

Potential of Red Sandalwood (*Adenanthera pavonina L.*) as an Antibacterial Agent against Clinical Isolates

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Abstract: The emergence of resistant pathogens is a leading cause of morbidity worldwide. For the treatment of diseases caused by these resistant pathogens, the use of medicinal plants as an alternative to synthetic drugs is increasing. Therefore, the aim of this study was to evaluate the efficacy of hexane and ethanolic extracts of a natural plant *Adenanthera pavonina L.* Antibacterial activity of *Adenanthera pavonina L.* leave extracts and pure compound (β -sitosterol glucoside) against *Enterococcus spp.*, *S. aureus*, *P. aeruginosa*, *S. typhi*, *E. coli*, *Proteus spp.*, *K. pneumonia* and *A. baumannii*, was carried out by well-diffusion assay and micro-dilution technique. Moreover, time-kill tests were carried out to assess the antimicrobial activity of the *Adenanthera pavonina L.* extracts against *E. coli*. The results showed good antibacterial potential of *Adenanthera pavonina L.* extracts against the clinical isolates tested.

Keywords: Antibacterial potential, β -sitosterol glucoside, *Adenanthera pavonina L.*

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INTRODUCTION

Antibiotics, which are either derived from microorganisms or synthesized chemically, provide the base of clinical therapy. Every year, two to three antibiotics were sprung into the global market, but due to the emergence of multi-drug resistant microbial strains, scientist are looking forward for the development of alternative and novel drugs [1]. Natural sources such as plants, offer a wide variety of natural medicinal compounds for therapeutic treatments [1, 2]. Their use for the treatment of diseases is increasing day by day because of their added advantages such as low toxicity, good therapeutic performance, cost-effectiveness and eco-friendliness [3-6]. In order to understand the antimicrobial efficacy of these medicinal plants, their properties should be thoroughly investigated.

The most common plants used for therapeutic purposes worldwide are *Lannea kerstingii*, *Curcuma xanthorrhiza*, *Senna alata*, *Moringa oleifera*, *Adenanthera pavonina L.* and *Pangium edule*. The plant *Adenanthera pavonina L.*, native to the subcontinent was chosen in this study. *Adenanthera*

pavonina L. is also known as Red Bead Tree (Red sandal wood) and belongs to the family Fabaceae [7]. Various parts of this plant have been traditionally used for treating diarrhea, gout and inflammations [8]. Its bark is typically used for the treatment of gonorrhoea, haematuria, ulcers and it is also used as an ointment [9-16].

Several documented phytochemical studies on this plant revealed the presence of secondary metabolites, mainly flavonoids, triterpenoids, tannins, saponins and sterols [5, 10, 11]. The chief constituents are flavonoid compounds [12, 13]. It is used as an aseptic paste and also used to treat boils and inflammation [12, 14]. Its wood is red in color and extremely hard wood timber is used for building purposes and furniture making [12]. It is also able to fix nitrogen [5, 15].

Plants generally synthesize ethyl sterols (sitosterols) while fungi, algae and protozoa, synthesize methyl sterols (ergosterols) [17]. Among the phytosterols, predominant is β -sitosterol which are structurally similar to cholesterol. β -sitosterols are present in natural foods and are considered as the health promoting constituents. They also possess pharmacological activities such as anti-inflammatory activity, immunomodulatory activity, chemoprotective effects, inducing apoptosis and angiogenic effect [18].

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Owing to the therapeutic properties of *Adenanthera pavonina L.*, this study was carried out to evaluate the antibacterial potential of leave extracts and a pure compound- β -sitosterol glucoside, isolated from *Adenanthera pavonina L.*

MATERIALS AND METHODS

Microbial Strains

Total 8 clinical isolates were used in this study which were provided by a clinical laboratory. Out of these 8 isolates, two were Gram-positive (*Enterococcus spp.*, *S. aureus*) and six were Gram-negative (*P. aeruginosa*, *S. typhi*, *E. coli*, *Proteus spp.*, *K. pneumonia* and *A. baumannii*).

Preparation of 0.5 McFarland

McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions. It was prepared by mixing 0.05 ml of 1% $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ with 9.95 ml of 1% H_2SO_4 . A_{600} was taken by using a spectrophotometer and stored in a refrigerator until use [19].

Preparation of Inoculum

Pure microbial isolates were streaked onto nutrient agar medium. Subsequently, isolated colonies were transferred in sterile PBS and turbidity of the suspension was adjusted to 0.5 McFarland standard [20].

Preparation of Extracts

Leaves of *Adenanthera pavonina L.* were soaked in hexane and ethanol for fifteen days, filtered and filtrate was evaporated under reduced pressure by means of rotary evaporator, while the pure compound was isolated from the leaves of *Adenanthera pavonina L.* [21].

Determination of Antimicrobial Potential

The antimicrobial activity of the extracts and pure compound *i.e.* β -sitosterol glucoside was evaluated against the clinical isolates. The procedures, well-diffusion assay and minimal inhibitory concentration (MIC) were carried out as per Clinical Laboratories Standards Institute (CLSI) guidelines to check the antibacterial sensitivity [20, 21]. Time-kill assay of the extracts and compounds against *E. coli* was also investigated. *Berberis vulgaris* extract was used as a positive control throughout the study.

Well-Diffusion Assay

Sensitivity test was performed using agar well-diffusion method. A lawn of bacterial inoculum (matched with 0.5 McFarland turbidity standard) was made on Mueller-Hinton agar plate. The plant extract or β -sitosterol glucoside (100 μl) was added in a 7 mm well punched aseptically in the agar plate with a sterile cork borer and was incubated at 37°C for

24 h. The sensitivity was determined based on the diameter of the zone of inhibition around the well [20-24].

Minimum Inhibitory Concentration (MIC)

The sensitivity of microbes to ethanolic extract was measured by micro dilution method using 96-well microtiter plate. The extract was serially diluted (two-fold) in Mueller-Hinton broth, 10 μl culture was added in each well along with the positive and negative controls and the plates were incubated at 37°C for 24 h. A_{540} was read by ELISA plate reader (Tecan sunrise, Switzerland). MIC's were calculated in percentages and in mg/ml.

Time-Kill Assay

To determine at which time of bacterial growth the test compound exerted its killing effect, a time kill assay was carried out. The experiment was performed by inoculating test organism (*E. coli*) in Nutrient broth and incubating at 37°C for 24 h. It was matched with 0.5 McFarland and broth culture was diluted to 10^{-3} dilution. 1 ml of culture was added with 0.1 ml of extract in dry cuvettes. A_{600} was measured after different time interval at 0 min, 30 min, 60 min, 120 min, 240 min and 1440 min.

Statistical Analysis

Data was organized and tabulated using Microsoft word and Excel 2007. Pearson's correlation was calculated for all three extracts vs. natural antimicrobial agent *Berberis vulgaris*. Time-kill assay graphs were made by using online DMFit, web edition (Dynamic modeling fit).

RESULTS AND DISCUSSIONS

Many infectious diseases have been known from history to be treated with herbal remedies due to their antibacterial activity against a large number of microbes as well as their reasonable cost [25-29]. The activity of plant extracts against bacteria is due to the presence of phytoconstituents, which may vary due to certain factors such as locality of plant growth, harvesting and extraction methods.

In this study two methods were used to determine the efficacy of *Adenanthera pavonina L.* against the bacterial strains *i.e.* the well-diffusion method and the microdilution method (Minimum Inhibitory Concentration).

Antibacterial Activity Determination by Well-Diffusion Assay

The results indicate that the *Adenanthera pavonina L.* leave extracts showed antibacterial activity against the respective clinical isolates with different diameter of zone of inhibition and only *Proteus spp.* showed resistance to all extracts (Table 1). The ethanolic extract was effective against *Enterococcus spp.*, *E. coli*, *S. aureus* and *A. baumannii* with zone of inhibitions of 7 mm, 17.5 mm, 8 mm and 17 mm respective-

ly. *S. aureus*, *P. aeruginosa* and *S. typhi* showed sensitivity with zones of inhibition of 10 mm and *Enterococcus spp.* with 11 mm diameter to hexane extract of *Adenanthera pavonina* L. Similar results have been reported by several workers [30, 31].

The antibacterial activity showed by *Adenanthera pavonina* L. pure compound (β -sitosterol glucoside) to *S. aureus*, *E. coli* and *A. baumannii* measured in diameter was 15 mm, 9

mm and 11 mm respectively. The potential of β -sitosterol as an antibacterial agent from *Senecio lyratus* is documented by Kiprono *et al.* in 2000 [32]. In a study by Sen *et al.* (2012), the antimicrobial activity of pure β -sitosterol is reported ranging from 10 to 14 mm for *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *S. aureus* which is nearly equal to the standard Gentamicin by well-diffusion method.

Table 1. Zone of inhibition. (-) indicates no inhibition *Berberis vulgaris* as a positive control, correlations were significant for hexane ($r = 0.808$, $r^2 = 0.654$) and pure compound (β -sitosterol glucoside) ($r = 0.252$, $r^2 = 0.0063$) and insignificant for ethanolic extract ($r = -0.084$, $r^2 = 0.0071$).

Clinical Isolates	Pure Compound (mm)	Ethanolic Extract (mm)	Hexane Extract (mm)	<i>Berberis Vulgaris</i> (Positive Control) (mm)
<i>S. aureus</i>	15	8	10.5	21
<i>Enterococcus</i>	-	7	11	16
<i>P. aeruginosa</i>	-	-	10	19
<i>E. coli</i>	9	17.5	-	15
<i>S. typhi</i>	-	-	10	15
<i>A. baumannii</i>	11	17	-	10
<i>K. pneumoniae</i>	-	-	-	11
<i>Proteus spp</i>	-	-	-	11

Table 2. Minimum Inhibitory Concentration presented in percentages and in mg/ml, significant correlation for hexane ($r = 0.0922$, $r^2 = 0.0085$) and pure compound ($r = 0.0315$, $r^2 = 0.001$) vs. positive control (*Berberis vulgaris*) while insignificant for ethanolic extract ($r = -0.674$, $r^2 = 0.4553$).

Clinical Isolates	Ethanolic Extract	Hexane Extract (mg/ml)	Pure Compound (mg/ml)	<i>Berberis Vulgaris</i> (Positive Control)
<i>S. aureus</i>	5%	25	25	25%
<i>P. aeruginosa</i>	10%	12.5	25	6.25%
<i>S. typhi</i>	10%	25	50	6.25%
<i>E. coli</i>	10%	25	25	3.1%
<i>Enterococcus</i>	10%	25	25	6.25%
<i>Proteus</i>	0.625%	25	25	12.5%
<i>K. pneumoniae</i>	10%	25	25	1.5%
<i>A. baumannii</i>	10%	25	12.5	3.1%

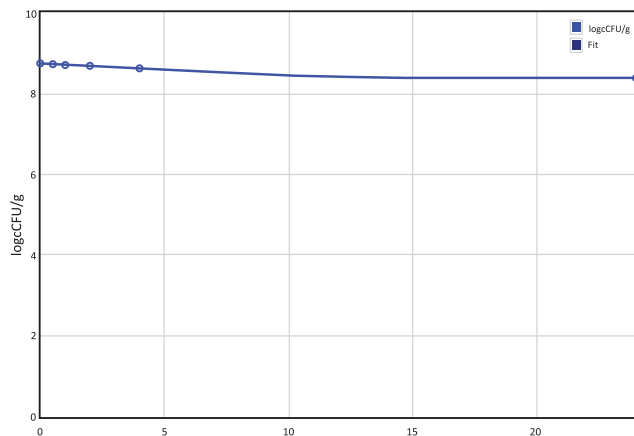


Fig. (1). Time kill of *Berberis vulgaris* positive control fitted in DmFit (Dynamic modeling fit) $R^2=0.997$ and SE of fit 0.00748.

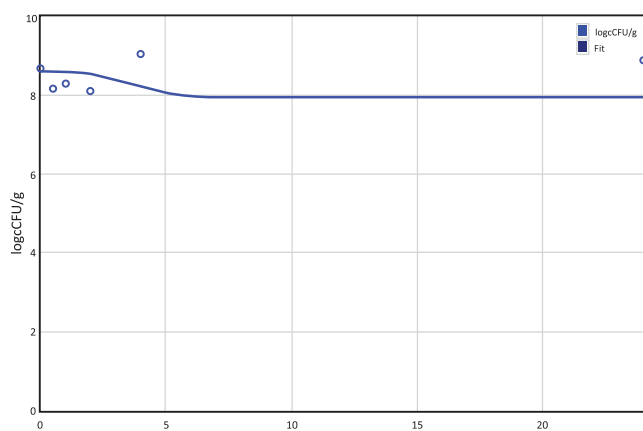


Fig. (2). Time kill of hexane extract of *Adenanthera pavonina* fitted in DmFit (Dynamic modeling fit) $R^2=0.947$ and SE of fit 0.0638.

3.2 Antibacterial Activity Determination by Minimum Inhibitory Concentration (MIC)

In clinical laboratories, the micro-dilution method is used routinely as a quantitative reference method therefore it was used in this study. The minimum inhibitory concentration calculated for *Adenanthera pavonina L.* pure compound (β -sitosterol glucoside) was 12.5 mg/ml to 50 mg/ml (Fig.2), whereas the MIC of the hexane and ethanolic extracts ranged from 12.5 mg/ml to 25 mg/ml and 0.625% to 10% respectively. Some clinical isolates failed to show results in well-diffusion assay while minimum inhibitory concentration was determined against them which might be due to reasons like; heavy bacterial growth, large inoculum size or the insolubility of extracts in agar.

3.3. Time-Kill Assay

Time-kill assay of hexane and ethanolic extracts as well as of β -sitosterol glucosidase was calculated against *E. coli*. The

results were compared with the positive control *Berberis vulgaris* (Fig. 1). Time kill of hexane extract of *Adenanthera pavonina L.* showed the bactericidal activity till two hours after which the extract lost its activity (Fig. 2). Ethanolic extract showed decrease in bacterial count till one hour and stationary phase was observed after that as shown in (Fig. 3). The pure compound β -sitosterol glucoside also exerted its killing effect and a decrease in bacterial count was observed as shown in Fig. (4). The results demonstrated that the test compounds exerted their killing effect by acting on the growing cells.

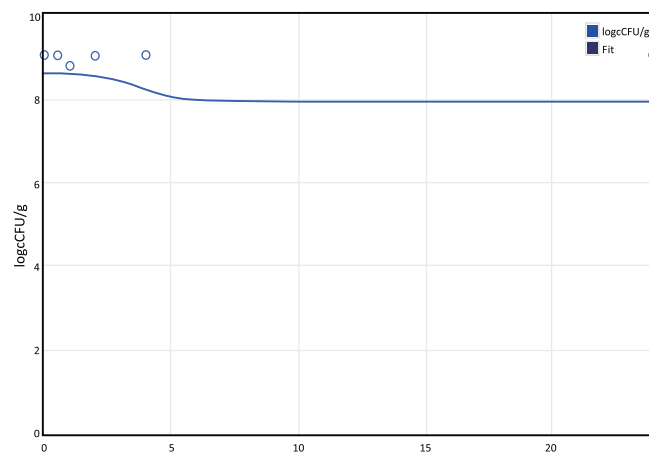


Fig. (3). Time kill of ethanolic extract of *Adenanthera pavonina* fitted in DmFit (Dynamic modeling fit) $R^2=0.947$ and SE of fit 0.0638.

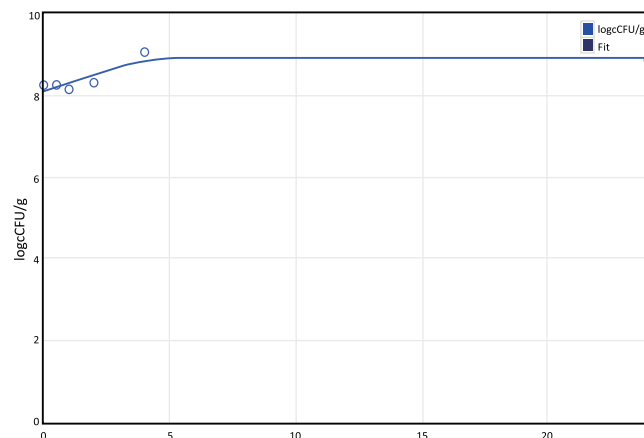


Fig. (4). Time kill of pure compound β -sitosterol glucoside of *Adenanthera pavonina L.* fitted in DmFit (Dynamic modeling fit) $R^2=0.701$ and SE of fit 0.208.

4. CONCLUSIONS

This study demonstrated that *Adenanthera pavonina L.* extracts or their bioactive components, if found non-toxic in animal studies and clinical trials, can be effectively used against the most common bacterial infections.

CONFLICT OF INTEREST

Declared none.

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