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ANTIBACTERIAL PROPERTIES OF SELECTED MEDICINAL PLANTS AGAINST PATHOGENIC BACTERIA

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ABSTRACT
Plants are rich source of antibacterial agents, which could be exploited in human disease management.The present investigation focused on antibacterial activity of *Acacia nilotica, Catharanthus roseus, Sida cordifolia, Euphorbia hirta* against four bacterial pathogen among which three were gram negative (*Escherichia coli, Pseudomonasaeruginosa, Enterococcoous faecalis)* and one was gram positive (*Staphylococcus aureus*).The antibacterial activities of Methanol, ethanol and acetone extract of four medicinal plants,*Acacia nilotica, Catharanthus roseus, Sida cordifolia ,Euphorbia hirta* were evaluated by using agar well diffusion method against four bacterial pathogen with measurement of minimum inhibitory concentration of these plants.From the result obtained *Acacia nilotica, Catharanthus roseus, Sida cordifolia* and *Euphorbia hirta* showed potent antibacterial activity against selected bacterial pathogens. Methanol extract of these medicinal plants exhibit greater inhibitory effect over the micro organisms.

Key words : Antibacterial activity ,Medicinal Plants.

INTRODUCTION

Medicinal plants are nature's gift to humans to make disease free healthy life. Infectious diseases continue to represent a significant challenge to human medicine. Clearly there is an urgent need for new and efficient drugs to treat the life threatening diseases. Large preparations of drugs currently used to treat infectious diseases are mostly natural products. Despite the progress in the field of Microbiology, incidence of epidemics due to drug resistant microbes and the emergence of unknown disease causing agents still occur. Herbal products remain highly effective instruments in the fight against microbial infections. Plant based antimicrobials represent a vast untapped source for medicine and they have enormous therapeutic potential. They are effective in treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs **(Hammer *et al*., 1999).**

Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country.

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are asource of many potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated. Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation will undertaken to screen the local flora for antibacterial activity from *Acacia spp., Sida cordifolia, Euphorbia hirta and Catharanthus roseus,*

Traditional medicine is an important source of potentially useful compounds for the development of phytotherapeutic agents. Antimicrobials of plant origin have enormous the rapeutic potential in the treatment of infectious diseases while simultaneously migitigting many of the side effects that are often associated with synthetic antimicrobials (**Lwu *et al.*, 1999**).

The antimicrobial research is geared towards the discovery and development of novel antibacterial and antifungal agents. A number of plants from different families of angiosperms have been reported to show antimicrobial activity **(Palombo *et al.*, 2001)**. The spread of multi drug resistant strains of microorganisms necessitates the discovery of new classes of antimicrobial and compounds that inhibit these resistance mechanisms. Natural products continue to play a major role as active substances.

Catharanthus roseus is an important medicinal plant of family Apocynaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities **(Jaleel *et al*.,2009). Muhammad *et al*. (2009)** reported that the antibacterial potential in crude extracts of different parts (*viz.*, leaves, stem, root and flower) of *C. roseus* against clinically significant bacterial strains. Emerging and reemerging infections and spread of deadly, drug-resistant strains of organisms pose a challenge to the global public health for their treatment. Bacterial resistance to antibiotics is a major therapeutic problem and the rate at which new antibiotics are being produced is slowing, **(Russell *et al*., 2002**). Thus, the search for novel antimicrobial agents is of the utmost importance **(Gootz *et al*., 1990)**. Global attention has been shifted towards finding new chemicals, specifically herbals, for the development of new drugs. These natural products can provide unique elements of molecular diversity and biological functionality, which is indispensable for novel drug discovery **(Nisbet and Moore, 1997).**

Euphorbia hirta Linn. is one of such herbs belonging to the family *Euphorbiaceae* which is frequently seen occupying open waste spaces and grasslands, road sides,

and pathways. The plant is an annual broad-leaved herb that has a hairy stem with many branches from the base to the top. The stem and leaves produce white or milky juice when cut. The medicinal usefulness of the herb has been the subject of numerous chemical and microbiological studies. Some of the reported phytoconstituents of the herb included triterpenoids, sterols, alkaloids, glycosides,

flavonoids, tannins, phenols, choline and shikimic acid, while some of the reported scientific uses include its use as an antispasmodic, antiasthmatic, expectorant, anticatarrhal and antisyphilitic (**Burkill, 1994, Adedapo et al., 2005; Falodun *et al.*,2006)**. Most of the activities of the plant are believed to be due to the presence of choline, shikimic acid and the quercetin (**Bala,** ***2006,*** **Falodun *et al.,*** *2006)*. *E. hirta* is a very popular herb amongst practitioners of traditional medicine. In Mauritius, a decoction of the plant is used to treat respiratory tract infections, vomiting, fever, bronchitis and pulmonary disorders (**Darwish *et al.*, 2002; Bala, 2006**). In Nigeria, exudates of the stem is used to treat eye and ear infections (Igoli et al., 2005), while a decoction of the plant is used to treat enteric infections including diarrhea and dysentery, constipations and other stomach problems, asthma. Because of its wide usage and easy availability, this study was undertaken to investigate the phytochemical properties and antibacterial activities of the plant against some economically important bacteria that cause a variety of intestinal and extra intestinal diseases.

MATERIALS AND METHODS

Place of work:

The present study entitled **"Antibacterial properties of selected medicinal plants against pathogenic bacteria"**was conducted in the Department of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences (Deemed-To-Be-University) Allahabad.

Sample collection:

The following test microorganisms (bacteria) were procured from MCCB laboratory and cultured on nutrient agar media and MRS media. (App 1.1-1.3)

1. **Gram positive bacteria:** *Staphylococcus aureus(MCCB 0250)*

2. **Gram negative bacteria:** *Escherichia coli(MCCB 0176) Pseudomonas aeruginosa(MCCB 0252), Enterococcus faecalis(MCCB0163)*

Collection of Plant Material:

Fresh leaves of four different plants Acacia *nilotica., Sida cordifolia, Euphorbia hirta, Catharanthus roseus* free from disease were collected from field of Sam Higginbottom Institute of Technology and Sciences (Deemed-To-Be-University) Allahabad. The leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water, leaf material was then air-dried on sterile blotter under oven.

Solvent Extraction:

Thoroughly washed dried leaves of *Acacia nilotica, Sida cordifolia, Euphorbia hirta,* and *Catharanthus roseus* plant material were dried in shed for five days and then powdered with the help ofWaring blender. The crushed leaves were taken in mortar and pestle and to which different solvents were added individually and mixed well. The solvents used were ethanol, acetone and methanol. The extracts thus obtained were stored in airtight screw capped vials at -10°C until used.

Growth and Maintenance of Test Microorganism for Antimicrobial Studies:

Bacterial cultures of *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus* (*S. aureus*) were obtained from laboratory. The bacteria were maintained on nutrient broth (NB) and MRS broth at37°C.

Preparation of Inoculum:

The Gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli, Pseudomonasaeruginosa, Enterococcus faecalis)* were pre-cultured in nutrient broth overnight in a incubator at 37°C for 48 hrs.

Anti-bacterial Activity:

Antibacterial activity of extracts was tested by using Agar well diffusion technique as described. Melted and cooled sterile nutrient agar media was poured into sterilized petridishes and allowed to solidify. Using the sterilized cotton swabs, overnight broth culture of the test organisms were swabbed on the nutrient plates uniformly. Wells of 5mm diameter were cut on the inoculated nutrient agar plates with the aid of a sterile stainless steel cork borer. About 0.1ml extract was filled into each of the wells. These plates were then be incubated at 37±1°C. The zone of inhibition (mm) was recorded after 24 hrs of incubation. The presence of zone of inhibition was regarded as the presence of antibacterial action.Eachextract was used in Duplicate.

RESULTS AND DISCUSSION

Plants are important source of potentially useful component for the development of new chemotherapeutic agents. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

The findings of the present study revealed that *Acacia nilotica ,Catharanthus roseusSida cordifolia, Euphorbia hirta,* contain potent antimicrobial property against tested microbes. Herbal medicine is still the mainstay of about 70-80% of world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with human body with lesser side effects. The World Health Organization (WHO) has listed more than 21,000 plants, which are used for many medicinal purposes around the world. They observed that about 74% of 119 plant-derived pharmaceutical medicines are used in modern medicine. It also estimates that 4 billion people (80 percent of the world population) presently use herbal medicine for health care (**Malviya*et al*., 2011).**

As a result, the antimicrobial activity of different medicinal plant parts extracts of four plants was screened against the most common pathogens.

In the present study, antibacterial activity of different plants extracts was observed against different pathogenic bacteria. The study showed that antibacterial activity of medicinal plant between methanol, ethanol and acetone extract of *Acacia nilotica* was found to be effective against all gram positive and gram negative organisms. Methanolic extract of *Acacia nilotica* was found to be more effective against *P.aeruginosa* (30mm), *S.aureus* (29mm), *E.coli (*25mm)while least was shown by *E.faecalis* (15mm). Ethanolic extract of *Acacia nilotica* was found to be more effective against,*S.aureus* (33mm), *E.coli (*29mm), *P.aeruginosa* (26mm) while least was shown by *E.faecalis* (20mm). Acetone extract of *Acacia nilotica* was found to be more effective against,*S.aureus* (32mm), *E.coli (*30mm), *P.aeruginosa* (30mm) while least was shown by *E.faecalis* (20mm). (Table 4.1, Figure 4.1,Plate 4.1-4.4). The data was statistically analysed and found to be significant or non significant. (App-2.1)

Methanol – *P.aeruginosa* (3o) >*S.aureus* (29) > *E.coli* (25) > *E. faecalis* (15)
Ethanol- *S.aureus* (33) > *E.coli* (29) > *P.aeruginosa* (26) > *E. faecalis* (20)
Acetone-*S.aureus (32)* > *E.coli,P.aeruginosa* (30) >*E. faecalis* (20)

Table 4.1:- Antibacterial activity of *Acacia nilotica* against Pathogenic bacteria.

Name of organisms	Zone of inhibition (mm)		
	Methanol	Ethanol	Acetone
Staphylococcus aureus	29	33	32
Escherichia coli	25	29	30
Pseudomonas aeruginosa	30	26	30
Enterococcus faecalis	15	20	20

Due to extract-$F_{cal}=2.389<F_{tab}=5.14$, C.D=3.728, NS=Non significant
Due to Pathogen-$F_{cal}=20.90>F_{tab}=4.76$, C.D=3.728, S= significant

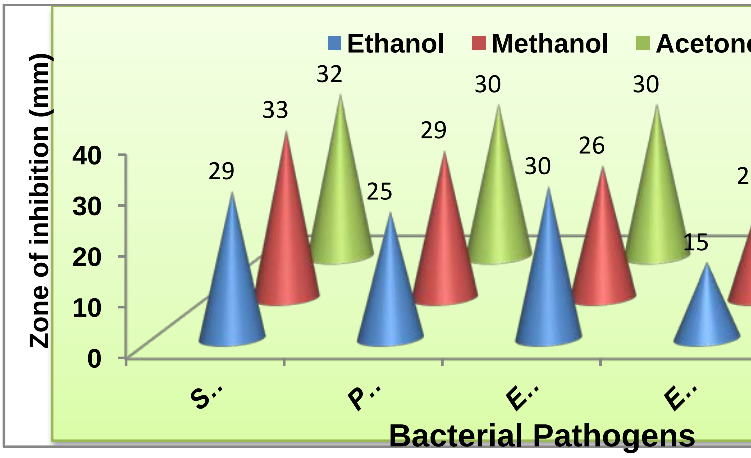


Figure 4.1: Antibacterial activity of *Acacia nilotica* against pathogenic bacteria.

Similarly results were reported by **Satish*et al.*(2008)**Showed that Highly significant degree of activity was observed against all the test bacteria in case of *Acacia nilotica* .The zone of inhibition in case of *Acacia nilotica* varied between 9mm to 35.5mm Highest degree of zone of inhibition was observed against *Staphylococcus aureus by Acacia nilotica*. More than 20mm of zone of inhibition was recorded by *Acacia nilotica*.Another report showed that the methanol leaf extracts of *Acacia nilotica, Sida cordifolia* showed the activity against *E. coli, S. aureus.* **Mahesh *et al.* (2008).** Methanol extracts of *A. nilotica* exhibited good activity in the range of 18.75-75.0 µg/ml had been reported by ***Dabur et al* (2004).**These finding are in fair correlation with the study carried out for *S.aureus* how ever against *E.coli, P.aeruginosa E.faecalis*.

In the present study, antibacterialactivity of different plants extracts was observed against different pathogenic bacteria.The study showed that antibacterial activity of medicinal plant between methanol ,ethanol and acetone extract of *Catharanthus roseus* was found to be effective against all gram positive and gram negative organisms.Methanolic extract of *Catharanthus roseus*was found to be more effective against *S.aureus* (15mm) *E.faecalis* (14mm), *E.coli* (10mm) while least was shown by *P.aeruginosa* (5mm) . Ethanolic extract of *Catharanthus roseus*was found to be more effective against *S.aureus* (10mm), *E.faecalis* (10mm), *E.coli (*7mm) while least was shown by *P.aeruginosa* (7mm). Acetone extract of *Catharanthus roseus*was found to be more effective against *S.aureus* (10mm) *E.faecalis* (10mm), *E.coli (*7mm) while least was shown by *P.aeruginosa*

(5mm). (Table 4.2, Figure 4.2, Plate- 4.5-4.8). The data was statistically analysed and found to be significant or non significant.
(App-2.1)

Methanol – *S.aureus* (15) >*E. faecalis*(14) > *E.coli* (25) >*P.aeruginosa*(15)
Ethanol- *S.aureus* (10), *E. faecalis* (10)> *E.coli* (7) , *P.aeruginosa* (7)
Acetone-*S.aureus (10)* >*E. faecalis* (10)>*E.coli* (7) > *P.aeruginosa* (5)

Name of organisms	Zone of inhibition (mm)		
	Methanol	Ethanol	Acetone
Staphylococcus aureus	15	10	10
Escherichia coli	10	7	7
Pseudomonas aeruginosa	5	7	5
Enterococcus faecalis	14	10	10

Table 4. 2: Antibacterial activity of *Catharanthus roseus* against pathogenic bacteria.

Due to extract-F_{cal}=4.043<F_{tab} =5.14, C.D=2.767, NS=Non significant
Due to Pathogen-F_{cal}=9.60>F_{tab} =4.76, C.D=2.767, S= significant

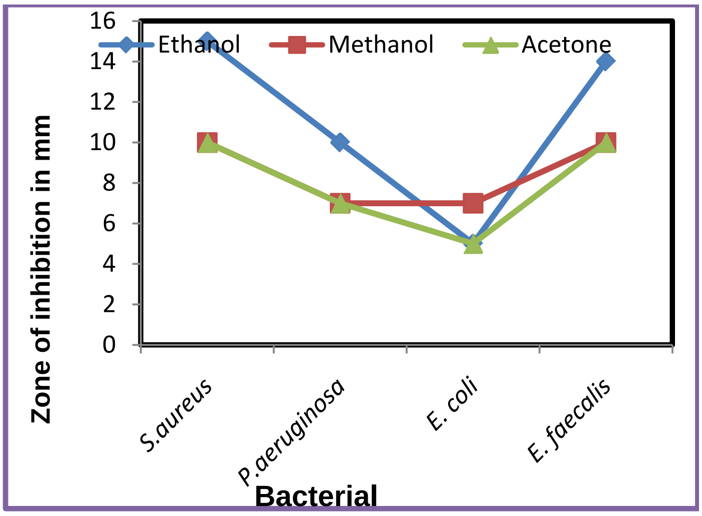


Figure 4. 2:- Antibacterial activity of *Catharanthus roseus* against pathogenic bacteria.

Similarly results were reported by ***Koppula et al. (2010)*** *examined that* the methanol extracts were showed strong activity against *S.aureus*. Hence, these medicinal plants can be used to discern bioactive natural products and new pharmaceutical molecules that serve in the development of unmet therapeutic needs**.** Another experiment showed that *C. roseus*have shown a good zone of inhibition.*Staphylococcus aureus*at 10µg/mL formed a zone of 9mm in diameter and 20µg/mL formed a zone of 16mm in diameter. **Vijayalakshmidevi *et al*. (2011)Patil *et al.* (2010)** examined that these extracts may not find a therapeutic use in immediate future but definitely it can be used as aprophylactic agent in regions where certain diseases can occur as endemic if not in pandemic scale.

The study showed that antibacterial activity of medicinal plant between methanol, ethanol and acetone extract of *Sida cordifolia* was found to be effective against all gram positive and gram negative organisms. The study showed that antibacterial activity of medicinal plant between methanol, ethanol and acetone extract of *Sida cordifolia* was found to be effective against all gram positive and gram negative organisms. Methanolic extract of *Sida cordifolia*was found to be more effective against *P.aeruginosa (17mm),E.faecalis* (15mm), *S.aureus* (14mm) while least was shown by *E.coli* (7mm). Ethanolic extract of *Sida cordifoliar*was found to be more effective against *S.aureus* (15mm), *P.aeruginosa* (14mm), *E.faecalis*(10mm)while least was shown by *E.coli* *(*8.5mm).Acetone extract of *Sida cordifolia*was found to be more effective against *S.aureus* (15mm) *E.faecalis* (10mm), *P.aeruginosa* (9mm) while least was shown by *E.coli* (7mm). (Table 4.3,Figure 4.3, Plate 4.9-4.12). The data was statistically analyzed and found to be significant or non significant. (App- 2.1)

Methanol – *P.aeruginosa* (17) >*E. faecalis* (15) *S.aureus* (14) > *E.coli* (8)
Ethanol- *S.aureus* (15) >*P.aeruginosa* (14) > *E. faecalis* (10) > *E.coli* (8.5) **Acetone**-*S.aureus (15)* >*E. faecalis* (10) *P.aeruginosa* (9) *E.coli* (7)

Table 4. 3: Antibacterial activity of *Sida cordifolia* against pathogenic bacteria.

Name of organisms	Zone of inhibition (mm)		
	Methanol	Ethanol	Acetone
Staphylococcus aureus	14	15	15
Escherichia coli	8	8.5	7
Pseudomonas aeruginosa	17	14	9
Enterococcus faecalis	15	10	10

Due to extract-F_{cal}=2.109<F_{tab} =5.14, C.D=7.87, NS=Non significant

Due to Pathogen-F_{cal}=5.252>F_{tab} =4.76, C.D=7.87, S= significant

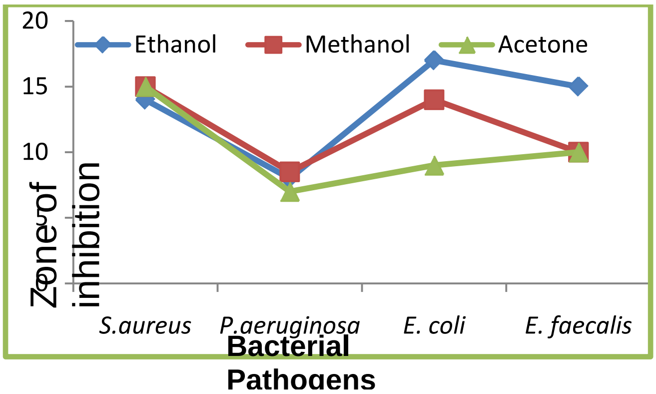


Figure 4. 3: Antibacterial activity of *Sida cordifolia* against pathogenic bacteria.

Similarly results were reported by**Kalaiarasan *et al.* (2010**) reported that the ethanolic leaf extract have was very large activity at 75 mL/disc concentration *Escherichia coli*, (9.5 mm) zone of inhibition at this 75 mL/disc concentration. *Pseudomonas aeruginosa* (11 mm) zone of inhibition at this 75 mL/disc concentration. Antibacterial activity of the plant extracts was determined against selected bacteria showing activities. The above results revealed that the methanolic extract have significant activity against , *Staphybococcus aureus* (8 mm) at 75 mL/ disc concentrations. *Escherchia coli* (8.5mm), *Pseudomonasaeruginosa* and respectively at 75 mL/ disc concentration. Zone of inhibition was recorded against respectively at 50 mL/disc concentration. Partially zone of inhibition was recorded against *Escherchia coli, Pseudomonasaeruginosa* (7 mm). These finding are in fair correlation with the study carried out for *S.aureus* however against *E.coli, P.aeruginosa, E.faecalis*. Another report showed that the zones of inhibition different extracts of S. cordifolia found 12 to 40 mm against bacteria and 12 to 22 mm. **Wake *et al.* (2012)**

The study showed that antibacterial activity of medicinal plant between methanol ethanol and acetone extract ofwas found to be effective against all gram positive and gram negative organisms. The study showed that antibacterial activity of medicinal plant between methanol, ethanol and acetone extract of *Euphorbia hirta* was found to be effective against all gram positive and gram negative organisms. Methanolic extract of *Euphorbia hirta* was found to be more effective against *E.coli* (17mm) *P.aeruginosa (16mm)*., *E.faecalis* (16mm),while least was shown by *S.aureus* (15mm).Ethanolic extract of *Euphorbia hirta*was found to bemore effective against *E.coli* (15mm) *P.aeruginosa* (15mm), *E.faecalis*(15mm),while least was shown by *S.aureus (*8.5mm).Acetone extract of*Euphorbia hirta* was found to be more effective against *E.faecalis* (15mm), *P.aeruginosa (1*5mm), *E.coli* (14mm)while least was shown by *S.aureus* (11mm) .and Methanolic extract of this plant showed more inhibitory effect against *E.coli* (6.25mm), *S.aureus* (12.5mm*) P.aeruginosa* (12.5mm), *E.faecalis* (12.5mm).(Table 4.4, Figure 4.4,Plate 4.13-4.16). The data was statistically analysed and found to be significant or non significant. (App-2.1)

Methanol – *E.coli* (17) >*P.aeruginosa*(16), *E. faecalis* (16)> *S.aureus* (15)

Ethanol-*E. faecalis* (15), *E.coli* (15) , *P.aeruginosa* (15) > *S.aureus* (12)

Acetone- *E. faecalis* (15), *P.aeruginosa* (15) > *E.coli* (14) > *S.aureus* (11)

Table 4.4: Antibacterial activity of *Euphorbia hirta* against pathogenic bacteria.

Name of organisms	Zone of inhibition (mm)		
	Methanol	Ethanol	Acetone
Staphylococcus aureus	15	12	11
Escherichia coli	17	15	14
Pseudomonas aeruginosa	16	15	15
Enterococcus faecalis	16	15	15

Due to extract-$F_{cal}=9.57>F_{tab}=5.14$, C.D=1.32, S= significant
Due to Pathogen-$F_{cal}=9.14>F_{tab}=4.76$, C.D=7.87, S= significant

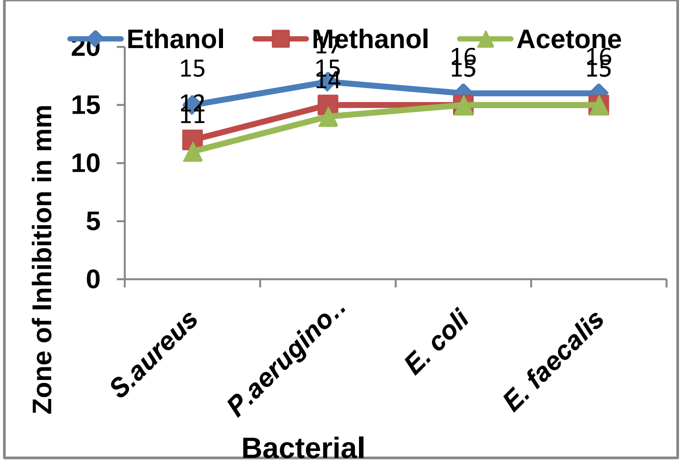
Figure 4.4: Antibactrial activity of *Euphorbia hirta* against pathogenic bacteria.

Similarly results were reported by **Abubakar *et al.* (2009)** reported that Methanol extract gave inhibitory zones of (13 mm) for *E. coli*, Another report showed that *E.hirta* revealed antibacterial activity *S.aureus*. **Parekh *et al.*, (2005)**

In the present study comparison of methanol extract was observed against different pathogenic bacteria. The study showed that Methanolic extract of *Acacia nilotica* was found to be more effective against *P.aeruginosa* (30mm) ,*S.aureus* (29mm), *E.coli* (25mm)while least was shown by *E. faecalis* (15mm). Methanolic extract of *Catharanthus roseus*was found to be more effective against *S.aureus* (15mm) *E.faecalis* (14mm), *E.coli* (10mm) while least was shown by *P.aeruginosa*(5mm) Methanolic extract of *Sida cordifolia*was found to be more effective against *P.aeruginosa* (17mm), *E.faecalis* (15mm), *S.aureus* (14mm) while least was shown by *E.coli*(7mm).(Table 4.5,Figure 4.5)

Table 4.5:- Comparison ofAntibacterial activity of methanolic extract of medicinal plants

Name of organisms	*Acacia nilotica*	*Catharanthus roseus*	*Sida cordifolia*	*Euphorbia hirta*
S. aureus	29	15	14	15
E.coli	25	10	8	17
P.aeruginosa	30	5	17	16
E.faecali	15	14	15	16



s

Due to extract-$F_{cal}=3.86<F_{tab}=o.420$, C.D=0.90, S= Significant
Due to Pathogen-$F_{cal}=3.86<F_{tab}=5.88$, C.D=3.728, NS= Non significant

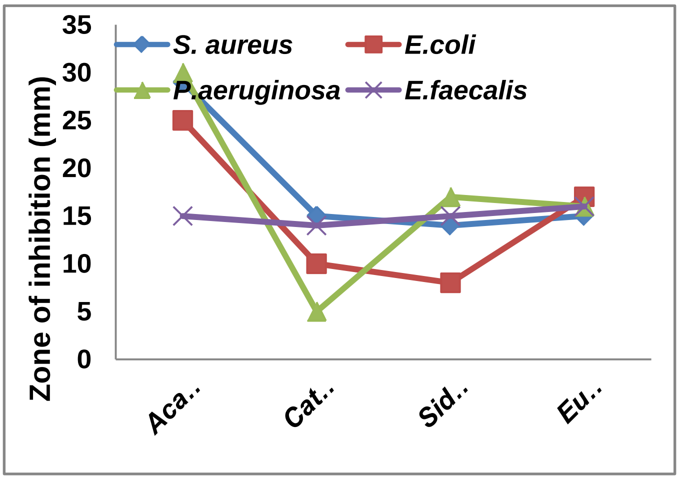


Figure: 4.5:-Comparison ofAntibacterial activity of methanolic extract of medicinal Plants

Table 4.6. Initial Minimum inhibitory concentration of Methanolic extract of selected medicinal plant

Name of organisms	Initial Minimum inhibitory concentration (µl/ml)			
	Acacia nilotica	*Catharanthus roseus*	*Sida cordifolia*	*Euphorbia hirta*
Staphylococcus aureus	12.5	6.25	12.5	12.5
Escherichia coli	6.25	3.12	6.25	6.25
Pseudomonas aeruginosa	12.5	12.5	12.5	12.5

In the present study minimum inhibitory concentration of different plants were observed against different pathogenic bacteria. Methanolic extract of *Acacia nilotica* showed more inhibitory effect against *P.aeruginosa* (6.25mm), S.*aureus* (12.5mm), *E.coli* (12.5mm), *E.faecalis* (12.5mm).

and Methanolic extract of this *Catharanthus roseus* showed more inhibitory effect against *E.coli* (3.12), *S.aureu s(*6.25*), P.aeruginosa* (12.5), *E.faecalis* (12.5) and Methanolic extract of this plant showed more inhibitory effect against *S.aureus* (6.25*), P.aeruginosa* (6.25)*, E.coli* (12.5), *E.faecalis* (12.5). Methanolic extract of *Euphorbia hirta* was found to be more effective against *E.coli* (17mm,) *P.aeruginosa* (16mm), *E.faecalis* (16mm),while least was shown by *S.aureus* (15mm) .(Table 4.6)

Another report showed that the quantity of the plant extracts used for antimicrobial susceptible experiments were suggested to be as lower as 1mg/ml and are considered to possess significant antimicrobial activity in the case where MIC values are lower than 100µg/ml by **(Gibbons, 2004; Rios and Recio, 2005).Sudhakar *et al.*, (2006)** reported the MIC values of ethanol extract of *E.hirta* lower than1mg/ml against few microorganisms using tube dilution method.

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