Evaluation on anti microbial potential of root extracts *Plumbago zeylanica* L against human intestinal microflora

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Abstract
There is increasing interest both in the industry and in the scientific research for aromatic and medicinal plants because of their potential application. The ethanol root extracts of *Plumbago zeylanica* L showed significant antimicrobial activity against *E.coli*, *Staphylococcus aureus* and *H.pylori* recorded. These properties were mainly due to active principle found in the plant root which plays a significant role in pharmaceuticals industry. The isolated compounds were screened for their antimicrobial potential against wide spectra of organism, these properties are mainly due to many active constituents including flavonoids, terpenoids, alkaloids etc and the alkaloids are natural nitrogen containing secondary metabolites mostly derived from amino acids and found in about 20% of plants.

Plumbagin is a naturally occurring naphthoquinone isolated from roots of *PLUMBAGO ZEYLANICA.L*. Plumbagin exhibited relatively specific antimicrobial activity specific antiulcer activity which is traditionally used in the treatment of peptic ulcer towards *H.pylori*. These properties are mainly due to active substances Plumbagin from the ethanol root extract of *Plumbago zeylanica* confirmed its significant antibacterial activity against human intestinal pathogen, which is a deep gastrointestinal erosion disorder that involves the entire mucosal thickness and can even penetrate the muscular mucosa. There has been considerable pharmacological research into the antiulcer activity of these compounds. It has been shown that the presence of infection by *Helicobacter pylori* is strongly associated with gastric cancer and peptic ulceration. These results suggest the naphthoquinone plumbagin as a promising antimicrobial agent had potent remedy from plant origin will be a great advancement in bacterial infection therapies.

Key words: Antimicrobial activity, *H.pylori*, Phytochemical analysis, *PLUMBAGO ZEYLANICA.L*

Introduction:
Pharmacological evaluation of traditionally known medicinal herbs for obtaining standardized extracts suitable for therapies (with several active components) and for identification of pharmacologically active constituents are important goals of drug discovery. A major problem arises from the fact that newly discovered compounds might occur in rare or endemic plant species, thus limiting the re-supply for drug development. Consequently, the pharmaceutical industry primarily seeks for new active lead structures, which can be remodeled afterwards, using advance technologies like molecular modeling or combinatorial chemistry and biochemistry respectively. A most characteristic feature of plants is their capacity to synthesize an enormous variety of new molecular weight compounds called secondary metabolites. Secondary metabolites produced by plants constitute a source of bioactive substances and nowadays the scientific interest has increased due to the search for new drugs from plant origin.

The plant species *Plumbago zeylanica* (known vernacularly as Chitraka, Chitramulamu, Tellachitramulamu, Agnichela, Agnimala or by its trade or popular names of “Lead wort-white flowered” and “Ceylon Lead wort”) of the Plumbaginaceae, is distributed as a weed in throughout the tropical and subtropical countries of the world. The family Plumbaginaceae consists of 10 genera and 280 species. The genus *Plumbago* includes 3 species, namely *Plumbago indica* L. (*P. rosea* L.) *P. capensis* L., and *P. zeylanica* L., which are distributed in several parts of India. Among these species *Plumbago zeylanica* grows all districts of plains in Andhra Pradesh, common, wild or in cultivation due to its more therapeutic uses.

*Plumbago zeylanica* belongs to *Plumbaginaceae* family, is a well known herb grown all over India. Several medicinal properties have been attributed to the plant in the traditional system of ayurvedic medicine. The chemical profile of the genus is marked by the presence of naphthoquinones, flavonoids and terpenoids [1]. Plumbagin (2-methyl-5-hydroxy-1,4 naphthoquinone ) is a naturally occurring yellow pigments, produced by members of plumbaginaceae accumulated mostly in roots [2]. The roots of the plants were posses to be used as antimicrobial properties. The present study was undertaken to evaluate the antimicrobial activity of the Plumbagin isolated from root of *Plumbago zeylanica* against human intestinal bacteria including *Helicobacter pylori*, a peptic ulcer disease causing agent. Biological activities of crude plant extracts from different *Plumbago*
species as well as of Plumbagin have been studies. Plumbagin showed anticancer [3]. Leishmanicidal [4] and bactericidal [5] activities were being also effective against insects [6, 7].

Peptic ulcer disease is a deep gastrointestinal erosion disorder that involves the entire mucosal thickness and can even penetrate the mucosa. Numerous natural products have been evaluated as therapeutics for the treatment of a variety of diseases [8]. For decades it was believed that gastrointestinal ulcers were caused by the excessive secretion of gastric acid, but many patients presenting such ulcerations had normal acid secretion rates [9]. Then, researchers reported that peptic ulcers were been caused by an imbalance between the aggressive factors and a number of known defense mechanism. Exogenous aggressive factors such as smoke, anti-inflammatory drugs, alcohol, stress, fatty foods. Helicobacter pylori infections triggered tissue necrosis through mucosal ischemia, free radicals generation and cessation of nutrient delivery, hydrochloric acid together mucosa such as the intercellular junctions, local blood flow, mucus/bicarbonate secretion and cellular growth [10]. In recent years, a large advance in chemical and pharmacological studies has contributed to the knowledge about new therapeutically active compounds obtained from the natural products [11]. These compounds can be used directly as leads for the development of new medicines the quality of life in long lasting diseases [12]. However, the incorrect use of the natural products offers dangers to society, so it is important to identify the active compounds, linking its structure with the biological activity and report the correct manner to use them with regards to dose, route of administration and frequency of use [13].

The natural active compounds classes or secondary metabolites as alkaloids, flavonoids, terpenoids, tannins and others have attracted researchers to investigate their chemical toxicological and pharmacological features. The alkaloids represent a group of natural products that had a major impact throughout history on the economic, medical, political and social affairs of humans. They are a diverse group of low molecular weight nitrogen containing compounds derived mostly from amino acids [14]. Several alkaloids and other natural compounds have complex activities and it is necessary to analyze pharmacological activities in the general tissues, linking the structure with the activity presented. It is common to find pharmacological results where a single experimental model generalizes a biological answer, but these can’t be accepted because all the pathologies in question are also complex and it is necessary to investigate specific experimental model [15]. Pharmacological testing, modifying, derivatising and research on these natural products represent a major strategy for discovering and developing new drugs [16].

The root and leaves of Plumbago zeylanica contain the naphthoquinone plumbagin. Other compounds that have been isolated are mainly plumbagin-derivatives, biplumbagin-derivatives and coumarins. Plumbagin possesses several pharmacological activities, e.g. antimicrobial, antiplasmodial, anticancer and antifertility actions. It is also a powerful irritant. In small doses, it is a sudorific and stimulates the central nervous system; large doses may cause death from respiratory failure and paralysis [17]. Plumbagin showed anti-implantation and abortifacient activities in rats and produced testicular lesions and testis-weight reduction in dogs. Because of its toxicity, the use of plumbagin in traditional medicine is a dangerous practice, and casualties have been recorded. In low concentrations, plumbagin has an antimitotic activity comparable to that of colchicine. In larger doses, it also has nucleotoxic and cytotoxic effects. Plumbagin significantly suppresses growth of several tumour cell lines in vitro and in vivo in mice, especially in combination with gamma-irradiation [18].

The microorganisms used for detecting antimicrobial activity were chosen for the following reasons: the bacterium Staphylococcus aureus was used due to its clinical relevance as a major cause of hospital acquired infections of surgical wounds and infections associated with indwelling medical devices. Besides, S. aureus rapidly develops resistance to many antimicrobial agents. [19]. The primary route by which humans acquire infection is by consumption of contaminated animal origin food. Escherichia coli is the best-known member of the normal microbiota of the human intestine and a versatile gastrointestinal pathogen. The varieties of E. coli that cause diarrhea are classified into named pathotypes, including enterotoxigenic, enteroinvasive, enteropathogenic, and enterohemorrhagic E. coli. Individual strains of each pathotype possess a distinct set of virulence-associated characteristics that determine the clinical, pathological and epidemiological features of the diseases they cause [20]. The ability of bacteria to deceive any kind of conventional therapy has become apparent and pathogens resistant to one or more antibiotics are emerging and spreading worldwide. Resistant pathogens lead to higher expenditure on treatments due to extended stay in hospitals and expensive medicines. There is an urgent need for a sustainable supply of new, potential and safer antibacterial drugs having no cross-resistance to currently used antibiotics.

Medicinal plants represent a rich source of antimicrobial in different countries and are a source of many potent and powerful drugs [21]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. In the present studies focused to identify the active compound found in root of Plumbago zeylanica and its therapeutic uses.
Materials and Methods:

Collection of Plant Material:
The Roots of Plumbago zeylanica were collected at the campus of Government Siddha Medical College, Palayamkottai, Tamil Nadu. A voucher of this plant was deposited at this college of Herbarium under the number 2210. The fresh plant root were collected properly washed in tap water, rinsed in sterile distilled water and then air dried in the hot air oven to remove moisture and air dried on sterile blotter under shade.

Solvent Extraction:
The dried roots were powered with the help of warming blender 10 g of shade dried power was filled in the thimble and extracted successively with 70% ethanol solvent in soxhlet extractor for 48h. The solvent extracts were concentrated under pressure and preserved at 5°C in airtight bottle until further use.

Phytochemical screening
Qualitative assay of ethanol root extract of Plumbago zeylanica, for the presence of plant phytoconstituents such as flavonoids, phytosterol, alkaloids, glycosides, tannins and saponins was carried out following standard procedure methods as described by [22].

Thin layer chromatography (TLC)
TLC is performed using silica gel 60 F254 percolated on alumna sheets. The metabolites were applied point wise as different spots on TLC plates and must be eluted with different solvent system. The plate was viewed under ultra violet (UV) lamp at 254 nm. For further clarity the plates were derivatised, using PUNCAL-D solution

(A solution of Cerisulphate (1.6 g) and Ammonium hepta molybdate (21.6 g) Conc. Sulphuric acid (50 ml) in 450 ml of water. Spraying the reagent on TLC plate followed by drying and heating did derivitisation at 130°C in a hot air oven. Blue colored spots appear indicates the presence of organic molecules.

Bacterial strains, stocks and growth in vitro
Five bacterial strains namely, Bacillus subtilis, Staphylococcus aureus (Gram-positive), Pseudomonas aeruginosa (Gram-negative), Proteus vulgaris and Escherichia coli (Gram-negative) were used to assess the antibacterial activity of Plumbago zeylanica root extract. These bacterial strains were obtained from the Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), New Delhi, India and the microbiologist of the department confirmed the identity based on microscopic examination, Gram’s character and biochemical test profile. Bacterial stocks were maintained and stored as 1 ml aliquots at -80°C in LB broth for all the five bacterial strains. Bacterial stocks were revived from -80°C and grown in Luria Bertani (LB) broth for all the five bacterial strains. All cultures were grown overnight at 37°C ± 0.5°C, pH 7.4 in a shaker incubator (190-220 rpm). Their sensitivity to the reference drug standard Amikacin (Sigma-Aldrich, New Delhi, India) was also checked. All selected bacteria were pre-cultured in LB broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A 610 nm).

Identification and culture methods of H. pylori
Helicobacter pylori is a spiral bacterium observed in the gastric mucosa of animals and human. Colonization of the gastric mucosa by H pylori is an important factor in the pathogenesis of gastritis, duodenal ulcer and gastric carcinoma. Gastric antral and duodenal biopsy specimens were obtained from patients exhibiting clinical symptoms of peptic ulcer diseases. Biopsy was taken by a professional gastroenterologist after endoscope examination. To prevent contamination, endoscope and biopsy forceps were carefully cleansed with 2% gluteraldehyde solution before and after the procedure. The biopsy specimen was transported to the laboratory in a transport medium (sterile brain heart infusion agar) in sterile screw cap tubes or in eppendorf tubes. Biopsies were immediately transported to the laboratory in an ice pack. In the laboratory, the biopsy specimens were impregnated into sterile modified brain heart infusion agar plates. Plates were incubated under microaerophilic condition in a candle jar apparatus for 48 – 72 hours at room temperature. After the completion of incubation period bacterial outgrowth from the biopsy specimen were streaked for purity on modified brain heart infusion medium plates and the pure culture was stored in respective slants. One loopful of the pure culture of H. pylori human isolate was inoculated into sterile brain heart infusion broth and incubated overnight. This was subjected to various identification procedures. The bacterial cultures isolated from human biopsy specimen were pure cultured and examined microscopically for Gram’s stain reaction and motility.

Growth on Triphenyl Tetrazolium Chloride 0.04% (TTC):
A loopful of fresh test culture was inoculated on blood agar plate which was incorporated with 0.04% TTC. Plates were incubated under microaerophilic condition for 24 – 48 hours. Bacterial growth was observed.

Human intestinal micro flora culture was maintained properly. Specific Media was prepared aseptically. To investigate antimicrobial activity in vitro of Plumbagin Isolated from Plumbago zeylanica, Biopsy Specimen
was used as clinical sample for culturing H. Pylori. The human isolates of H. Pylori isolated and identified in this study was used as inoculum. The culture was maintained in Brain heart infusion broth and was used in microbial assay

Anti microbial activity:
The ethanol root extracts of Plumbago zeylanica were tested by the disc diffusion method [23]. Different concentration of the extracts (100 µg ml⁻¹) was prepared by reconstituting with ethanol. The test microorganisms were seeded in to respective medium by spread plate method 10µl (10⁶ cells/ml) with the 24h culture of bacteria growth in nutrient broth. After solidification the filter paper discs (5mm in diameter) impregnated with the extracts were placed on test organism-seeded plants. Standard Plumbagin (1mg/1ml benzene) used as positive control and ethanol solvent (100 µg ml⁻¹) was used as negative control. The antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm. The active compound possess antimicrobial potential was identified by TLC and confirmed by using HPLC.

High performance liquid chromatography (HPLC)
To check the purity of isolated compound [23] and crude extract of dichloromethane, High resolution HPLC was performed using Shimadzu LC-10AT up chromatograph provided with isocratic pump and UV visible detector. The crude dichloromethane extracts were filtered through 2 µm-membrane filters and used for analysis. Column of C18 ODS, Gemini 5 µ, 110A of dimensions 250 x 4.5 mm with mobile phase 70:30:1 (methanol :water : acetic acid), was used at flow rate of 0.5 ml / min. The detection wavelength was 339 nm and injection volume was 20 µl and flow rate 0.9 ml / min , range 0.0100 AUFS

Results and Discussion:
Plumbago zeylanica, a rambling subscendent perennial herb or under shrub with green branches, stems somewhat woody, spreading, terate, striate, glabous. Leaves alternate, ovate or oblong, petiole narrow, amplexicaul at the base and often dialted into stipule like auricles. Flowers white, in axillary and terminal elongated spikes, bisexual. Calyx densely covered with stalked, sticky glands. Corolla white, very slender, tubular. Stameus 5, free. Ovary superior, 5-connate, one celled, ovule one, basal. Roots are light yellow coloured when fresh, reddish brown when dry, found in the form of tauge pieces, straight unbranched or slightly branched with or without secondary roots, with uniform and smooth texture, strong and characteristic odour with acrid and bitter taste [Figure 1].

Results obtained in the present study revealed that plant root extracts possess potential antibacterial activity against the tested organism such as Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Helicobacter pylori. Figure 2 showed the zone of inhibition data of examined microorganisms, The highest antimicrobial activity of 30 mm H.pylori and Staphylococcus aureus and least activity recorded in Escherichia coli measured 15 mm. Root extract of Plumbago zeylanica exhibit highest activity against Staphylococcus aureus and Proteins vulgaris (27 mm) and lowest in Pseudomonas aeruginosa (18mm).

Plumbagin (10 µg /disc) did not interfere with the growth of lactic acid producing bacteria Lactobacillus acidophilus. But Chloramphenicol (10 µg/disc.) which affected the normal growth of Lactobacillus acidophilus. Plumbagin does not affect Lactidophilus in contrast Chloramphenicol has moderate effect on growth of it. In order to determine the minimum inhibitory concentration of Plumbagin specific concentration was prepared by means of a twofold serial dilution technique in an enriched broth medium. For Staphylococcus aureus the MIC was 1.95 µg/ml whereas in Pseudomonas aeruginosa it was 0.97µg/ml. The MIC of combination drug (Streptomycin and Plumbagin) against E.coli was 31.25 µg./ml. The minimum inhibitory concentration of Plumbagin is compared with standard Amikacin.

Sample extract 1ml was passed on column chromatography and isocratic elution technique was followed to elute yellow pigment. The compound was identified by Thin Layer chromatography where Retardation factor value [Rf value] of both standard plumbagin and extract were same [plumbagin standard 0.79 & plumbagin extract 0.78]. Since the Rf value of both was same, the compound plumbagin from the extract was identified by compared with its standard. The purity of the compound was analyzed by subjecting the compound to High performance Liquid chromatography (Figure 3 & 4). Under identical condition, the retention time of standard plumbagin and sample plumbagin and was 5.37 & 5.71 respectively. This was shown in fig 3 & 4. Presence of additional peaks in sample plumbagin revealed presence of impurities.

The percentage of plumbagin in the extract was calculated.

<table>
<thead>
<tr>
<th>Weight of sample injected</th>
<th>0.02 mg</th>
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<tbody>
<tr>
<td>Weight of standard injected</td>
<td>0.02 mg</td>
</tr>
<tr>
<td>Area of the peak of sample</td>
<td>241269771</td>
</tr>
<tr>
<td>Area of the peak of standard</td>
<td>367602385</td>
</tr>
</tbody>
</table>
% of plumbagin in the extract = \frac{\text{Area of sample} \times \text{Weight of standard}}{\text{Area of standard} \times \text{Weight of sample}} \times \text{Purity of std}

\begin{align*}
&= \frac{0.02 \times 241269771}{0.02 \times 367602385} \times 93.69 \\
&= 61.49\%
\end{align*}

The isolated Plumbagin from root of *Plumbago zeylanica* was tested (zone of inhibition) against various intestinal bacteria and the results are shown in Table 1. Plumbagin has greater effect on *Staphylococcus aureus* and *Proteus vulgaris* whereas no effect against *E.coli*. (Table 1). The effect of Plumbagin on *Staphylococcus aureus* and *Proteus vulgaris* were high but no effect on *Escherichia coli* due to its resistance against Plumbagin. *Pseudomonas aeruginosa* had moderate antibacterial activity. No growth was observed when *Escherichia coli* was inoculated into the antibiotic (Streptomycin) medium, due to development of resistance in some of the cells. However, the growth was completely prevented when *E.coli* was grown in the medium containing antibiotic and Plumbagin (Streptomycin 10 µg. and Plumbagin 10 µg/disc.) together. The inhibition zone is shown in Table 2. Broad spectrum antibiotic Amikacin10 µg./disc used as control.

**CHEMICAL IDENTIFICATION**

- **Registry Number**: 481-42-5
- **Chemical Abstracts Service Name**: 1,4-Naphthalenedione, 5-hydroxy-2, Methane -(9CI)
- **Synonyms and Trade Names**: 5-Hydroxy-2-methyl -1-1-4 Naphthoquinone; 2-methyl-Juglone; plumbagin;Plumbagone.
- **Structural Class**: Bicyclic;naphthoquinone.

**Structure, Molecular Formula and Molecular Weight:**

![Structure of Plumbagin](image)

- **C_{11}H_{8}O_{3}**  
  **Mol. Wt**: 188.18

**Chemical and Physical Properties:**

- **Description**: Yellow needles (Merck, 1997)
- **Melting Point**: 78-79°C (Merck, 1997)
- **Solubility**: Slightly soluble in hot water; soluble in alcohol, Acetone, chloroform, benzene, and acetic acid
- **Reactivity**: Highly toxic, corrosive (Sigma Aldrich,1999)

Technical Products and Impurities: Plumbagin is available at a purity of 95+% from TCI America (1998) and 99+% from Across Organics 1997).

Traditionally, Plumbagin from *Plumbago Zeylanica* has been used to treat many diseases, some of them caused by bacteria. Plumbagin could be the main constituent from root bark responsible for its activity. The results showed that Plumbagin exhibited relatively specific antibacterial activity against various intestinal bacteria *S.aureus*, *P.aeruginosa* and *P.vulgaris*. However it was ineffective against Gram negative bacteria *E.coli* demonstrating the specificity of Plumbagin activity. The growth of *E.coli* was completely prevented when *E.coli* was grown in the medium containing streptomycin and plumbagin together [24]. The results obtained for *Lactobacillus acidophilus* were interesting. According to [25], plumbagin, a naturally occurring selective growth inhibiting agent could be useful as new preventive agents against various diseases caused by harmful
intestinal bacteria. No growth inhibition was observed against L. acidophilus at 10mcg/disc. While 10mcg/disc of Chloramphenicol showed moderate growth inhibition L. acidophilus.

All the bacterial strains used in this work demonstrated susceptibility to the root extract of Plumbago Zeylanica. Root extract gave the highest zone of inhibition 40mm in diameter. The observation that 38mm has good inhibition against H. pylori tends to prove worthy remedy to the problem of drug resistance against these pathogen which are already known to be resistant to the most of the standard antibiotics (Amikacin). The results of these investigation showed that plant extracts of Plumbago Zeylanica possess appreciable and potential antimicrobial activity against commonly encountered microorganisms in humans. Grayish brown colour colonies were observed on a sterile blood agar medium containing 0.04% Triphenyl Tetrazolium Chloride (TTC) (Figure 5). After the isolation of H. pylori from the biopsy specimen zone of inhibition of Plumbagin against H. pylori was recorded. The Plumbagin effects correlated with the effect of known standard cephalothin 30µg/disc and the minimum inhibitory concentration of Plumbagin against H. pylori was 0.97µg/ml. H. pylori is a helix-shaped bacterium which can inhabit the stomach mucosa. The infection route is thought to be the oral infection. The infection rate of H. pylori tends to be higher in developing countries and lower in developed countries.

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. Antimicrobial agents are often categorized according to their principal mechanism of action. Acquired resistance genes may enable a bacterium to produce enzymes that destroy the antibacterial drug, to express efflux systems that prevent the drug from reaching its intracellular target, to modify the drug’s target site, or to produce an alternative metabolic pathway that bypasses the action of the drug. Bacterial cell killing action by the diversity of structure within antimicrobial peptides results in an essential, innate defense system for both simple and complex organisms [26]. Although the antimicrobial peptides have unique structural properties, they can be simply categorized by their killing action. Antimicrobial peptides typically use one of the following killing mechanisms[27] cell membrane interference, cell wall synthesis interference, protein synthesis inhibition, protein inhibition and nucleic acid inhibition.

Plumbago zeylanica is very popular throughout Africa and Asia as a remedy for skin diseases, infections and intestinal worms, especially leprosy, scabies, ringworm, dermatitis, acne, sores, ulcers of the leg, haemorrhoids and hookworm. All parts of the plant are used, but the root is considered to have the highest activity. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. In vitro antibacterial activity observations have helped in identifying the active principle responsible for such activity and in the developing drugs for the therapeutic use in human beings. However, ethanol extract was subsequently fractioned and monitored by bioassay leading to the isolation of active fraction by further phytochemical analysis [28]. Further, isolated active principle compound plumbagin from Plumbago zeylanica root extracts revealed antibacterial activity against intestinal pathogen. Which found to be inhibits the severity of gastric ulcer induced by Helicobacter pylori. It is speculated that the extract was able to inhibit electrolyte permeability or the intestine due to plumbagin. [29] studied that plumbagin had highest inhibitory effect against Helicobacter pylori. The result revealed that isolated plumbagin from Plumbago zeylanica (root bark) has also the same effect and Plumbagin could be considered as a promising antimicrobial agent for gastro intestinal diseases including peptic ulcer disease[30]. The results of present investigation clearly indicate that the antibacterial activity vary with the species of the plants and plant material used in ayurveda which could be of considerable interest to the development of new drugs.

Antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infections. The active component has a significant role in interfere with growth and metabolism of microorganisms in a negative manner and was quantified by determining the minimum inhibitory concentration and minimum bactericidal activity [31]. In recent years drug resistance to several human pathogenic bacteria has been reported world over for various diseases. The situation may become more alarming due to indiscriminate use of antibiotics for a different length of time [32]. Thus there is a need for newer antimicrobial compounds having broad spectrum activity. The natural products from medicinal plants which can either the growth of disease and may have no host cells are the potential candidate for the systemic studies on the development of antimicrobial drugs to replace antibiotics or other drugs for which microbes have developed resistance [33]. The study of synergistic interaction of active phytochemicals with antibiotics is required to be done. This may help in exploiting infectious disease caused by multi drug resistant microbes.

Conclusion:
The Infectious diseases are the leading causes of death worldwide. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multi drug resistance pathogens. Many infectious diseases have been known to treat with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts provides unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Plumbagin is a naturally occurring naphthoquinone isolated from roots of Plumbago zeylanica. The plant was
collected at the campus of Government Siddha Medical College, Palayamkottai, TamilNadu used as a traditional medicine for the treatment of several diseases. The antimicrobial activity of plumbagin was evaluated using the zone of inhibition method. The compound exhibited relatively specific activity against bacteria and the minimum inhibitory concentration test showed the growth inhibition of *Staphylococcus aureus*, *Bacillus subtilis*, and *Helicobacter pylori.* These results suggest the naphthoquinone plumbagin as a promising antimicrobial agent. Plumbagin showed an interesting activity against the both Gram-positive and Gram-negative bacteria and a very high inhibitory activity against these microbes. These results indicate the naphthoquinone plumbagin as a potential antibiotic to be considered in this moment of great spread of infectious diseases. The spectacular research in medicinal field is progressing from system biology and employing nano systems biotechnology towards individual medication may help to controlling diseases in human and increase the human life span.

**References:**


Legends:

**Figure 1a** Morphology of *Plumbago zeylanica* is a flowering herb belonging to the Plumbaginaceae family and commonly known as White Leadwort and vernacular name as Kodivelli. This herb is found throughout India.

**Figure 1b**: Ethanol root extract of *Plumbago zeylanica* separated by using TLC and identified active compound as Plumbagin compared with standard (Plumbagin Practical Grade p.7262) Solvent used for separation were Benzene: n-Hexane 1:1 ($\frac{V}{V}$) after the development of TLC plates a yellow spot was visualized as Plumbagin.
Ethanol root extract of *Plumbago zeylanica* was investigated for antimicrobial activity at 100µg/ml concentration by using disc diffusion method against human pathogen such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Lactobacillus acidophilus* and *Helicobacter pylori*. After incubation for 24 hrs, the zone of inhibition was compared with standard antibiotics Streptomycin (10µg/disc) served as positive controls for antimicrobial activity. Filter discs impregnated with 10µl of distilled water were used as negative control solvent control disc was also placed with the test, positive and negative control.
Figure 3 & 4: HPLC of Standard Plumbagin and Sample Root extract of *P. zeylanica*
Legend: HPLC identification for Ethanol root extracts of *Plumbago zeylanica* showed similarity in the recorded (Fig 3 Standard and Fig 4 Plant extract) when compared with standard Plumbagin that confirm the presence of Plumbagin in the root extracts of sample.
Figure 5: *Helicobacter pylori* growth on Triphenyl tetrazolium chloride test

**Legends:**

Figure d: Showed the growth of *H. pylori* on Triphenyl tetrazolium chloride agar medium.

Figure e: The growth-inhibitory zone (halo) which shows inhibition of *H. pylori* proliferation was recognized and it was showed that *Plumbago zeylanica* extract has the antibacterial activity against *H. pylori*.
Figure 6: Comparative analysis of antimicrobial activity of root extracts of *Plumbago zeylanica*

**Legends:**

Antimicrobial activity of ethanol root extracts ((100 µg ml⁻¹) and standard (10 µg/ml) against bacterial sp tested by disc diffusion assay. After incubation, all dishes were observed for microbial inhibition. Streptomycin sulphate (10 µg/ml) used as positive control and methanol solvent (100 µg/ml) used as negative control. The antibacterial assay plates were incubated at 37°C for 24 hours. The diameters of the inhibition zone were measured in mm.

Blue color box indicates Standard drug
Red color box indicates *Plumbago zeylanica* root extract