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# Antimicrobial Activities of the Leaf Extract of *Sansevieria Liberica* Ger. and Labr. (Fam: Dracaenaceae)

***Eze, C.C.; Inya-Agha, S.I.; Ezugwu, C.O.; Ezea, S.E.**

Department of Pharmacognosy and Environmental Medicine. University of Nigeria, Nsukka.

**ABSTRACT**

The antimicrobial activity of the leaf extract of *Sansevieria liberica* Ger. and Labr. has been studied. The antimicrobial investigation carried out in this study involved the determination of the sensitivity pattern of some microorganisms (bacteria and fungi) to the leaf extracts and the determination of the minimum inhibitory concentration (MIC) of the leaf extracts against sensitive microorganisms. The toxicity test was done to ascertain the toxicity profile and the phytochemical tests were carried out to determine the secondary metabolites present in the extracts. The juices from the fresh leaves were expressed manually and lyophilized (freeze-dried). The toxicity and phytochemical tests were carried out using standard procedures. The crude extract (CE) was then fractionated into n-hexane fraction (HF), chloroform fraction (CF), ethylacetate fraction (EF) and methanol fraction (MF). The crude extract (CE) and the fractions were screened for antimicrobial activity using agar diffusion method. Only the crude extract (CE) and the methanol fraction (MF) exhibited antibacterial activities against Gram –ve *(P. aeruginosa, E. coli*) and Gram +ve (*B. subtilis, S. aureus*) organisms which were comparable to that of chloramphenicol. All were inactive against *Candida albicans* and *Aspergillus niger*. Acute toxicity test on the CE established an oral and intraperitoneal $LD_{50}$ of > 5000 mg/kg in mice. Phytochemical analysis of the crude extract (CE) and the fractions showed the presence of various bioactive substances such as alkaloids, saponins, flavonoids, terpenoids, steroids, glycosides, reducing sugars, tannins, resins, carbohydrates, proteins, acidic compounds, fats and oils. The results of the study, showed that the leaves of *Sansevieria liberica* Ger. and Labr. possess antibacterial activity.

**keywords**:Antimicrobial activity*, Sansevieria liberica,* phytochemical screening.

## INTRODUCTION

Many microorganisms have potentials that can be exploited for human benefits while some can cause infections to man, animals and plants. A good number of microorganisms have been implicated in some disease conditions like eczema, dysentery, cholera, and urinary tract infections [1]. Antimicrobial agents interfere with the growth or activity of these microorganisms and they are named based on the class of organisms they attack like antibacterial, antifungal, antiprotozoal and antiviral agents when they attacks bacteria, fungi, protozoa and virus respectively.Infection by microorganisms has been implicated as one of the initiators of inflammatory response [2-3]. Hence, it follows that elimination of the pathogenic microorganisms by antimicrobial agents will certainly attenuate the inflammatory response.

Today, about one-quarter of the prescription drugs dispensed by community pharmacists contain at least one active ingredient derived from plant material [4].

*Sansevieria libericaGer.* and *Labr.* is a tropical, West African perennial, rhizomatous plant and an erect herb with several stiffred-edged, elliptic leaves, arising from the rhizome with one to three or more leaves in a clump. The plant grows wide green mottled leaves which are upright, up to 60 cm long and 6 – 10 cm broad, transversely marked with dark and light green bands, and the margins are marked with red and white lines. The inflorescence is longer than the leaves with abundant white flowers which are borne on interrupted common stalks. The fruits are reddish, almost round about 1.25 cm long and each fruit contains one seed. It is used in traditional

medicine to cure numerous ailments like cough, hemorrhoids, infections, inflammation, tooth pain, feverish headache, cold, convulsions, as vermifuge, as stimulating tonic, ulcer, small pox sores, asthma, sexual weakness, hypertension, diarrhoea, abdominal pains, colic, gonorrhoea, eczema, piles, snake and dog bites, jaundice, auria, palpitation, viral hepatitis and malaria [5 - 8]. The cure characteristics and physicomechanical properties of natural rubber vulcanizates filled with fibres of *S. liberica* Ger. and Labr.and carbon black have been investigated and compared [9]. Its amino-acid, mineral and vitamin composition as well as antidiarrhoeal effect have also been studied [10,11].

The present study seeks to evaluate the antimicrobial activity of the leaf extract of *S. liberica* Ger. and Labr. against isolates of some pathogenic fungi, Gram negative and Gram positive bacteria, determine the safety of the plant and identify the phytochemical constituents responsible for its effect.

## MATERIALS AND METHODS

### Materials

### Chemicals and Drugs

All the chemicals used for the extraction and fractionation, phytochemical screening, toxicity profile and antimicrobial analysis were of analytical grade and used as procured from the manufacturers; methanol (sigma-aldrich, Germany), n-hexane (sigma-aldrich, Germany), ethylacetate (sigma-aldrich, Germany), chloroform (sigma-aldrich, Germany), chloramphenicol (Hovid).

### Collection and identification of plant material

Fresh leaves of *Sansevieria liberica* Ger. and Labr.were collected in August, 2009 from People's Flowering Garden in Nsukka, Nsukka Local Government Area, Enugu-State, Nigeria and identified by Mr. A. O. Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu-State, Nigeria. The herbarium specimen number is 167 and it is deposited in Pharmacognosy herbarium, University of Nigeria, Nsukka.

### Animals and Microorganisms

The animal experimental protocols were approved by our institution's Animal Ethics Committee and were in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC) [12]. These includes the in–bred albino mice of either sex (24 – 36g), obtained from the animal house, Department of Zoology, University of Nigeria, Nsukka. The mice were fed on standard pellets marketed by Pfizer Livestock Feeds (Nig.) Limited. Microorganisms used were Gram –ve organisms (*P.aeruginosa, E. coli)*, Gram +ve organisms (*B. subtilis, S. aureus)*, and fungi (*A. niger and C. albicans)* which were obtained from the Pharmaceutical Microbiology laboratory, Department of Pharmaceutics, University of Nigeria, Nsukka.

### Preparation of Plant Material

The juice from the fresh leaves was expressed manually (crude extract), sieved and stored in containers which was later lyophilized (with Amsco-Finn-Aqua, Lyovac GT3 freeze dryer) at NIPRD Abuja and stored in air-tight containers in the refrigerator for subsequent use. These samples were brought out and allowed to assume room temperature prior to use for analysis.

### Extraction and Fractionation

Sixty six grammes of the crude extract (CE) was adsorbed on silica gel and eluted in succession with n – hexane, chloroform, ethylacetate and methanol to yield hexane (HF), Chloroform (CF), ethylacetate (EF) and methanol (MF) fractions. The CE and the fractions HF, CF, EF and MF were screened for antimicrobial activity.

### Phytochemical Screening

Chemical tests were carried out on the CE and on the fractions using standard procedures and by characteristic colour changes as described by other authors [13-16].

### Acute toxicity test

The acute toxicity profile of the extract was assessed using standard procedures [17].

### Test for antimicrobial activity

### Preparation of Culture Media

The growth media employed were nutrient agar, nutrient broth and Saboraud's dextrose agar and all were prepared using the methods specified in the oxoid manual.

### Preparation of standard suspensions of microorganism

The bacterial and fungal suspensions used were prepared using standard procedures [18].

**Determination of sensitivities of microorganisms to crude extract (CE) and the fractions**

The ability of the CE and the fractions to inhibit the microorganisms was evaluated using agar diffusion method [19].

Ten milliliters of molten nutrient agar at 45° C was inoculated with 0.1ml of each of the bacteria and fungal suspension and poured into a sterile Petri dish. The content was thoroughly mixed and was allowed to set. Six cups were bored onto the seeded agar media using a sterile cork borer of 8mm in diameter. Standard solutions of the extract/fractions were prepared in dimethylsulfoxide (DMSO) and 0.5 ml of each transferred to a cup. This was done with plates containing each of the organisms. The plates were allowed to stand at room temperature for 15 min to allow for pre-diffusion and incubated at 37° C for 24 h for bacteria and 27° C for 48 h for fungi.

The inhibition zone diameters (1ZD) were measured.

**Determination of Minimum Inhibitory Concentration (MIC)**

The MIC was determined using serial dilution method [20]. A 2 – fold serial dilution of each of the extract/fractions was carried out to obtain 100, 50, 25 and 12.5 mg/ml in DMSO. Each of the broth cultures was mixed with 15 ml of molten agar at 45° C by transferring 0.1 ml of each culture into the nutrient agar using a sterile pipette. The molten seeded nutrient agar was poured into a petri-dish and was allowed to set. Holes of about 8.0 mm in diameter were bored onto the seeded plates with a sterile cork borer.

The prepared different concentrations of each of the crude extract and the fractions were transferred into the cups of the seeded plates with each microorganisms. The plates were allowed to stand at room temperature for 15 min to allow for pre-diffusion before incubating at 37° C for 24 h.

After 24 hours, the IZDs were measured. IZD was obtained by subtracting the hole diameter from the zones of inhibition. A plot of the square of the IZD ($IZD^2$) of the crude extract and the fractions against the corresponding log concentration was made. The minimum inhibitory concentration (MIC) was calculated from the graph and recorded. Chloramphenicol was used as the control.

**RESULTS**

The extraction process yielded 240 g of the CE, and fractionation process yielded 0.16 g (0.24 % $^{W}/_{W}$) of n – hexane fraction, 1.10 g (1.7 % $^{W}/_{W}$) of chloroform fraction, 0.70 g(1.06 % $^{W}/_{W}$) of ethylacetate fraction and 10.52 g (16.0 % $^{W}/_{W}$) of methanol fraction. Phytochemical Screening revealed the presence of fats and oils, flavonoids, saponins, proteins, steroids, terpenoids, tannins, reducing sugars, carbohydrates, acidic compounds, alkaloids and glycosides for CE, resins, fats and oils, flavonoids, saponins, proteins, steroids, terpenoids, tannins, reducing sugars, carbohydrates, acidic compounds, alkaloids and glycosides for MF, resins, fats and oils, flavonoids, steroids, and terpenoids for EF, resins, fats and oils, flavonoids, steroids and terpenoids for CF, and resins, fats and oils, steroids and terpenoids for HF (Table 1).

**Table 1**:Results of phytochemical analysis

| S/N | Phytochemical Constituents | Relative abundance CE | MF | EF | CF | HF |
|---|---|---|---|---|---|---|
| 1 | Resins | + | + | ++ | +++ | +++ |
| 2 | Fats and Oils | + | + | + | ++ | +++ |
| 3 | Flavonoids | ++ | + | +++ | + | - |
| 4 | Saponins | +++ | +++ | - | - | - |
| 5 | Proteins | +++ | +++ | - | - | - |
| 6 | Steroids | + | + | ++ | ++ | +++ |
| 7 | Terpenoids | + | + | ++ | ++ | +++ |
| 8 | Tannins | +++ | +++ | - | - | - |
| 9 | Reducing sugars | ++ | ++ | - | - | - |
| 10 | Carbohydrates | +++ | +++ | - | - | - |
| 11 | Acidic compounds | +++ | +++ | - | - | - |
| 12 | Alkaloids | ++ | ++ | - | - | - |
| 13 | Glycosides | ++ | +++ | - | - | - |

Key

CE = Crude extract
MF = Methanol fraction
EF = Ethylacetate fraction
CF = Chloroform fraction
HF = n-hexane fraction

\- = Not present
\+ = Present
++ = Present in moderately high concentration.
+++ = Present in very high concentration

Acute toxicity and lethality tests indicated no death in the two phases of the tests and the $LD_{50}$ was thus

established to be > 5000 mg/kg. The activities of the crude extract (CE), the fractions and chloramphenicol against the used microorganisms are listed in Table 2.

**Table 2**: Sensitivity Patterns for the Test Organisms

| Microorganisms | CE (100mg/ml) | HF (100mg/ml) | CF (100mg/ml) | EF (100mg/ml) | MF (100mg/ml) | Chloramphenicol (5ng/ml) |
|---|---|---|---|---|---|---|
| *B. subtilis* | + | - | - | - | + | + |
| *S.aureus* | + | - | - | - | + | + |
| *P. aeruginosa* | + | - | - | - | + | + |
| *E. coli* | + | - | - | - | + | + |
| *A. niger* | - | - | NT | NT | NT | NT |
| *C. albicans* | - | - | NT | NT | NT | NT |

Key : - = Not sensitive, + = Sensitive, NT = Nottested, CE = Crude extract, HF = n-hexane fraction, EF = ethylacetate fraction, MF = Methanol fraction.

The inhibition zone diameters IZD (mm) against the respective concentrations (mg/ml), log concentration and $IZD^2$ ($mm^2$) are shown in Table 3 while their plots of the square of inhibition zone diameters ($IZD^2$) (mm) against the log concentration for the crude extract (CE), methanol fraction (MF) and chloramphenicol are shown in Figs.1, 2 and 3 respectively.

The minimum inhibitory concentration (MIC) of the crude extract (CE), methanol fraction (MF) and chloramphenicol (mg/ml) are shown to be 8.76, 3.98, 0.55, against *Bacillus subtilis,* 11.84, 7.26, 0.50 against *Staphylococcus aureus*, 8.50, 4.01, 0.54 against *Pseudomonas aeruginosa* and 11.23, 4.66, 0.50 against *Escherchia coli* respectively as shown in Table 4.

## DISCUSSION

Phytochemical screening of the crude extract and the fractions revealed the presence of biologically active constituents such as glycosides, alkaloids, tannins, carbohydrates, flavonoids, steroids, saponins, terpenoids, etc. The antimicrobial activities of most plant extracts can be traced to these bioactive constituents hence the presence of tannins, alkaloids, flavonoids and saponins suggests possible antimicrobial activity by a plant as proposed by earlier workers [21, 22]. More specifically, the presence of fats and oils, alkaloids, saponins, tannins and reducing sugars is a further indication of the probable antidiarrhoeal activity of the aqueous root extract of *S. liberica* and that supports the medicinal use of the plant as observed by earlier workers [11, 23, 24]. The results of the toxicological studies established the $LD_{50}$ of the CE to be >5000mg/kg and that implies that the leaves of *S. liberica* are safe. Possibilities are remote for acute intoxication of the leaves in humans.

In the sensitivity tests, the Gram +ve (*B. subtilis, S. aureus*) and Gram –ve *(P. aeruginosa, E. coli*) organisms were sensitive to the CE and MF and were not sensitive to HF, CF, and EF. The insensitivity of these organisms to HF, CF, and EF may be due to the absence of saponins, proteins, tannins, reducing sugars, carbohydrates, acidic compounds, alkaloids, and glycosides in them, as some of these constituents are known to possess antimicrobial activity [21-24] (Table 1). *A. niger* and *C. albicans* were also not sensitive to both the crude extract (CE) and all the fractions. The antibacterial activity of the CE and the MF at 100 mg/ml against these sensitive organisms were comparable to that of chloramphenicol at 5 ng/ml.

Chloramphenicol produced the highest IZD (mm) at lower concentration followed by the methanol fraction (MF) and lastly the crude extract (CE). This antibacterial activity has justified the traditional uses of the plant in treating gonorrhea, diarrhea, UTIs, eyes and ears infections [5-7, 23, 24]. It is evident, therefore that the non antifungal effects of the leaf extract from *S. liberica* do not appear to justify the ethno-medicinal uses of the plant as a remedy for eczema [7]. It may be reasonable to infer that factors such as geographical source, seasonal variation and climatic conditions may have affected the plant's antifungal principles as reported by earlier workers [25-29].

**Table 3**: Concentrations (mg/ml), log concentration, IZD (mm) and $IZD^2$ (mm) for the crude extract (CE), methanol fraction (MF) and chloramphenicol.

| CE, MF and Chloramphenicol | Microorganism | Concentration (mg/ml) | Log-concentration | IZD(mm) | $IZD^2$ ($mm^2$) |
|---|---|---|---|---|---|
| | *B. subtilis* | 100.00 | 2.0000 | 16.00 | 256.00 |
| | | 50.00 | 1.6990 | 12.00 | 144.00 |
| | | 25.00 | 1.3979 | 10.00 | 100.00 |
| | | 12.50 | 1.0969 | 7.00 | 45.00 |
| CE | | | | | |
| | *S. aureus* | 100.00 | 2.0000 | 12.00 | 144.00 |
| | | 50.00 | 1.6990 | 8.00 | 64.00 |
| | | 25.00 | 1.3979 | 6.00 | 36.00 |
| | | 12.50 | 1.0969 | 4.00 | 16.00 |
| | *P. aeruginosa* | 100.00 | 2.0000 | 19.00 | 361.00 |
| | | 50.00 | 1.6990 | 16.00 | 256.00 |
| | | 25.00 | 1.3979 | 12.00 | 144.00 |
| | | 12. 50 | 1.0969 | 8.00 | 64.00 |
| | *E. coli* | 100.00 | 2.0000 | 15.00 | 225.00 |
| | | 50.00 | 1.6990 | 11.00 | 121.00 |
| | | 25.00 | 1.3979 | 8.00 | 64.00 |
| | | 12.50 | 1.0969 | 5.00 | 25.00 |
| | *B. subtilis* | 100.00 | 2.0000 | 20.00 | 400.00 |
| | | 50.00 | 1.6990 | 18.00 | 324.00 |
| | | 25.00 | 1.3979 | 15.00 | 225.00 |
| | | 12.50 | 1.0969 | 12.00 | 144.00 |
| | *S. aureus* | 100.00 | 2.0000 | 15.00 | 225.00 |
| MF | | 50.00 | 1.6990 | 14.00 | 196.00 |
| | | 25.00 | 1.3979 | 10.00 | 100.00 |
| | | 12.50 | 1.0969 | 7.00 | 49.00 |
| | *P. aeruginosa* | 100.00 | 2.0000 | 17.00 | 289.00 |
| | | 50.00 | 1.6990 | 15.00 | 225.00 |
| | | 25.00 | 1.3979 | 13.00 | 169.00 |
| | | 12.50 | 1.0969 | 10.00 | 100.00 |
| | *E. coli* | 100.00 | 2.0000 | 17.00 | 289.00 |
| | | 50.00 | 1.6990 | 15.00 | 225.00 |
| | | 25.00 | 1.3979 | 12.00 | 144.00 |
| | | 12.50 | 1.0969 | 10.00 | 100.00 |
| | *B. subtilis* | 0.02 | -1.6990 | 40.00 | 1600.00 |
| | | 0.01 | -2.0000 | 35.00 | 1225.00 |
| | | 0.005 | -2.3010 | 24.00 | 576.00 |
| | | 0.0025 | -2.6021 | 16.00 | 256.00 |
| | *S. aureus* | 0.02 | -1.6990 | 35.00 | 1225.00 |
| | | 0.01 | -2.0000 | 28.00 | 784.00 |
| chloramphenicol | | 0.005 | -2.3010 | 19.00 | 361.00 |
| | | 0.0025 | -2.6021 | 14.00 | 196.00 |
| | *P. aeruginosa* | | | | |
| | | 0.02 | -1.6990 | 24.00 | 576.00 |
| | | 0.01 | -2.0000 | 19.00 | 361.00 |
| | | 0.005 | -2.3010 | 14.00 | 196.00 |
| | | 0.0025 | -2.6021 | 10.00 | 100.00 |
| | *E. coli* | 0.02 | -1.6990 | 27.00 | 729.00 |
| | | 0.01 | -2.0000 | 18.00 | 324.00 |
| | | 0.005 | -2.3010 | 15.00 | 225.00 |
| | | 0.0025 | -2.6021 | 11.00 | 121.00 |

$IZD^2$(mm)

Vol. 3, pp. 14-21, 2011

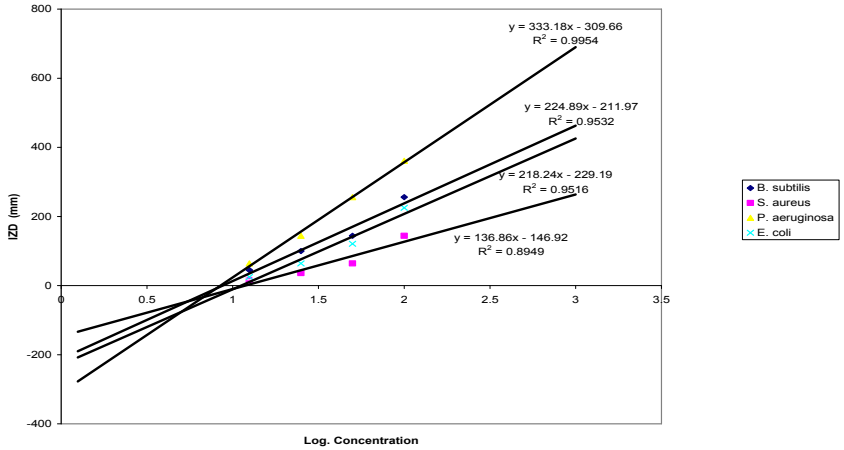


**Fig. 1**: Plots of IZD$^2$ (mm) Versus Log. Concentration of the crude extract (CE) for the test organisms

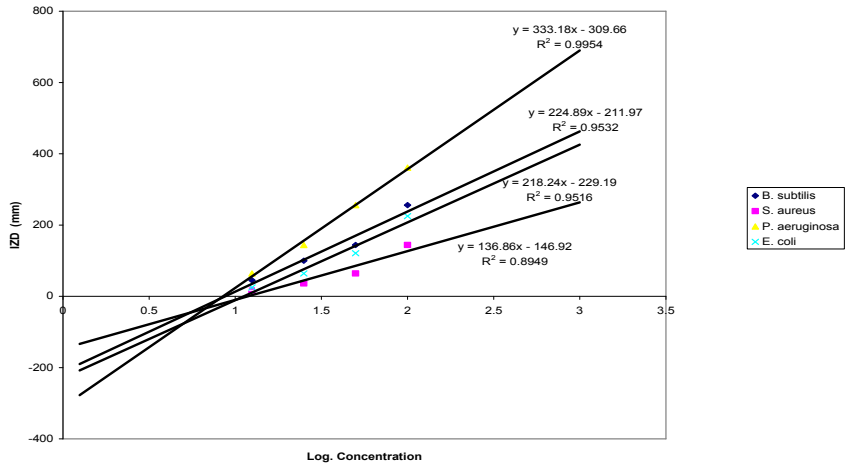


**Fig**. 2: Plots of IZD$^2$ (mm) Versus Log. Concentration of the methanol fraction (MF) for the test organisms

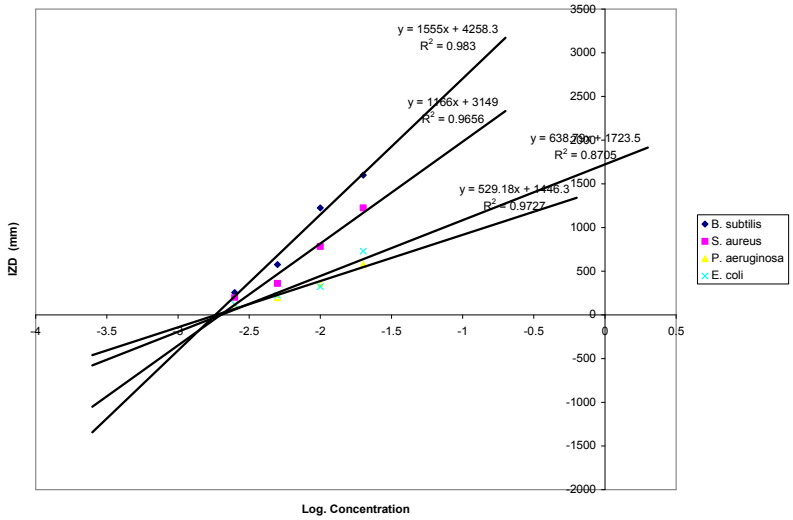


**Fig.** 3: Plots of IZD$^2$ (mm) Versus Log. Concentration of the chloramphenicol for the test organisms

**Table 4**: Minimum Inhibitory Concentration (MIC)

| MICROORGANISMS | MIC (mg/ml) | | |
|---|---|---|---|
| | CE | MF | Chloramphenicol |
| *B. subtilis* | 8.76 | 3.98 | 0.55 |
| *S. aureus* | 11.84 | 7.26 | 0.50 |
| *P. aeruginosa* | 8.50 | 4.01 | 0.54 |
| *E. coli* | 11.23 | 4.66 | 0.50 |

CE = crude extract, MF = methanol fraction.

The antibacterial activity could be related to the constituents as both the CE and the MF contained almost the same constituents (Table 1).

It is obvious that the antibacterial property of the plant resides in the bioactive constituents and any further purification on these bioactive constituents responsible for the observed antibacterial effect may result in the development of potent antibacterial agent with low toxicity and better therapeutic index.

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Vol. 3, pp. 14-21, 2011