t/M_a=Kt^n$, where M_t/M_a is the fraction of drug released after time 't' and 'K' is kinetic constant and 'n' is release exponent which characterizes the drug transport mechanism. The values 'n' was in the 0.928. The formulation indicating Class II transport release mechanism ('n' values is n>0.89).

An accelerated stability study as per ICH norms was performed for the formulation for 60 days at, 40^{0}C/75% RH. The stability of the tablet was found be in limit and observed no change in the physical appearance, release nature and drug content.

In summary, by the above experiment, tablets with herbal extracts could be formulated with no significant drug excipient interaction and kept stable for longer time. Further clinical evidence is required to study in animals and in humans.

CHAPTER 7

CONCLUSION

8. CONCLUSION

The presence study was designed to primarily to identify the bio-enhancing property selected plant extracts in presence of ciprofloxacin as a model antibiotic ciprofloxacin on *K. pneumonae*, *staphylococcus aereus, Escherichia coli*, and *Pseudomonas aeruginosa*organisms. Secondly, to formulate the identified plant extract along with drug into tablet dosage form followed by its complete evaluation including compatibility and stability studies. The following outcomes are obtained by this project.

1. Ethanolic extracts of Pepper, Turmeric,zinger and Cow urine distillate are potential candidates to enhance the activity of ciprofloxacin. Hence these can be named as bioenhancers
2. Bioenhancing activity is concentration dependent; as concentration increases the antimicrobial activity of ciprofloxacin increases.
3. The antibacterial activity improved in the following order for extracts Pepper > CUD > Turmeric > Zinger.
4. Tablet formulation can be made effectively using the extracts and ciprofloxacin with no significant drug excipient interaction
5. Flow property of the granules, pre and post compression parameters were within the specified limits indicting that tableting process is easy.
6. *In vitro* stability of the tablets at 40^0 C / 75% RHfor two months was found to be stable with no significant change in physical and chemical properties.
7. Finally, it can be concluded that, drug can be suitably given with herbal drugs for their activity potentiation using bioenhancer

CHAPTER 8

SUMMARY

8. SUMMARY

This project is designed with an objective to enhance the antibacterial activity of Ciprofloxacin. In this project, activity of ciprofloxacin was determined alone and in combination with selected herbal extracts. Further, dose of selected herbal extracts are optimized and formulated into tablets followed by its evaluation of properties along with stability and compatibility studies. This project is summarized as below

- Calibrations curve of ciprofloxacin in water showed good linearity with the regression co-efficient of 0.998.
- Powdered roots of *Curuma longa* and *Zingibar officinale,* dried powdered seeds of *Piper nigrum,* and dried powdered leaves *of Moringa olifera,* are extracted using 99% ethanol and concentrated using rotator evaporator. Cow urine distillate is also used as a test sample.
- Antibacterial activity of Ciprofloxacin observed alone and in combination with four different concentrations such as 50,100,150 and 200µg/ml of extracts in 10% DMSO solution. 10% DMSO solution and ciprofloxacin 10µg/ml acts as negative and positive control.
- Test samples in all concentrations and negative control showed no activity
- In combination with drug showed increased activity compared to positive control in all four organisms.
- The activity was found depended on the concentration of the extract.
- Bioenhancing property was in the following order pepper> CUD> Turmeric> Zinger extracts. Drum stick extract did not show any bioenhacing property.

- Zone of inhibition in K. *Pneumonae*was increased more than 40 % in case of Pc, Pd, Cd, and Td. Zone of inhibition of *E. coli, S. aereus& P. aurigenosa*was more than 30 % in case of Pd.
- Using herbal extracts of pepper, turmeric, zinger, CUD and ciprofloxacin tablet formulation was prepared
- FTIR study confirmed no significant interaction between drug and excipients.
- Pre and post compression parameters were well within the specified limits.
- Accelerated stability study as per ICH norms at 40^0C/75% RH for 60 days indicated no significant variation in physical appearance, release nature and drug content.

In summary, by the above experiment, tablets with herbal extracts could be formulated with no significant drug excipient interaction and kept stable for longer time. Further clinical evidence is required to study in animals and in humans.

CHAPTER 9

BIBLIOGRAPHY

9. BIBLIOGHAPHY

1. Bennett P, Brown M. Clinical Pharmacology. 9th ed. London: Churchill Livingstone; 2003. 202 - 9.
2. Zafarullah M. Irrational use of antibiotics in children leads to antimicrobial drug resistance; a case report. 2014. 1–5.
3. WHO's first global report on antibiotic resistance reveals serious, worldwide threat to public health Geneva: The World Health Organization; 2014. Available from: http://www.who.int/mediacentre/news/releases/ cited 2014
4. Atal N, BediL. K. bioenhancer revolutionary concept to market. J Ayurveda Integr Med. 2010;1(2):96–9.
5. Singh G, Maurya S. Antimicrobial , antifungal and insecticidal investigations on essential oils ó An overview. Nat Prod Radiance. 2005;4(3):179–92.
6. Cowan MM. Plumbago zeylinica Linn . (Chitrak) - Review as Rasayan (Rejuvenator / Antiaging). International Journal of Research in Pharmaceutical and Biomedical Sciences. 2012;3(1):250–67. Am Socity Microbiol. 1999;12(4):564–82.
7. Thipathi KD. Essentials of Medical Pharmacology. 6th ed. New Delhi: Jaypee Brothers Medical Publishers; 2010. 500-699 .
8. Hawkey PM. The growing burden of antimicrobial resistance. J Antimicrob Chemother. 2008 Sep;62:1–9.
9. Hardman JGG& G. Sulfonamides, Trimethoprim Sulfamethoxazole, Quinolones, And Agents For Urinary Tract Infections. In: William A, Petri J, editors. The Pharmacological Basis of Therapeutics. 10th edn. NewYork: McGraw-Hill; 2006.

10. Bertram G. Katzung. Basic and Clinical Pharmacology. 7th Ed. New York: Lange Medical Books/McGraw-Hill; 1998.
11. Sibanda T, Okoh AI. The challenges of overcoming antibiotic resistance:Plant extracts as potential sources of antimicrobial and resistance modifying agents. African J Biotechnol. 2007;6(25):2886–96.
12. Frieden T. Antibiotic resistance threats. CDC. USA: Department of Health and Human Services; 2013. 22-51.
13. Benayache S, Benayache F, Benyahia S. Leaf Oils of some Eucalyptus Species Growing in Algeria. J Essent Oil Res. 2001;13:210–3.
14. Benhassaini H, Enabderrahmane K, Chi. Contribution to the assessment of the antiseptic activity of essential oils and oleoresin of Pistacia tial Atlas on some microbial sources: Candida albicans (ATC 20027), Candida albicans (ATCC 20032) and s accharomyces cerevisiae: ethnopharmacology. 2003;30:38–46.
15. Benjilali B, Elaraki-Tantawi A, Ismaili-Alaoui M and, Ayadi. A Study method antiseptic oils essntielles direct contact agar. Phyther Med Plant. 1986;20:155–67.
16. Chanda S, Rakholiya K. Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas (Ed.). 2011.
17. Adwan. G, Abu-Shanab B and AK. In vitro Interaction of Certain Antimicrobial Agents in Combination with Plant Extracts Against Multidrug-resistant Pseudomonas aeruginosa Strains. Middle-East J Sci Res. 2009;4(3):158–62.

18. Darwish R, and Aburjai A. Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on Escherichia coli. BMC Complement Altern Med. 2010;1:1–8.

19. Adwan M, Mhanna G. Synergistic Effects of Plant Extracts and Antibiotics on Staphylococcus aureus Strains Isolated from Clinical Specimens. Middle-East J Sci Res. 2008;3(3):134–9.

20. Randhawa G., Kullar S. R. Bioenhancers from mother nature and their applicability in modern medicine. Int J Appl Basic Med Res. 2011;1(1):5–10.

21. Jubie S, Nilesh P, Dhanabal P, Kalirajan R, Muruganantham N, Shanish A. European Journal of Medicinal Chemistry Synthesis , antidepressant and antimicrobial activities of some novel stearic acid analogues. Eur J Med Chem. Elsevier Masson SAS. 2012;54:931–5.

22. Harris M. Pharmaceutical Microbiology. 6th ed. Hugo B, Russell D, editors. Academic Medicine. Cardiff: Blackwell Science Ltd.1998. 3-34 .

23. Prescott LM, Harley JP, Klein DA. Microbilogy. 5th ed. Graaff KM Van De, Johnson FB, editors. USA: The McGraw−Hill Companies; 2002. 42-53 .

24. Annamalai AR, Manavalan R. Effects of “Trikatu” and its individual components and piperine on gastro intestinal tracts: trikatu: a bioavailable enhancer. Indian Drugs. 1990;27(12):595–604.

25. Johri RK, Thusu N, Khajuria A, Zutshi U. Piperine mediated changes in the permeability of rat intestinal epithelial cells. The status of γ-glutamyl transpeptidase activity, uptake of amino acids and lipid peroxidation. Biochem Pharmacol. 1992;24(7):1407–992.

26. Hayeshi R, Masimirembwa C, Mukanganyama S, Ungell AL.Hayeshi R, Masimirembwa C, Mukanganyama S. The potential inhibitory effect of antiparasitic drugs and natural products on P-glycoprotein mediated efflux. Eur J Pharm Sci. 2006;29:70–81.

27. Tsai T, Lee C, Yeh P. Effect of P-glycoprotein modulators on the pharmacokinetics of campothecin using microdialysis. Br J Pharmacol. 2001;134:1245–52.

28. Chan K, Liu X, Jiang Z, Zhou H, Wong Y, Xu H. The effects of sinomenine on intestinal absorption of paeoniflorin by the everted rat gut sac model. J Ethnopharmacol. J Ethnopharmacol. 2006;20:425–32.

29. Bajad S, Bedi K, Singla A, Johri R. Piperine inhibits gastric emptying and gastrointestinal transit in rats and mice. Planta Med. 2001;67:176–9.

30. Kumar A, Khan I, Koul S, Koul J, Taneja S, Aki I et al. Novel structural analogs of piperine as inhibitors of NorA efflux pump of Staphylococcus aureus. J Antimicrob Chemother. 2008;61:1270–6.

31. Khajuria A, Thusu N, Zutshi U. Piperine modulates permeability characteristics of intestine by inducing alterations in membrane dynamics:Influence on brush border membrane fluidity , ultrastructure and enzyme kinetics. Phytomedicine. 2002;9:224–31.

32. Kang M, Cho J, Shim B, Kim D, Lee J. Kang MJ, Cho JY, Shim BH, Kim DK, Lee J. Bioavailability enhancing activities of natural compounds from medicinal plants. J Med Plants Res. 2009;3:1204–11.

33. Esposito G. of intestinal epithelial cell: Permeability of border and basolateral membranes. In: Csaky T, editor. Pharmacology of Intestinal Permeation. Berlin Heidelberg: Springer-Verlag; 1984. 283–308.

34. Breedveld P, Beijnen JH, Schellens JHM. Use of Pglycoprotein and BCRP inhibitors to improve oral bioavailability and CNS penetration of anticancer drugs. Trends Pharmacol Sci. 2006;27(1):17–24.

35. Kang MJ, Cho JY, Shim BH, Kim DK, Lee J. Bioavailability enhancing activities of natural compounds from medicinal plants. J Med Plant Res. 2009;3(13):1204–11.

36. Tatiraju D V, Bagade VB, Karambelkar PJ, Jadhav VM, Kadam V. Natural Bioenhancers. J P P. 2013;2(3):55–60.

37. Tablet. The International Pharmacopoeia [Internet]. 4th ed. WHO; 2013. Available from: http://apps.who.int/phint/en/p/docf/ cited 6/3/2015

38. Ghanshyam B, Mody D, K S, Awale MM, Patel HB, Modi CM, et al. A Comprehensive Review on Pharmacotherapeutics of Herbal Bioenhancers. Sci World J. 2012;1–33.

39. Singha R, Devib S, Patela J, Patela U. Review Article Indian Herbal Bioenhancers. PhconNet. 2009;3(5):80–2.

40. Rakshitha M, Nandini K, Martis R, Shruthi J, Tr PK. Bioenhancing effect of Cow urine distillate on antibacterial activity of Capsicum frutescens (L .) var . longum fruit. Res Rev Biomed Biotechnol. 2010;1:64–7.

41. Randhawa GK. Cow urine distillate as bioenhancer. J Ayurveda Integr Med. 2010 Oct;1(4):240–1.

42. Jantarat C. bioavailability enhancement techniques of herbal medicine: a case example of curcumin. Int J Pharma Sci. 2013;5(1):493–500.

43. Pattanaik S, Hota D, Prabhakar S, Kharbanda P PP. Pharmacokinetic interaction of single dose of piperine with steady-state carbamazepine in epilepsy patient. Phytother Res. 2009;23(9):1281–6.

44. Kasibhatta R NM. Influence of piperine on the pharmacokinetics of nevirapine under fasting conditions: a randomised, crossover, placebo-controlled study. Drugs R D. 2007;8(6):383–91.

45. Mekala P, Arivuchelvan A. Bioenhancer for Animal Health and Production:a Review. nato-are. 2012;1–5.

46. Drabu S, Khatri S, Babu S, Lohani P. Use of Herbal Bioenhancers to Increase the Bioavailability of Drugs. Res J Pharm Biol Chem Sci. 2011;2(4):107–19.

47. Basalious EB, Shawky N, Badr-eldin SM. SNEDDS containing bioenhancers for improvement of dissolution and oral absorption of lacidipine . I: Development and optimization. Int J Pharm. Elsevier B.V.; 2010;391(1-2):203–11.

48. Thanou M, Verhoef JC, Junginger HE. Oral drug absorption enhancement by chitosan and its derivatives. Adv Drug Deliv Rev. 2001 Nov 5;52(2):117–26.

49. Singh A, Deep A. Piperine:A Bioenhancer. Int J Pharm Technol. 2011;1(1):1–5.

50. Naidu MUR. Influence of Piperine on the Pharmacokinetics of Nevirapine under Fasting Conditions. Drugs R D. 2007;8(6):383–91.

51. Kesarwani K, Gupta R. Bioavailability enhancers of herbal origin: An overview. Asian Pac J Trop Biomed. 2013;3(4):253–66.

52. kiran RS, Bhargava VK GS. effect of trikatu on the pharmacokinetic profile of indomethacin in rabbits. indian J Pharmacol. 1999;31:160–1.

53. Singh M, Varshneya C, Telanga RS SA. effect of Trikatu pretreatment on the pharmacokinetics of pefloxacin administered orally in mountain Gaddi goats. J Vet Sci. 2001;80(10):1302–5.

54. Atal CK, Dubey RK SJ. Biochemical Basis of Enhanced Drug Bioavailability by Piperine: Evidence that Piperine Is a Potent Inhibitor of Drug Metabolism. JPET. 1985;232:258–62.

55. Surabhi K, Swarnalatha S, Preethi H, Kekuda TP, Mukunda S. Cow urine distillate as a Bioenhancer of antibacterial activity of Polyalthia longifolia Thw fruit pericarp. Res Rev Biomed Biotechnol. 2011;2(4):18–20.

56. Santos I, Fawaz F, Lagueny AM, Bonini F. Improvement of norfloxacin oral bioavailability by EDTA and sodium caprate. Int J Pharm. 2003;260:1–4.

57. Yan Y, Hwan D, Ho J, Soon C, Gon H. Enhanced oral bioavailability of docetaxel in rats by four consecutive days of pre-treatment with curcumin. Int J Pharm. Elsevier B.V.; 2010;399(1-2):116–20.

58. Junginger HE. Trimethylated chitosan as polymeric absorption enhancer for improved peroral delivery of peptide drugs. Eur J Pharm Biopharm. 2004;58:225–35.

59. Acharya SG, Momin AH, Gajjar AV. Review of Piperine as a Bio-enhancer. Am J Pharmtech Res. 2012;2(2):32–44.

60. Patil UK, Singh A, Chakraborty AK. Role of Piperine As A Bioavailability Enhancer. Int J Recent Adv Pharm Res. 2011;4(1):16–23.

61. Vance-Bryan K, Guay DRP, Rotschafer JC. Clinical Pharmacokinetics of Ciprofloxacin. Clin Pharmacokinet. 2012;19(6):434–61.

62. Davis R, Markham A, Balfour J. Ciprofloxacin: an updated review of its pharmacology, therapeutic efficacy and tolerability. Drugs. 1996;51:1019–74.

63. Crump B, Wise R, Dent J. Pharmacokinetics and tissue penetration of ciprofloxacin. Antimicrob Agents Chemother. 1983;24:784–6.

64. Lettieri J, Rogge M, Kaiser L, Echols R, Heller A. Pharmacokinetic profile of ciprofloxacin after single intravenous and oral doses. Antimicrob Agents Chemother. 1992;36:993–6.

65. ciprofloxacin (Cipro) [Internet]. eMed Expert; Available from: http://www.emedexpert.com/facts/ciprofloxacin-facts.shtml 11/02/2015

66. Information on Cipro (Ciprofloxacin Hydrochloride) [Internet]. US Food and Drug Administration; 2001. Available from: http://www.fda.gov/Drugs/EmergencyPreparedness/BioterrorismandDrugPreparedness/ucm130711.htm 11/02/2015

67. Stuart MC, Kouimtzi M, Hill SR, editors. WHO Model Formulary. 2008.

68. Dimethyl sulfoxide [Internet]. Wikipedia; Available from: http://en.wikipedia.org/wiki/Dimethyl_sulfoxide#Applications 13/02/2015

69. Rowe RC, Sheskey PJ, Owen SC. Dimethyl Sulfoxide. In: Rowe raymond C, Sheskey PJ, Owen SC, editors. Handbook of Pharmaceutical excipient. 2006.

70. Polyvenyl pyrrolidone [Internet]. [cited 2015 Mar 19]. Available from: http://en.wikipedia.org/wiki/Polyvinylpyrrolidone

71. Jinjiang L, Yongmei W. Lubricants in Pharmaceutical Solid Dosage Forms. Lubricants. 2014;2:21–43.

72. Rowe RC, Sheskey PJ, Owen SC. Talc. Handbook of Pharmaceutical excipient. 6th ed. USA: Pharmaceutical Press Publications division of the Royal Pharmaceutical Society and the American Pharmacists Association; 2006.767–8.

73. Rowe RC, Sheskey PJ, Owen SC. Lactose. Handbook of Pharmaceutical excipients. 6th ed. London: Pharmaceutical Press and American Pharmacists Association; 2006. 385–6.

74. Holt J, Krieg N, Sneath P, Staley J, Williams. Bergey's MUnual of Determinative Bacteriology. 9th ed. Willian R, Henary, editors. Maryland: lippincot William And Wilkins; 1994.

75. Freeman-Cook L, Cook K freeman-. Staphylococccus Areuus infection. Koelhoffer T, Reger B, Dzani K, editors. USA: Chelsea House; 2006. 10-21.

76. Tsai S-S, Huang J-C, Chen S-T, Sun J-H, Wang C-C, Lin S-F, et al. Characteristics of Klebsiella pneumoniae bacteremia in community-acquired and nosocomial infections in diabetic patients. Chang Gung Med J. 2010;33:532–9.

77. Stock I, Wiedemann B. Natural antibiotic susceptibility of Klebsiella pneumoniae, K. oxytoca, K. planticola, K. ornithinolytica and K. terrigena strains. J Med Microbiol. 2001;50(5):396–406.

78. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Williams and Wilkins, editor. Baltimore; 1985;1.

79. Murray PR, Baron JH, Pfaller MA, Jorgensen JH, Yolken R. Manual of Clinical Microbiology. 8th ed. Washington D.C.: American Society for Microbiology; 2003.

80. Dehydrated Culture Media (MUELLER-HINTON AGAR). Thermo scientific oxoid microbioloty product; Available from: http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0337&c=UK&lang=EN 13/ 02/2015

81. Wood, G. L. and JA. Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of clinical microbiology. 6th ed. Washington D.C.: American Society for Microbiology,; 1995. p. 1327–51.

82. Distilled Cow Urine. Vedic Cow Product; Available from: http://vediccowproducts.com/30-distilled-cow-urine.html 13/02/2015

83. Go Ark (Distilled Cow Urine). VG Product; Available from: http://www.vedicgiftshop.com/product/cow-urine-ark/ 13/02/2015

84. Gomata Ark (Medicinal Distilled Cow Urine). Matchless-Gifts.com.; Available from: http://www.matchless-gifts.com/store/products/Gomata-Ark-(Medicinal-Distilled-Cow-Urine).html 13/02/2015

85. Vasavirama K, Upender M. Piperine:a valuable alkaloid from piper species. Int J Pharm Pharm Sci. 2014;6(4).

86. Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: A Food Plant with Multiple Medicinal Uses. Phyther Res. 2006;25:17–25.

87. Mallam A, Angothu S, Gurajala S, Khuddus GA. Antimicrobial Activity of Sarcostemma Acidum Voigt. (Apocynaceae) Stem. Int J Biol Pharm Res. 2012;3(6):752–7.

88. Chandra D, Maurya JK, Singh AP, Mishra P, Pradesh U. fast dissolving tablet with pierine. Int J Univers Pharm Life Sci. 2013;3:82–106.

89. Moses Prabhu, Subramanian L., Palanichamy S. JS and T a. T. Formulation and evaluation of ciprofloxacin controlled release matrix tablets. Der Pharm Lett. 2010;2(2):237–43.

90. Timilsina P, Josh V, Dhakal P, Sachin A, Mohammad GA. Formulation and Evaluation of Diclofenac Sodium Dual Type Mini Tablets for Extended Action. Americian J Pharm Res. 2014;4(5):721–35.