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Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria

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ABSTRACT

The methanol, ethanol and aqueous extracts of seven medicinal plants were evaluated for activity against medically important bacteria such as Staphylococcus sp., Escherichia coli, Klebsiella sp., Pseudomonas sp. The invitro antimicrobial activity was performed by agar well diffusion method and disc diffusion method. antimicrobial activity was performed by agar well diffusion and disc diffusion method. The ethanolic and aqueous extracts showed minimum antimicrobial activity when compared to methanolic extracts. The methanolic extract Phyllanthus niruri (stone breaker) showed the maximum activity against Staphylococcus sp. The use of plant extracts with known antimicrobial properties, can be of great significance in therapeutic treatments.

Keywords: Medicinal plants, ethanol, methanol and aqueous extracts, human pathogens, Antimicrobial activity.

INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Now a days, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. [1]

The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tanning.

The screening of plant products for antimicrobial activity have shown that the higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants [2].

Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 4,00,000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities. [3]

Plants and plant based medicaments are the basis of many of the modern pharmaceuticals we use today for our various oilments. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of much intractable disease. [4]

Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season climate and particular growth phase. Leaf is one of the highest accumulated plant part of such compounds and people are generally preferred it for therapeutic, purposes some of the active compounds inhibit the growth of disease causing microbes either singly or in combination. [5]

Medicinal plants have been tested for biological, antimicrobial and hypoglycemic activity. They have also tested for antiulcerogenic, antihelminthic, hepatoprotective, analgesic, antipyretic, antileishmania and insecticidal activities. [6]
Some plants such as, *Ocinum gratissimum* and *Eugenia uniflora* have been reported to be rich in volatile oils. It which contain up to 75% thymol which has antimicrobial effect and mainly used in the treatment of diarrhea and ear infection in human beings, besides it also has antimicrobial properties against *Staphylococcus* sp., *Escherichia coli* and *Shigella* sp. [7]

Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care. The primary benefits of using plant-derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. [8]

A lot of supplementary treatment strategies have been tried. Current social trends in health care show a definite movement towards the use of natural remedies like medicinal plants and away from chemotherapectic regimens [9]. Hence in the present study, an attempt has been made with the antimicrobial activity of extracts of certain selected medicinal plants on some human pathogenic bacteria.

MATERIALS AND METHODS

Collection of medicinal plants

There are seven medicinal plants (*Aloe vera, Phyllanthus emblica, Phyllanthus niruri, Cynodon dactylon, Murrya koenigii, Lawsonia inermis, Adha-thoda vasica*) have been used in the present study and they were collected from Ayurvedic medical shop at Nagercoil, Tamilnadu, India.

Processing of medicinal plants

The whole plant or parts of plants were used to prepare extracts for the study. The plants collected were washed with water to remove the soil and dust particles. Then they were dried in thoroughly shaded place, and blended to form a fine powder and stored in airtight containers.

Human pathogenic bacterial species

The human pathogenic bacteria such as *Staphylococcus* sp., *Klebsiella* sp., *Pseudmonas* sp., *Escherichia coli* were obtained from Vivek Laboratory, Nagercoil, Tamilnadu and were maintained in Nutrient agar slant at 4^{o}C for experimental studies.

Preparation of plant extract

The ethanolic, methanolic and aqueous extracts of al the seven medicinal plants were prepared by dissolving plants were prepared by dissolving 10gm of fine powder of each medicinal plants separately in 50 ml of ethanol, methanol and water respectively. The contents were kept in orbitary shaker for 48 h. Then the extract was filtered and it is dried in hot air oven at 40^{o}C. Then the extract was stored under refrigeration at 4C for further studies.

Preparation of sterile disc

Whatman's No.3 filter paper was punched into 5 mm disc form and they sterilized, each sterile disc was incorporated individually with 20 - 60l of extracts using micropipette. Precautions were taken to prevent the flow of the solvent extract from the discs to the outer surface. The condensed extracts were applied in small quantities on discs and they were allowed to dry in air. After sometimes another doses of extracts were applied on discs. Then they were stored at 4^{o}C.

Assay of antimicrobial activity using Disc diffusion method

The 20 ml of sterilized Muller Hinton Agar was poured into sterile petriplates, after solidification, 100 µl of fresh culture of human pathogens were swabbed on the respective plates. The discs were kept over the agar plates using sterile forceps at various concentrations (20, 30, 40, 50 and 60 µl).

The plates were incubated for 24 hours at 37C. After incubation the diameter of inhibitory zones formed around each discs were measured (mm) recorded.

Assay of Antimicrobial activity using Agar well diffusion method:
The 20 ml of sterilized Muller Hinton Agar was poured into sterile petriplate, after solidification, 100 µl of fresh culture of human pathogens were swabbed on the respective plates. The wells were punched over the agar plates using sterile gel puncher at various concentration (20, 30, 40, 50 and 60) of each plant extract were added to the wells. The plates were incubated for 24 hours at 37C. [10] After incubation the diameter of inhibitory zones formed around each discs were measured in mm and recorded.

Antimicrobial activity of commercially available antibiotics
The antimicrobial activities of some selected plant extracts on human pathogenic bacteria were compared with the commercially available antibiotics. Sterile Muller Hinton Agar plates were prepared and the test organisms were swabbed over the surface of agar plates using sterile cotton swab. The antibiotic discs such as Gentamycin, Ciproflaxacin, Penicillin and Ampicillin were placed on the surface of the plates. The plates were incubated at 37^{o}C for 24 hours and after incubation the diameter of the inhibition zones were measured in mm and recorded [9].

RESULTS

The antimicrobial activity of ethanol methanol and aqueous extracts of seven Indian medicinal plants were invertigated using disc diffusion method (Tables 1 – 3) and agar well diffusion method (Tables 4 – 6) against selected human pathogens such as *Staphylococcus* sp., *Escherichia coli*, *Klebsiella* sp., *Pseudomonas* sp. These four different pathogens have also tested with commercially available four different antibiotics and results were indicated in Table 7. All the medicinal plant extracts used against the pathogenic organisms have showed varied degree of antimicrobial activity against the pathogens.

Fig 1 : Antimicrobial activity of Ethanol extract of medicinal plants against human pathogenic bacteria using Disc diffusion method

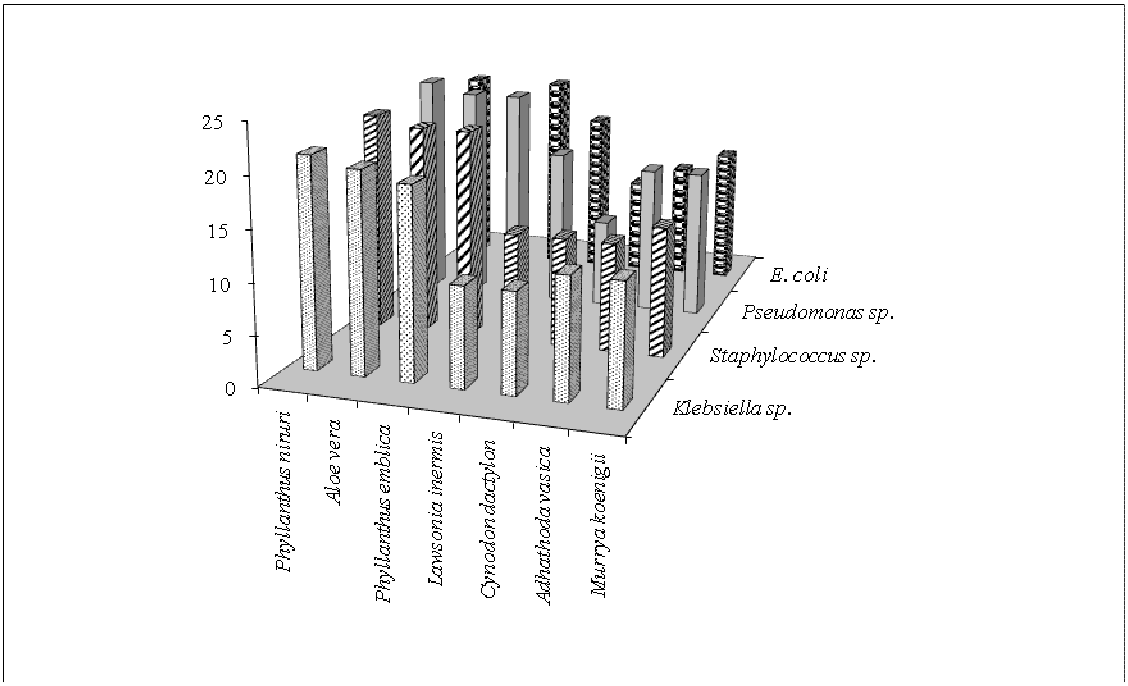


Fig 2 : Antimicrobial activity of Methanol extract of medicinal plants against human pathogenic bacteria using Disc diffusion method.

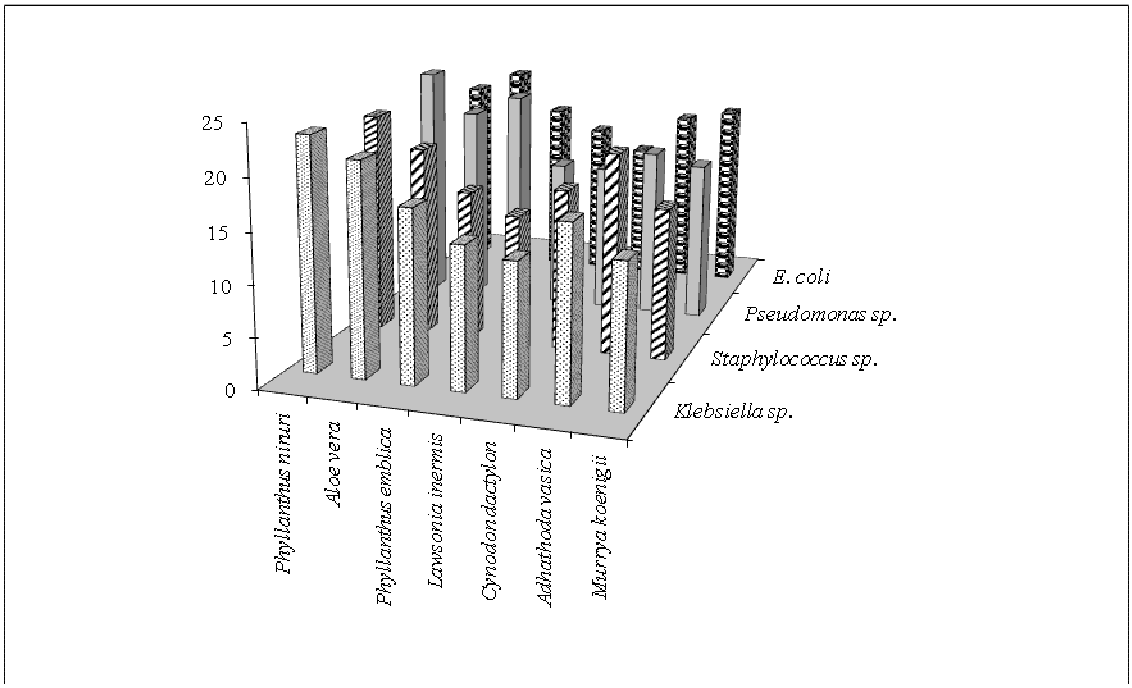


Fig 3 : Antimicrobial activity of Aqueous extract of medicinal plants against human pathogenic bacteria using Disc diffusion method.

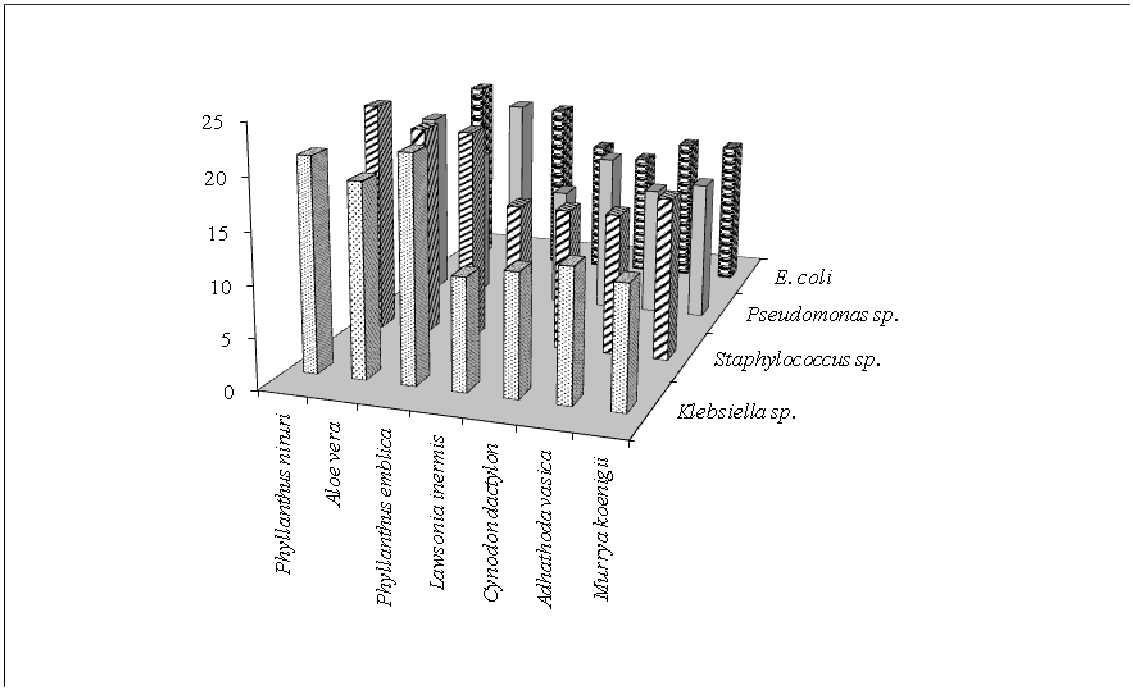


Fig 4 : Antimicrobial activity of Ethanol extract of medicinal plants against human pathogenic bacteria using Agar well diffusion method.

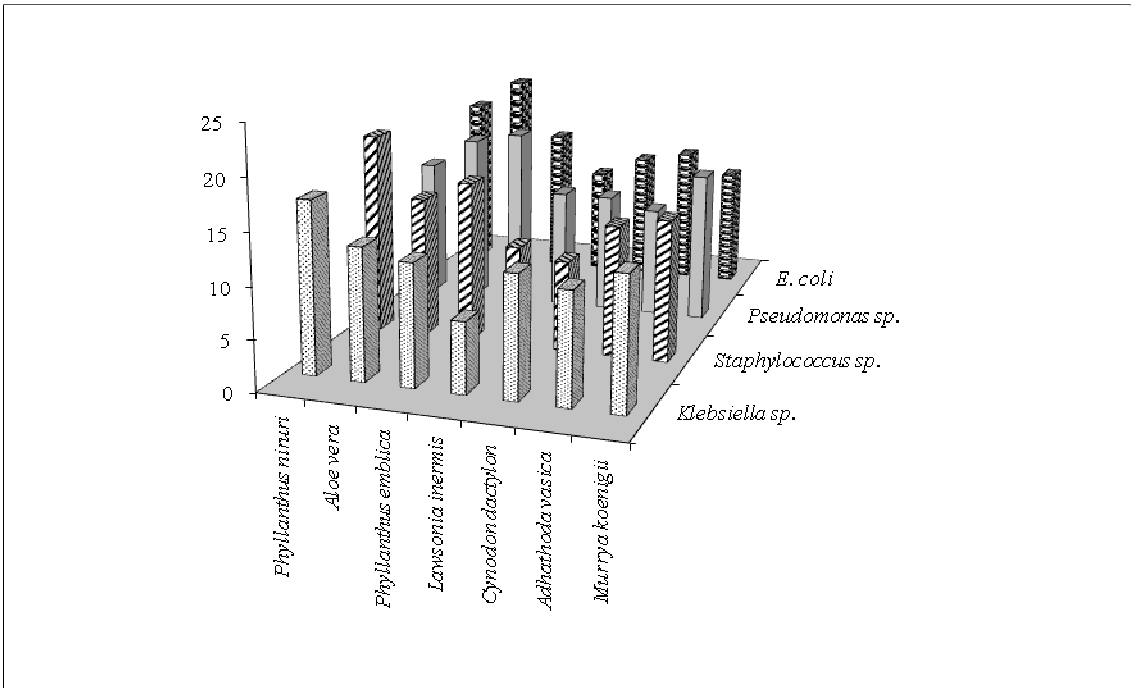


Fig 5 : Antimicrobial activity of Methanol extract of medicinal plants against human pathogenic bacteria using Agar well diffusion method.

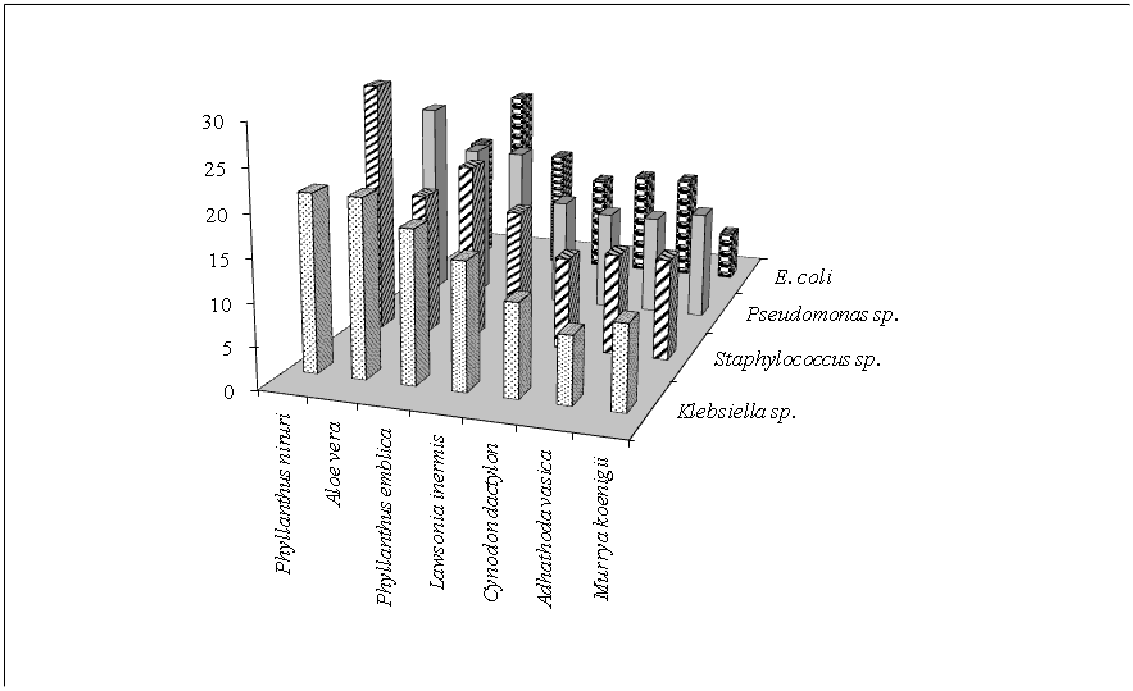


Fig 6 : Antimicrobial activity of Aqueus extract of medicinal plants against human pathogenic bacteria using Agar well diffusion method.

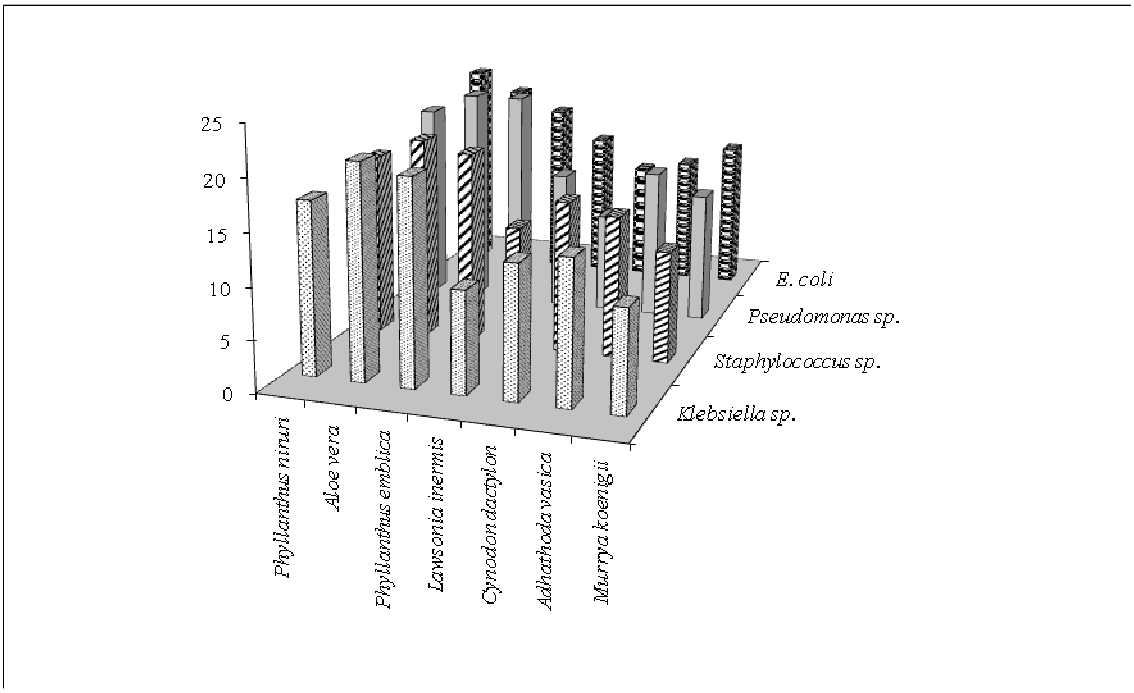
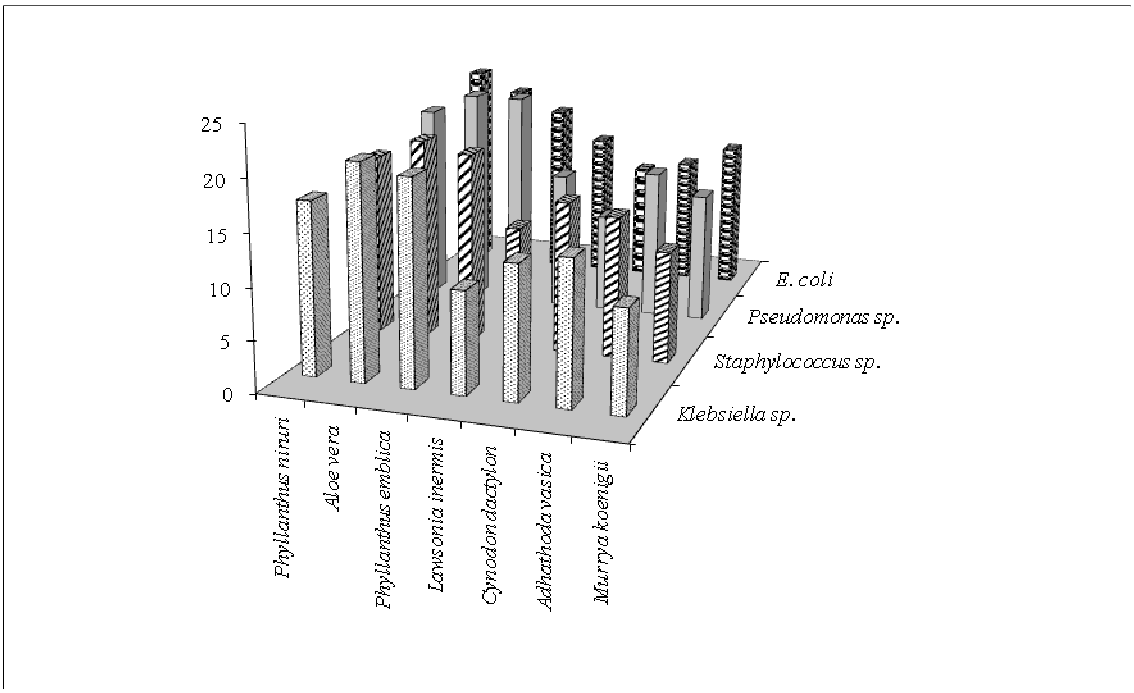


Fig 7 : Antimicrobial activity of commercially available antibiotics against human pathogenic bacteria.



Antimicrobial activity of ethanolic extracts

The amtimicrobial activity of ethanolic extracts of *Phyllanthus niruri* on four different human pathogenic organisms using disc diffusion method have showed maximum zone of inhibition (23 mm) (Table 1) against *Pseudomonas* sp. followed by *Staphylococcus* sp. (22 mm), *Klebsiella* sp. (21 mm) and *E. coli* (21 mm). The collective analysis of antimicrobial activity of ethanolic extract indicated that among the seven medicinal plants used in the study *Phyllanthus niruri*, *Aloe vera* and *Phyllanthus emblica* have better impact ranged from 19 to 23 mm on all the four species of pathogenic bacteria when compared to rest of the plant species such as *L. inermis*, *C. dactylon*, *A. vasica* and *M. koenigii* (ranged from 9 mm to 17 mm). Whereas, in case of agar well diffusion method (Table 4), the ethanolic extract of *Aloe vera* showed the maximum zone of inhibition (21 mm) against *E. coli* followed by *Staphylococcus* sp. (20 mm) with the extract of *Phyllanthus niruri*. The extract of *L. inermis* showed the minimum

zone of inhibition (7 mm) against *Klebsiella* sp. while the collective analysis indicated similar trend of impact obtained in case of disc diffusion method.

Antimicrobial activity of methanolic extract

The antimicrobial activity of methanolic extract using disc diffusion method, *P. niruri* showed maximum zone of inhibition (24 mm) against *Pseudomonas* sp. followed by *Klebsiella* sp. (23 mm), *Staphylococcus* sp. (22 mm) and *E. coli* (20 mm) (Table 2). Similarly, the methanolic extracts of *A. vera* and *P. emblica* have highest antimicrobial activity on *E. coli* (22 mm) and *Pseudomonas* sp. (22 mm) respectively. Whereas the *L. inermis* and *C. dactylon* have showed the minimum activity (13 mm) against *Staphylococcus* sp. and *Klebsiella* sp. respectively. The overall observation indicated that (Table 2) the methanolic extracts of *L. inermis*, *C. dactylon*, *A. vasica* and *M. koenigii* have lesser impact on the four different human pathogens when compared to other three species of medicinal plants (*P. niruri*, *A. vera* and *P. emblica*). Whereas in the case of agar well diffusion method, the methanolic extracts of *P. niruri* showed maximum zone of inhibition (30 mm) against *Staphylococcus* sp. followed by *Pseudomonas* sp. (24 mm), *Klebsiella* sp. (21 mm) and *E. coli* (16 mm) (Table 5). The extract of *M. koenigii* showed minimum zone of inhbition against *E. coli* (6 mm), moreover the collective analysis indicated that the extract of *L. inermis*, *C. dactylon*, *A. vasica* and *M. koenigii* have leser rate of antimicrobial activity when compared to other species of plants used in the study.

Antimicrobial activity of aqueous extracts

In disc diffusion method, the aqueous extracts of *P. niruri* showed maximum zone of inhibition against *Staphylococcus* sp. (23 mm) followed by *Klebsiella* sp. (21 mm), *E. coli* (20 mm) and *Pseudomonas* sp. (19 mm) (Table 3). Similarly the aqueous extract of *P. emblica* has high impact on the human pathogenic bacteria such as 22mm, 21 mm, 21 mm and 18 mm against *Klebsiella* sp., *Staphylococcus* sp., *Pseudomonas* sp. and *E. coli* respectively. The extract of *L. inermis* showed minimum zone of inhibition against *Klebsiella* sp. (11 mm) and the extract of *A. vera* showed moderate rate of antimicrobial activity on all the four human pathogenic bacterial species (Table 3).

The observation antimicrobial activity, aqueous extracts of medicinal plants on human pathogenic species using agar well diffusion method showed that the extracts of *P. niruri*, *A. vera* have more impact on *Pseudomonas* sp. (22 mm) and *P. emblica* on *E. coli* (22 mm) (Table 6). The aqueous extract of *L. inermis* and *M. koenigii* have showed minimum zone of inhibition on *Klebsiella* sp. (10 mm). The overall observation antimicrobial activity of aqueous extract of seven medicinal plants (using agar well diffusion method) indicated that the *P. niruri*, *A. vera* and *P. emblica* have more impact than the remaining four species of plants.

Antimicrobial activity of commercial available antibiotics

Among the four different types of antibiotics used in the study, Gentamycin has wide range of impact on all the four species of human pathogenic bacteria. The maximum zone of inhibition was observed against *Staphylococcus* p. and *Klebsiella* sp. (22 mm) (Table 7). The maximum zone of inhibition was obtained using Ciprofloxacin (29 mm) against *Staphylococcus* sp. and *Klebsiella* sp. whereas the minimum zone of inhibition was exhibited in Ampicillin (7 mm) against *E. coli*. Penicillin has impact only on *Staphylococcus* sp. and it has no antimicrobial activity on *E. coli*, *Klebsiella* sp. and *Pseudomonas* sp. Altogether, the antibiotics Gentamycin and Ciprofloxacin have higher antimicrobial activity on the selected test organisms.

DISCUSSION

Infections diseases are the major cause of morbidity and mortality worldwide. The number of multidrug resistant microbial strains and the appearance of strains which reduced susceptibility to antibiotics are continuously increasing. Such increase has been attributed to indiscriminate use of broad spectrum antibiotics, immunosuppressive agents, intravenous catheters organ transplantation and ongoing epidermis of human immunodeficiency virus (HIV) infections. This situation provided the impetus to the search for new antimicrobial substances from various source like medicinal plants.

The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well being. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment. [2]

In this present study, preliminary screening for antimicrobial activity showed, that the methanolic extract of *Phyllanthus niruri* exhibited maximum inhibitory zone (30 mm) against *Staphylococcus* sp. While the ethanol and aqueous extracts of *Murrya koenigii*, *Cynodon dachylon*, *Lawsonia inermis* and *Adha-thoda vasica* showed least inhibitory activity. The antimicrobial assay by agar-well diffusion method revealed that methanol extract of

medicinal plants exhibited broad spectrum activity against tested isolates as compared to ethanol and aqueous extracts.

Results obtained from this study, indicated that, the plant extracts showed the strongest antimicrobial activity than the commercially available antibiotics. For instance, Ciproflaxacin showed the maximum zone of inhibition (29 mm) against *Staphylococcus* sp. But the methanol extract of *Phyllanthus neurri* showed the maximum zone of inhibition (30 mm in 60 l) against *Staphylococcus* sp. The present investigbation corroborates with studies of many authors.

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