

ANTIBACTERIAL SCREENING OF THE BARK OF *ADENANTHERA PAVONINA*

(L.)

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ABSTRACT

Adenanthera pavonina L. syn. Red Sandalwood, (Fabaceae) is an unarmed deciduous tree and its bark is traditionally used for treatment of various disease conditions in gonorrhea, haematuria, ulcers, it is astringent, vulnerary and aphrodisiac in nature. The aim of the present study was to evaluate the qualitative analysis of phytochemicals and antibacterial activity of solvent extracts of *Adenanthera pavonina* bark. Antimicrobial activity of different solvent extracts of *Adenanthera pavonina* bark were tested against Gram positive and Gram negative bacterial strains by observing the zone of inhibition. The bacteria used in the study were *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Staphylococcus epidermidis*, and *Salmonella typhimurium*. Ethanolic and aqueous extracts showed the highest activity against all the tested bacteria. These results were compared with the Zones of inhibition produced by commercially available standard antibiotics. The inhibitory effects of extracts are higher or very close and comparable with the standard antibiotics used.

KEY WORDS: *Adenanthera pavonina*, Antibacterial activity, Phytochemical screening.

INTRODUCTION

Human infections particularly those involving microorganisms i.e., bacteria, fungi, viruses, nematodes cause serious damages in tropical and subtropical countries of the world.^[1] Over the past 20 years, there has been an increased interest in the investigation of natural materials as sources of new antimicrobial agents. Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects. As a result some natural products have been approved as new antimicrobial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance.^[2]

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. 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.^[3] Herbal medicine is still the mainstay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades advances in phytochemistry and in identification of

plant compounds, effective against certain diseases have renewed the interest in herbal medicines. [4] Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. [5] In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. [6] This situation forced to search for new antimicrobial substances. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. [7] Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body [8].

Adenanthera pavonina belongs to the family Leguminosae, subfamily Mimosoideae. The tree is known by a host of common names, including red-bead tree, red wood. The tree has been planted extensively throughout the tropics as an ornamental and has become naturalized in many countries. In India its origin is south; it has been cultivated in many parts in southern region. [9] In India, the plant is traditionally used for various medicinal purposes and its seeds are useful in vitiated conditions of vata and pitta. Besides this they are also used in curing gout, burning sensation, hyperdipsia,

vomiting, fever and giddiness. Its heartwood is used as an astringent, aphrodisiac, haemostatic, and is useful in dysentery, and haemorrhages. Leaves are used to treat gout and rheumatism. [10, 11] The plant *Adenanthera pavonina* has been reported to contain a new five-membered lactone named pavonin with an exo-cyclic double bond has been isolated from the methanol soluble part of *Adenanthera pavonina* [12], sterols (β -sitosterol, β -sitosterol-3 β -D- glucoside), triterpenes (nonacosane & hentriacontane) [13] and saponins (sapogenins). [14] Earlier scientific investigation of *Adenanthera pavonina* showed that the crude extract has blood pressure lowering effect [15] antifungal, antioxidant and cytotoxic [16] and anti-inflammatory activities [17]. The main objective of the research is to screen and evaluate antibacterial activity of crude extracts of *Adenanthera pavonina* bark and to find out minimum inhibitory concentration (MIC) of these extracts against both gram positive as well as gram negative bacteria.

MATERIAL AND METHODS

Description of plant Adenanthera pavonina

The scientific name is derived from a combination of two Greek words aden, "a gland," and anthera, "anther". It is found in Sub Himalayan tract, ascending upto an altitude of 1,200 meters in Sikkim, West Bengal Assam, Meghalaya, Gujarat, Maharashtra, and is commonly known as Red wood. The main important constituents are flavonoid compounds. [18] It is used as an antiseptic paste and also used to treat boils and inflammations. [19] A medium to large-sized deciduous tree, *Adenanthera pavonina* ranges in height from 6-15 m. It is generally erect, having dark brown to grayish bark, and a

spreading crown. The seeds are hard-coated, lens-shaped, vivid scarlet in color, and adhere to the pods. The seed coat is smooth, shiny, bony, and very hard and generally has no fracture lines. The pods are leathery, curve and twist upon dehiscence to reveal 8-12 showy seeds. The leaves are bipinnate. They are dark green in upper surface and blue green in lower surface. They become yellow with ageing. The bark is dark brown or grayish brown on outer surface and grayish white in inner surface. It is rough on old trees with longitudinal fissures. The small, yellowish flower grows in dense drooping rat-tail flower heads. They are small, creamy-yellow in color, and fragrant. Each flower is star-shaped with five petals. The wood is red in colour and extremely hard. It is durable and used for building purpose.

Collection and authentication

The fresh bark of the tree of *Adenanthera pavonina* L. was collected during the month of January 2009, from National Botanical Research institute, Lucknow, India. For identification and Taxonomic authentication, sample of plant material was given to National Botanical Research Institute (NBRI) Lucknow, India. The text report from National botanical research institute, Lucknow, India and confirmed the authenticity of plant material sample was *Adenanthera pavonina* L. with voucher specimen no. NBRI-SOP-202 Receipt no. and date 19/72, 24-02-09. The fresh bark was used for the study of macroscopic and microscopical characters. Whereas collected plant materials were shade-dried and coarsely powdered. This coarse powder was used for the determination of ash values, extractive values, and preliminary phytochemical investigation was studied as per standard methods.

Extraction of plant materials

100 gm coarsely powdered of air dried bark of *Adenanthera pavonina* L. were packed in muslin cloth and subjected to Soxhlet extractor for continuous hot extraction with distilled water, ethanol, petroleum ether and chloroform for 8 hrs separately. Then the each extracts were filtered and filtrate was evaporated to dryness. The percentage yield of the water, ethanol, petroleum ether and chloroform extracts was 4.18%, 2.72%, 1.68% and 1.15% respectively.

Preliminary phytochemical screening

Preliminary phytochemical screening for the detection of various was carried out by using standard procedures described by Harborne [20] and Khandelwal [21].

Thin layer chromatography and high performance thin layer chromatography (HPTLC)

Thin layer chromatography studies of the ethanol and chloroform extracts carried out in various solvents at 30^o C using Silica gel G as adsorbent and the R_f values were determined [22]. The same mobile phase was used for the HPTLC profiles of these extracts.

Screening of Antibacterial Activity of *Adenanthera pavonina* L

Pathogenic Bacteria Used for the Present Study

About five human pathogenic bacterial strains were used. Both the gram-negative (*Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) and gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus epidermidis*) were included. Axenic cultures of bacterial

strains were obtained from the Department of Biotechnology, Integral University, Lucknow.

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Agar well diffusion method

Antibacterial activity was screened by agar well diffusion method^[23, 24]. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with eight-hours-old broth culture of respective bacteria. Using the sterile cork borer, the well (6mm) was made into the each Petri-plate. Various concentrations of petroleum ether, chloroform, ethanol and aqueous extracts (25 mg, 50 mg and 75mg/ well) were used to assess the dose dependent activity of the extracts. The extracts were prepared in DMSO (Dimethyl sulphoxide) which showed no zone of inhibition and acts as a negative control and were added into the wells by using sterile micropipettes. Simultaneously the standard antibiotics (as positive control) were tested against the pathogens. Discs of Doxycycline (30 µg), Gentamicin (10 µg), Penicillin (10 units/disc), Streptomycin (300µg), Ampicillin (10 µg) and Tetracycline (10µg) were used as positive antibacterial controls. All product of Himedia Laboratories Mumbai (India) were used in this study. Then the plates were incubated at 37° C for 24 - 48 hours. After the incubation period, the diameter of the

inhibition zones of each well was measured. And the values were noted. Triplicates were maintained in each extract and the average values were calculated for the eventual antibacterial activity.

Broth Dilution Test

Broth dilution test is used to determine the Minimum Inhibitory Concentration (MIC) of the antimicrobial drugs. Freshly prepared nutrient broth was used as diluents. Overnight cultures of the test bacteria grown in nutrient broth cultures were diluted 100 folds in nutrient broth. (100 µl bacterial cultures in 10 ml NB). Increasing concentrations of the extract were added to the test tubes containing the bacterial cultures to know the inhibitory concentration in a particular tube inhibiting the bacterial growth. All tubes were incubated at 37°C for 24 hours. The tubes were examined for visible turbidity and optical density of cultures were determined at 620 nm using NB as a control. Control tubes without the tested extracts were assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC^[25, 26].

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The preliminary phytochemical investigation of the aqueous, ethanol, petroleum ether and chloroform extracts of *Adenantha pavonina* L. showed the presence of phytosterols, flavonoids, terpenoid saponins, carbohydrates, tannins, glycosides, alkaloids and proteins Table 1.

Table 1. Thin layer chromatography of *Adenanthera pavonina* Linn.

Extracts	Pet ether	Chloroform	Ethanolic	Aqueous
Sterols	+	+	+	+
Tannins	-	-	+	-
Flavonoids	-	+	+	+
Proteins and amino acid	-	-	-	+
Glycosides	-	-	+	+
Acidic compounds	-	-	-	-
Carbohydrates	-	-	+	+
Saponins	-	-	-	-
Alkaloids	-	+	+	+
Phenols	-	-	+	+

+ Present - Absent

Thin layer chromatography and high performance thin layer chromatography (HPTLC)

Thin layer chromatography of the aqueous and ethanolic extracts was carried out

separately using chloroform: ethanol (7:3) for aqueous extract and chloroform: ethanol: ethyl acetate (7:2:1) for ethanolic extract as mobile phase respectively and the R_f values were recorded Table 2.

Table 2. Qualitative phytochemicals analysis of bark of *Adenanthera pavonina* Linn.

S.No.	Test extract	Solvent system	Number of spots	R _f value
1	Aqueous extract	Chloroform: Ethanol (7:3)	2	0.75 0.41
2	Ethanolic extract	Chloroform: ethanol: ethyl acetate (7:2:1)	3	0.22 0.47 0.95

The visualizing reagent employed was anisaldehyde-sulphuric acid reagent to effect visualization of the resolved spots. TLC (Fig. 4) and HPTLC finger printing studies on ethanol extract showed presence of various phytoconstituents

with their respective R_f values. The ethanolic extract

was developed on chromatographic plates with many ratios of different solvents and the best eluent mixture was used further for HPTLC profile to minimize errors in TLC pattern. The preliminary HPTLC

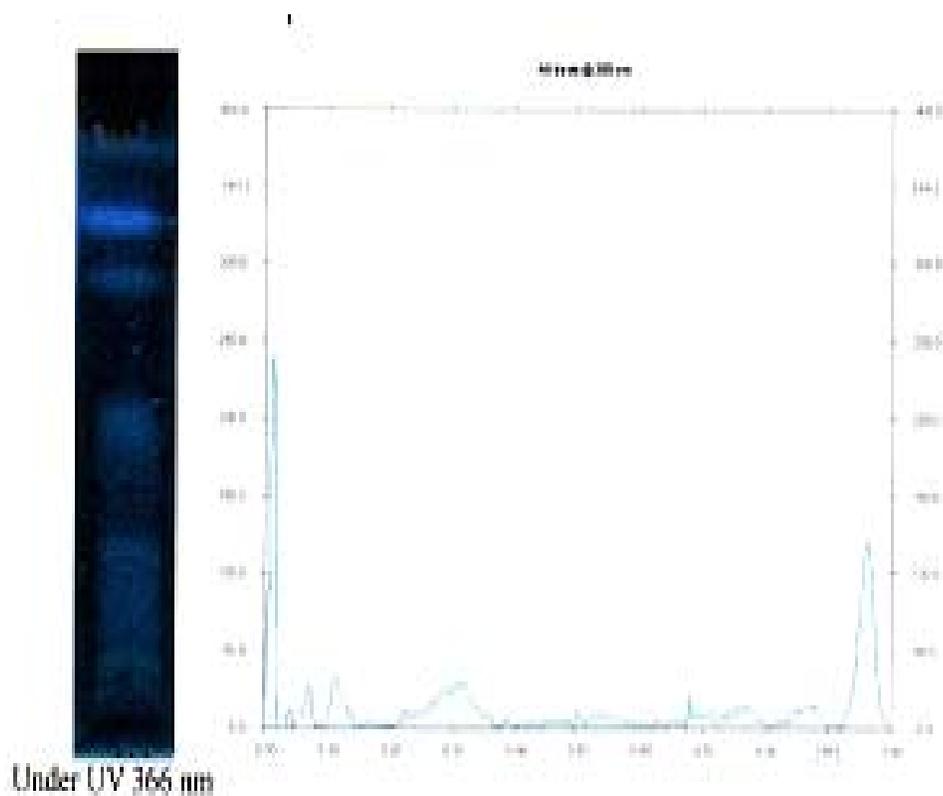
studies revealed that the solvent system Toluene: Ethyl acetate (8.5: 1.5) was ideal and gave well resolved sample peaks. The

spots of the chromatogram were visualized at 366 nm (Fig. 5).

Figure 4. TLC finger printing of ethanolic and chloroform extract of bark of *Adenanthera pavonina* Linn.



Figure 5. HPTLC Finger printing of ethanolic extract of bark of *Adenanthera pavonina* L. scanned at wavelength 366 nm.



Antibacterial Activity

The results of antibacterial activities by agar well diffusion method were presented in Fig-1 and minimum inhibitory concentration (MIC) values were tabulated in Fig-2. The antibacterial activity was tested on the basis of the magnitude of zones of inhibition (in mm) and minimum inhibitory concentration (in mg/ml). The activity of *Adenanthera pavonina* has also been compared with the broad spectrum commercially available antibiotics. Some bacteria were found to be resistant towards commercially used antibiotics while others were found to be sensitive Fig-3. The strain of *E.aerogenes* was shown resistance towards penicillin and ampicillin. *P.aeruginosa* was resistant to tetracycline, ampicillin, doxycycline and penicillin. *S.epidermidis* was resistant to ampicillin and streptomycin. *B.subtilis* was resistant to doxycycline and penicillin while *S.typhi* showed resistance towards ampicillin, doxycycline and penicillin.

The overall result showed that *E.aerogenes* was the most sensitive strain and *S.typhi* was the most resistant strain towards *Adenanthera pavonina* bark extracts. By analyzing the overall data it could be concluded that the gram negative pathogens were more susceptible towards extracts tested while gram positive bacteria showed little resistant towards extracts.

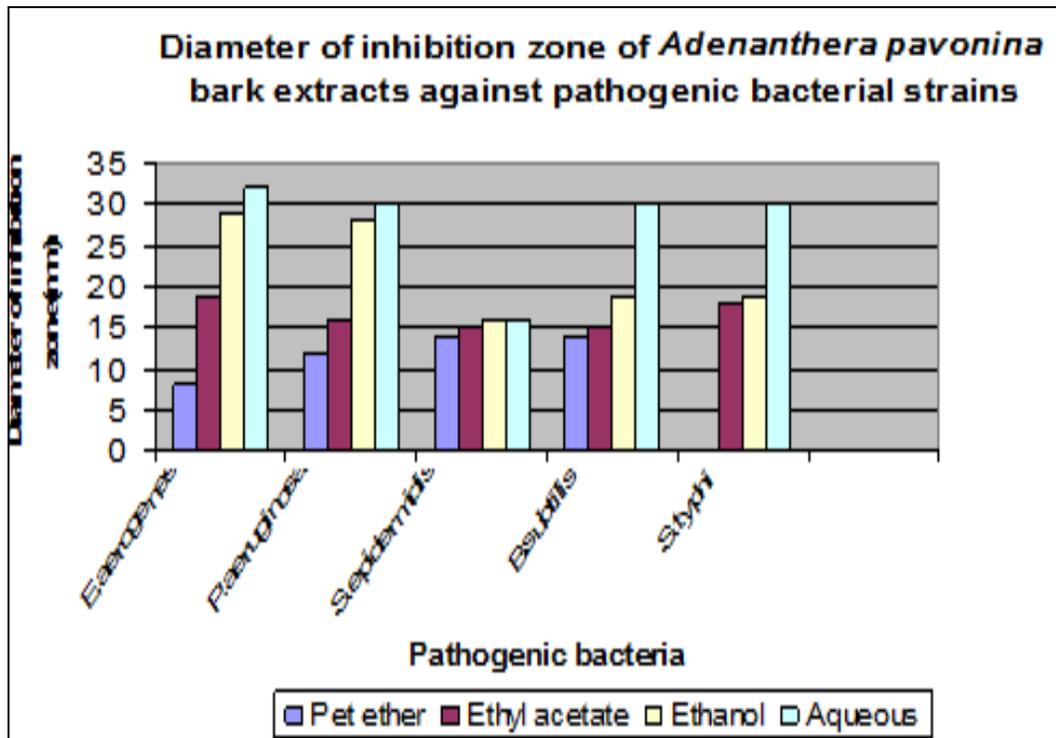
The detailed analysis of antibacterial activity of petroleum ether extract showed dose dependent activity *S.epidermidis* and *B.subtilis* showed maximum susceptibility which was found to be(14mm) followed by *P.aeruginosa*(12mm) and *E.aerogenes* (8mm) while *S.typhi* was found to be resistant to the extract. Gram positive bacteria *S.epidermidis* and *B.subtilis* were more susceptible towards this extracts than gram negative ones (*E.aerogenes*, *P.aeruginosa*, and *S.typhi*) Fig. 1.The

MIC (minimum inhibitory concentration) of the extract for *E.aerogenes* was found to be 2mg/ml for *P.aeruginosa* (3.75 mg/ml) for *S.epidermidis* (2.5 mg/ml), *B.subtilis* (2.25 mg/ml) while no activity was shown by this extract against *S.typhi* Fig. 2. Petroleum ether was when compared to the standard antibiotics it showed better performance in inhibiting bacterial growth than ampicillin, penicillin, and doxycycline against *E.aerogenes* (8mm), and *P.aeruginosa*(12mm).It also has better effective than penicillin, and doxycycline against *B.subtilis* (14mm),but less effective than gentamicin against all the pathogenic bacteria used for the study. When compared with streptomycin it was also found to be less effective against all the bacteria used except *S.epidermidis*, which was found to be resistant towards streptomycin. Similarly when compared with tetracycline it was observed less effective against all the bacteria except one gram negative bacteria i.e. *P.aeruginosa* Fig. 3. Ethyl acetate showed highest activity against *E.aerogenes* (19mm) followed by *S.typhi* (18mm) and *P.aeruginosa* (16mm) and almost similar zone of inhibition was observed in *S.epidermidis* and *B.subtilis* (15mm). Ethyl acetate showed good inhibitory effect in gram negative bacilli when compared with gram positive bacilli Fig1. The MIC of the extract for *E.aerogenes*, *P.aeruginosa* and *B.subtilis* was found to be (2mg/ml) *S.typhi* (1.75mg/ml) and *S.epidermidis* (1.5 mg/ml).Table:2When the extract was compared with the broad spectrum antibiotics used for the study it was found to have better inhibitory effect than tetracycline, ampicillin, penicillin, doxycycline and gentamicin against *E.aerogenes* and *S.typhi*. When compared with streptomycin ethyl acetate was also found less effective against all the pathogenic bacteria used for the study

except *S.epidermidis*. Similarly when compared with penicillin, ethyl acetate was found to have better effective against all the gram negative bacteria (*E. aerogenes*, *P.aeruginosa*, and *S.typhi*) and one gram positive bacteria i.e. *B.subtilis*, but less effective against *S.epidermidis* (23mm). Similarly when compared with tetracycline, revealed that it was effective against all the gram negative bacteria *E. aerogenes* (16mm), *P.aeruginosa*, and *S.typhi*(10mm), but less effective against gram positive bacteria *S.epidermidis* (28mm) and *B.subtilis* (23mm) Fig. 3. The analysis of ethanolic bark extract revealed that it possesses maximum activity against *E. aerogenes* (29mm) followed by *P.aeruginosa* (28mm) and lowest in *S.epidermidis* (16mm) and almost equal zone of inhibition was observed in *B.subtilis* and *S.typhi* (19mm). Gram negative bacteria were more sensitive towards this extract as compared with gram positive bacteria. The similar MIC was observed in *P.aeruginosa*, *B.subtilis* and *S.typhi* (1.5 mg/ml) followed by *E. aerogenes* (1.25 mg/ml) and *S.epidermidis* (1mg/ml). When the ethanol was compared with the broad spectrum antibiotics it was found to have better inhibitory effect than tetracycline, ampicillin, penicillin, and doxycycline against all gram negative bacteria (*E. aerogenes*, *P.aeruginosa*, and *S.typhi*) while against *B.subtilis* it was less effective than tetracycline, ampicillin

and streptomycin, and more effective than penicillin, and doxycycline. Similarly against *S.epidermidis* it was found to be more effective than ampicillin and streptomycin and less effective than tetracycline (28mm), doxycycline (25mm), gentamicin (23mm) and penicillin (23mm). The analysis of aqueous bark extract showed maximum activity against *E. aerogenes* (32mm) and the least zone of inhibition was observed in *S.epidermidis* (16mm) and similar zone of inhibition was observed in *B.subtilis* and *S.typhi* (30mm). Gram negative bacteria were more sensitive towards aqueous extract when compared with gram positive bacilli. Similarly the MIC of aqueous bark extract was found to be same in *P.aeruginosa*, *B.subtilis* and *S.typhi* (1mg/ml). In *S.epidermidis* it was observed to be (1.5 mg/ml) while in case of *E. aerogenes* it was (0.75mg/ml). When the aqueous bark extract was compared with the broad spectrum commercially used antibiotics it was found to have greater zone of inhibition against all the gram negative bacilli [*E. aerogenes* (32mm), *P.aeruginosa* (30mm), and *S.typhi* (30mm)] as well as one gram positive bacterial strain i.e. *B.subtilis* (30mm), but less effective than tetracycline (28mm), doxycycline (25mm), gentamicin (23mm) and penicillin (23mm) against *S.epidermidis* (16mm).

Figure 1. Antibacterial activity of *Adenanthera pavonina* against different pathogenic bacterial strains by agar cup diffusion method.



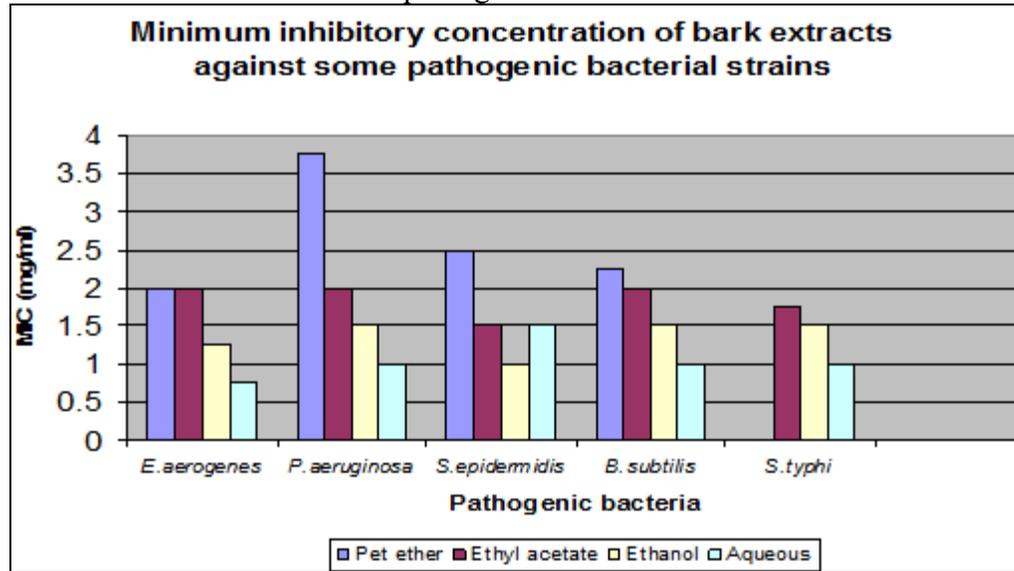
Data is a mean of two replications

“-” No inhibition observed

Antibiotics were used as positive control

10% DMSO were used as negative control (No inhibition observed)

Figure 2. Minimum inhibitory concentration of extracts of *Adenantha pavonina* against different pathogenic bacterial strains

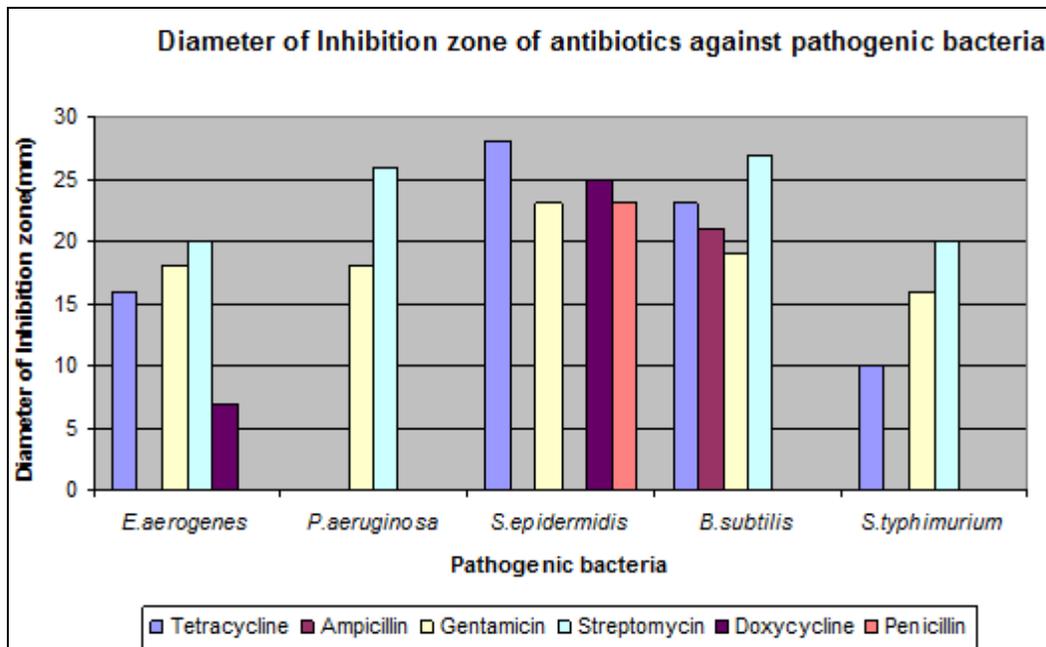


Data is a mean of two replications

“-“No inhibition observed

10% DMSO Negative control

Figure 3. Diameter of Inhibition zones of antibiotics against pathogenic bacterial strains.



Data is a mean of two replications

“-“No inhibition observed

Antibiotics Positive control

DISCUSSION

In vitro studies in the present work concluded that the plant extract inhibited bacterial growth but their effectiveness varied. The antimicrobial activity has been attributed to the presence of some active constituents in the extracts. This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. [27, 28] These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources. [29, 30] In general, the inhibitory activity of bark extracts was seen on both gram positive as well as gram negative strains. In most cases the antibacterial activity of bark extracts was more than the many of the antibiotics used. All the extracts are very effective antibacterial agents, except petroleum ether it was least effective against all the bacterial strains. The results of diameter of zones of inhibition showed that the bark extracts were effective in inhibiting the growth of both gram positive as well as gram negative bacteria up to varying extents, which may be due to the presence of alkaloids, glycosides, flavonoids, tannins, saponins, and triterpenoids.

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