

ANTIMICROBIAL ACTIVITIES OF EXTRACTS FROM *RHAMNUS PRINOIDES*

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Abstract

Hexane, chloroform, ethyl acetate and methanol extracts from leaves and stem-bark of *Rhamnus prinoides* were evaluated for their inhibitory effect against six bacterial isolates viz. *Escherichia coli* (wild), *Escherichia coli* (H0157), *Staphylococcus aureus*, *Listeria monocytogens*, *Pseudomonas aeruginosa*. and *Serratia marcescens* and two fungal isolates viz. *Penicillium digitatum* and *Candida albicans*. The zones of inhibition were found to be in the range of 8.8±3.0 to 15.8±1.4 mm diameter against bacterial isolates and 10.3±1.8 to 14.3±2.5 mm diameter against fungal isolates. Additionally, all these extracts were evaluated for their minimum inhibitory concentrations (MICs) and were found to be in the range of <31.25 to > 1000 µg/mL. *R. prinoides* have been used to treat variety of diseases such as TB, pneumonia, rheumatism, gonorrhoea, bladder and kidney problems etc.

Keywords: *Rhamnus prinoides*, *Rhamnaceae*, antibacterial activity, antifungal activity, hexane extract, chloroform extract, ethyl acetate extract, methanolic extract.

Introduction

Rhamnus prinoides belongs to the Rhamnaceae family [1]. It is also known by other names such as African Dogwood, Glossy-leaf etc. [1] and the vernacular name is mofifi (in Sesotho) [1]. *R. prinoides* grows in evergreen forests, in the wild, along streams etc. and reaches up to 4.5-meter height [2-5]. The leaves begin with pale green and turn to shiny and dark green on maturation. The roundish red berries attract bees and domestic fowl [6]. *R. prinoides* casts a very deep shade such that it will not be allowed other plants to grow around it. *R. prinoides* flowers towards the end of the year and fruits at the beginning of the year [7]. *R. prinoides* finds therapeutic applications in the traditional medicine. The decoction of roots has been used to treat pulmonary tuberculosis, pneumonia, bladder and kidney problems etc. [8, 9] The decorticated roots has been used to cleanse the blood, to relief muscular rheumatism and as a remedy for gonorrhoea. An extract of the root together with the bark of *Erythrina tomentosa* has been used to relief colic [10]. The bark has been used to induce vomiting [11]. The leaves have been applied as a liniment to simple sprains. *R. prinoides* has also been used to provide a special aroma and flavour [12, 13]. Secondary metabolites such as emodin, physcion, prinoidin, rhamnazin, naphthalenic glucoside, geshoidin and many other emodin-derived compounds have been reported from *R. prinoides* [14, 15]. The Kingdom of Lesotho is blessed with plant biodiversity. However, the biological and pharmacological activities of these plants have largely been unexplored. The aim of this study was to evaluate the antimicrobial activities of hexane, chloroform, ethyl acetate and methanol extracts from leaves and stem-bark of *R. prinoides* against six bacterial strains viz. *Escherichia coli* (wild), *Escherichia coli* (H0157), *Staphylococcus aureus*, *Listeria monocytogens*, *Pseudomonas aeruginosa* and *Serratia marcescens* and two fungal isolates viz. *Candida albicans* and *Penicillium digitatum*. The results are communicated in this article.

Methods

Plant materials

The leaves and stem-bark of *R. prinoides* were collected from the foothills of Popa and Popanyane mountains at Mokhokhong village, Roma, Maseru district, the Kingdom of Lesotho, Southern Africa. A voucher specimen viz. Santi/RPLS/2018 for leaves and Santi/ RPSB/2018 for stem-bark were kept separately in the Organic Research Laboratory, Department of Chemistry and Chemical Technology, Faculty of Science and Technology, National University of Lesotho, Roma Campus, Maseru district, Kingdom of Lesotho, Southern Africa.

Processing of materials

The leaves were allowed to air dry at room temperature for two weeks and then ground into powder using a commercial blender (Waring Blender, Blender 80119, Model HGB2WT93, 240V AC, 50-80 Hz, 3.6AMPs, Laboratory and Analytical Supplies). The chopped stem-barks were allowed to air dry at room temperature for two weeks and ground into powder using the blender.

Preparation of plant extracts

The powdered leaves (300.043g) of *R. prinoides* was extracted with methanol for 3 days. The solution was filtered using a filter paper (Boeco, Germany). The solvent was removed by *vacuo* and the extract was collected. The same procedure was repeated once again. Finally, the plant material was extracted with hot methanol. 40.1858g of combined methanol extract was obtained after removal of solvent. The same extraction procedure was followed to get hexane (3.2274g), chloroform (10.6285g) and ethyl acetate (11.4763g) extracts from 300.254, 300.131 and 299.921g of powdered leaves, respectively. The powdered stem-bark (299.530g) of *R. prinoides* was extracted first with methanol at room temperature for 3 days followed by a reflux condition for 6 hours. 32.2047g of combined methanol extract was obtained after removal of solvent. The same extraction procedures were followed to get hexane (2.5895g), chloroform (5.4327g) and ethyl acetate (8.1493g) extracts from 300.014, 300.157, 300.422g of powdered stem-bark, respectively.

Microorganisms

Six bacterial isolates viz. *Escherichia coli* (wild), *Escherichia coli* (H0157), *Staphylococcus aureus*, *Listeria monocytogens*, *Pseudomonas aeruginosa* and *Serratia marcescens* and two fungal isolates viz. *Penicillium digitatum* and *Candida albicans* were used for this study. All these microorganisms were available in the Department of Biology, Faculty of Science and Technology, National University of Lesotho, Roma campus, Maseru district, Kingdom of Lesotho, Southern Africa.

Evaluation of antimicrobial activity

The antimicrobial activities of various extracts were evaluated by *in vitro* by hole-plate diffusion method as described in literature [16,17]. Briefly, solutions of various extracts were prepared separately at a concentration of 100 mg of extract in 1 mL of DMSO. The solutions were then filtered separately using a 0.20µm filter and then used for both antibacterial and antifungal activities. A volume of 0.1 mL of the broth culture was spread on the NA (Nutrient Agar) plates. The agar wells of size 4.00 mm height and 6.00 mm diameter were punched on the agar plate using a sterile cork-borer and filled with 30 µL aliquots of the extract. The Petri plates were then incubated at 37°C for 24 hours. Tetracycline served as positive control for *E. coli* (wild), *E. coli* (H0157), *S. aureus* and *L. monocytogens*. Amoxicillin served as positive control for *P. aeruginosa* and *S. marcescens*. DMSO served as negative control. For antifungal assay, the Petri plates containing 25mL of Potato Dextrose Agar (PDA) were used. The agar plates were first spread with 0.1 mL of fungi and cylindrical cavities of size 4.00mm height and 6.00mm diameter were punched using a sterile cork-borer and filled with 30 µL aliquots of the extract. The plates were incubated at 24°C for 48 hours. Miconazole nitrate served as positive control for *C. albicans*. However, we did not maintain positive control for *P. digitatum*. DMSO served as negative control. The sensitivity of microorganism species to the various extracts of *R. prinoides* was determined by measuring the diameter of inhibition zones on the agar surface around the holes. All experiments were performed in duplicates and results were reported as the average of two experiments. A clear zone >

10 mm are considered as positive results [18]. Inhibition zones of <12, 12-20 and ≥20 mm diameter are expressed as weak, moderate and strong activities, respectively.

Determination of minimum inhibitory concentrations (MICs)

The MIC is the minimum inhibitory concentration of the sample needed to inhibit the growth of the microorganisms [19]. The MIC values of < 100 µg/mL, 100 to ≤ 625 µg/mL and > 625 µg/mL were considered as significantly active, moderately active and weakly active, respectively [20-22]. The MICs were determined as described in literature [19,23]. Briefly, a stock solution at a concentration of 1000 µg/mL of various extracts of *R. prinoides* were prepared separately. Two-fold serial dilutions such as 1000, 500, 250, 125, 62.5 and 31.25 µg/mL were made from the stock solutions. A suspension of the microorganisms was prepared at a concentration of 1 X 10⁶ to 2 X 10⁶ colony forming units (CFU) per mL by growing the strains in nutrient broth in an incubator with continuous shaking [22] and then used against various extracts. The cylindrical cavities of size 4.00 mm height and 6.00 mm diameter were punched on the agar plates using a sterile cork-borer and then filled with 30 µL aliquots of the extract. The plates were then incubated at 37°C for 24 hours.

Statistical analysis

The SPSS 17.0 statistic program by means of two-way analysis of variance was performed for data analysis and the differences were considered statistically significant when $p \leq 0.05$.

Results

The antibacterial and antifungal activities of various extracts of *R. prinoides* are summarized in Table 1. RPHELS, RPCHLS, RPEALS and RPMELS are *R. prinoides* hexane leaves extract, chloroform leaves extract, ethyl acetate leaves extract and methanol leaves extract, respectively. RPHEsb, RPCHsb, RPEASb and RPMESb are *R. prinoides* hexane stem-bark extract, chloroform stem-bark extract, ethyl acetate stem-bark extract and methanol stem-bark extract, respectively. Against *E. coli* (wild), RPHELS, RPCHLS, RPEALS and RPMELS

showed inhibition zones of 11.1 ± 2.1 , 13.4 ± 1.0 , 09.1 ± 1.1 and 12.6 ± 2.4 mm, respectively. RPHEBS, RPCHSB, RPEASB and RPMESB showed inhibition zones of 11.3 ± 2.1 , 13.3 ± 1.0 , 12.9 ± 2.0 and 12.9 ± 3.0 mm, respectively. RPHELS, RPEALS and RPHEBS were weakly active with inhibition zones of 11.1 ± 2.1 , 09.1 ± 1.1 and 11.3 ± 2.1 mm. while RPCHLS, RPEASB, RPMESB and RPCHSB were moderately active with the inhibition zones greater than 12.0 mm but less than 14.0 mm. The positive control, tetracycline, showed inhibition zone of 19.5 ± 3.5 mm against the same bacteria. Against *E. coli* (H0157), RPHELS, RPCHLS, RPEALS and RPMELS showed inhibition zones of 10.8 ± 2.0 , 09.8 ± 1.7 , 12.4 ± 1.4 and 10.3 ± 2.0 mm, respectively. RPHEBS, RPCHSB, RPEASB and RPMESB showed inhibition zones of 10.8 ± 1.9 , 13.3 ± 1.1 , 11.5 ± 1.6 and 13.0 ± 1.6 mm, respectively. RPHELS, RPCHLS, RPMELS, RPHEBS and RPEASB were weakly active with inhibition zones less than 12.0 mm. However, RPEALS, RPCHSB and RPMESB were moderately active with inhibition zones between 12 and 14 mm. The positive control, tetracycline, showed inhibition zone of 22.5 ± 2.1 mm against the same bacteria.

Against *S. aureus*, RPHELS, RPCHLS, RPEALS and RPMELS showed inhibition zones of 14.3 ± 2.9 , 14.3 ± 2.1 , 13.3 ± 2.4 and 13.8 ± 3.9 mm respectively. RPHEBS, RPCHSB, RPEASB and RPMESB showed inhibition zones of 13.7 ± 2.1 , 15.8 ± 1.4 , 11.5 ± 1.0 and 12.5 ± 2.4 mm. These results showed that all extracts were moderately active except RPEASB which was weakly active with inhibition zone of 11.5 ± 1.0 mm. The positive control, tetracycline, showed inhibition zone of 31.5 ± 0.7 mm. Against *L. Monocytogens*, RPHELS, RPCHLS, RPEALS and RPMELS showed inhibition zones of 14.4 ± 3.5 , 10.8 ± 1.5 , 12.8 ± 4.3 and 14.2 ± 1.5 mm, respectively. RPHEBS, RPCHSB, RPEASB and RPMESB showed inhibition zones of 12.2 ± 1.8 , 12.7 ± 1.4 , 09.3 ± 1.6 and 14.3 ± 1.8 mm, respectively. RPCHLS and RPEASB exhibited weak activity with inhibition zones of 10.8 ± 1.5 and 09.3 ± 1.6 mm, respectively and all other extracts showed moderate activity with inhibition zones greater than 12 mm. The positive control, tetracycline, showed inhibition zone of 22.5 ± 0.7 mm.

Against *P. aeruginosa*, RPHELS, RPCHLS, RPEALS and RPMELS showed inhibition zones of 11.8 ± 1.7 ,

12.8 ± 2.0 , 10.3 ± 1.1 and 14.3 ± 5.7 mm, respectively while RPHEBS, RPCHSB, RPEASB and RPMESB showed inhibition zones of 11.4 ± 1.7 , 12.6 ± 2.2 , 10.4 ± 3.2 and 08.9 ± 4.5 mm, respectively. RPCHLS, RPMELS and RPCHSB showed moderate activity with inhibition zones of 12.8 ± 2.0 , 14.3 ± 5.7 and 12.6 ± 2.2 mm, respectively. All other extracts showed weak activity with inhibition zones less than 12.0 mm. The positive control, amoxicillin, showed inhibition zone of 22.5 ± 2.1 mm. Against *S. marcescens*, RPHELS, RPCHLS, RPEALS and RPMELS showed inhibition zones of 8.8 ± 3.0 , 9.1 ± 1.7 , 12.1 ± 2.7 and 8.4 ± 2.2 mm, respectively. RPHEBS, RPCHSB, RPEASB and RPMESB showed inhibition zones of 8.9 ± 2.8 , 12.4 ± 4.7 , 12.7 ± 3.5 and 8.0 ± 1.5 mm, respectively. RPEALS, RPCHSB and RPEASB showed moderate activity with inhibition zones of 12.1 ± 2.7 , 12.4 ± 4.7 and 12.7 ± 3.5 mm, respectively while all other extracts were weakly active with inhibition zones less than 12 mm. The positive control, amoxicillin, showed inhibition zone of 9.0 ± 0.0 mm.

Against *P. digitatum*, RPHELS, RPCHLS, RPEALS and RPMELS showed inhibition zones of 11.4 ± 1.7 , 10.3 ± 1.8 , 13.7 ± 1.7 and 10.8 ± 2.0 mm, respectively. RPHEBS, RPCHSB, RPEASB and RPMESB showed inhibition zones of 12.6 ± 2.1 , 13.9 ± 1.3 , 14.3 ± 2.5 and 12.3 ± 2.9 mm, respectively. RPEALS, RPHEBS, RPCHSB, RPEASB and RPMESB exhibited moderate activity with inhibition zones ranging from 12.0-15.0 mm while RPHELS, RPCHLS and RPMELS exhibited weak activity with inhibition zones less than 12 mm. Against *C. albicans*, RPHELS, RPCHLS, RPEALS and RPMELS showed inhibition zones of 12.8 ± 2.4 , 13.8 ± 4.7 , 13.4 ± 3.8 and 14.1 ± 2.6 mm, respectively. RPHEBS, RPCHSB, RPEASB and RPMESB showed the inhibition zones of 12.0 ± 2.4 , 11.6 ± 2.4 , 12.3 ± 1.8 and 11.5 ± 2.0 mm, respectively. RPHELS, RPCHLS, RPEALS, RPMELS, RPHEBS and RPEASB were moderately active with inhibition zones greater than 12 mm but less than 15 mm diameter. RPCHSB and RPMESB showed weak activity with inhibition zones of 11.6 ± 2.3 and 11.5 ± 2.0 mm, respectively. The positive control, miconazole nitrate, showed inhibition zone of 25.8 ± 1.8 mm. In general, all extracts exhibited antimicrobial activity against all six bacteria isolates and two fungal isolates but their relative antimicrobial activity varied from one extract to another as shown in Table 1.

The minimum inhibition concentrations (MICs) of various extracts of *R. prinoides* are summarized in Table 2. The MIC value of RPMELS, RPEASB and RPMESEB was found to be with of 62.5 µg/mL for each extract against *E. coli* while RPCHLS, RPEALS, RPHELS, RPCHSB and RPHESEB exhibited MIC values of <31.25, >1000, 125, <31.25 and 250 µg/mL, respectively, against the same bacterial isolates. The MIC value of RPHELS, RPMELS and RPHESEB was found to be 500µg/mL for each extract against *E. coli* H0157. However, the MIC values of RPCHLS, RPEALS, RPCHSB, RPEASB and RPMESEB were found to be >1000, 62.5, 62.5, 250 and <31.25 µg/mL, respectively, against *E. coli* (H0157). The MIC value of RPHELS, RPCHLS, RPHESEB and RPCHSB was determined to be <31.25 µg/mL for each extract against *S. aureus* while the MIC values of RPEALS, RPMELS, RPEASB and RPMESEB were determined to be 62.5, 62.5, 500 and 62.5 µg/mL, respectively, against the same bacterial isolates. The MIC value of RPEALS, RPHESEB and RPCHSB was found to be 62.5µg/mL for each, against *L. monocytogenes*. The MIC value of RPHELS, RPMELS and RPMESEB was found to be <31.25 µg/mL for each and the MIC value of RPCHLS and RPEASB was determined to be 500 and >1000 µg/mL, respectively, against the same bacterial isolates. The MIC value of RPEALS and RPEASB was found to be 500µg/mL for each extract against *P. aeruginosa* while RPHELS, RPCHLS, RPMELS, RPHESEB, RPCHSB and RPMESEB showed MIC values of 125, 62.5, <31.25, 250, <31.25 and >1000 µg/mL, respectively, against the same bacterial isolates. The MIC value of RPHELS, RPMELS and RPHESEB was found to be >1000µg/mL for each against *S. marcescens* while RPCHLS, RPEALS, RPCHSB, RPEASB and RPMESEB exhibited a MIC values of 500, 62.5, 62.5, 62.5 and 500 µg/mL, respectively, against the same bacterial isolates. The MIC value of RPEALS, RPCHSB and RPEASB was found to be <31.25 µg/mL for each against *P. digitatum* while the MIC value for RPHELS, RPCHLS, RPMELS, RPHESEB and RPMESEB were found to be 250, 500, 500, 62.5 and 62.5 µg/mL respectively, against this fungal isolates. The MIC value for RPEALS, RPHESEB and RPEASB was found to be 62.5 µg/mL for each against *C. albicans* while RPCHLS and RPMELS showed an MIC of <31.25 µg/mL for each. However, the MIC values for RPHELS, RPMESEB and

RPCHSB were found to be 125, 250 and 125 µg/mL, respectively, against this fungal isolates.

Discussion

95% ethanolic and methanolic extracts have been obtained from leaves and shoots of *R. prinoides*. They have been evaluated for their antibacterial activity against *E. coli*, *L. monocytogenes*, *S. aureus*, *Staphylococcus sp.*, *Shigella dysenteriae*, *Shigella flexneri*, *P. vulgaris*, *Salmonella spp.* and *Streptococcus pneumonia* [24]. Both extracts showed a clear inhibition zones of >10 mm diameter against all tested bacterial isolates. Additionally, the MIC values of these two extracts were also evaluated and found to be in the range of 97.5 to 780 mg/mL [24]. The methanolic crude extract and the microorganisms, *E. coli*, *L. monocytogenes* and *S. aureus* were common to our study also. Our results showed a good agreement with literature report. In an another report, chloroform and methanol fractions were obtained from leaves of *R. prinoides* by successive extraction [25]. These fractions showed inhibitory effect against *S. aureus*, *Streptococcus pyogenes*, *S. pneumonia* and *Salmonella typhi* and the zones of inhibition were found to be in the range of 9 to 17 mm diameter against these bacterial isolates. The MIC values of chloroform fraction was found to be in the range of 8.13 mg/mL to 16.25 mg/mL and for methanol fraction it was in the range of 8.13 mg/mL to 32.5 mg/mL against these bacterial isolates [25].

We evaluated antibacterial and antifungal activities of hexane, chloroform, ethyl acetate and methanolic extracts from leaves and stem-bark of *R. prinoides*. Six bacterial isolates viz. *E. coli* (wild), *E. coli* (H0157), *S. aureus*, *L. monocytogenes*, *P. aeruginosa* and *S. marcescens* and two fungal isolates viz. *P. digitatum* and *C. albicans* were used in this study. The zones of inhibition were found to be in the range of 0.8±3.0 to 15.8±1.4 mm diameter against bacterial isolates and 10.3±1.8 to 14.3±2.5 mm diameter against fungal isolates. Additionally, these extracts showed minimum inhibitory concentrations (MICs) values in the range of <31.25 to > 1000 µg/mL. To conclude, *R. prinoides* showed significant antibacterial and antifungal activities. *R. prinoides* finds therapeutic applications in the traditional medicine in Southern Africa. Therefore,

further studies are required to commercialize products from this plant.

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Table 1. Inhibitory effect of hexane, chloroform, ethyl acetate and methanolic extracts from leaves and stem-bark of *R. prinoides* on selected bacterial and fungal isolates.

Extracts	Microorganisms/ Zones of inhibition (mm) (diameter)							
	<i>E. coli</i> (wild)	<i>E. coli</i> (H0157)	<i>S. aureus</i>	<i>L. mono</i> <i>cytogens</i>	<i>P. aeru</i> <i>ginosa</i>	<i>S. marc</i> <i>escens</i>	<i>P. digi</i> <i>tatum</i>	<i>C.</i> <i>albicans</i>
RPHELs	11.1±2.1	10.8±2.0	14.3±2.9	14.4±3.5	11.8±1.7	08.8±3.0	11.4±1.7	12.8±2.4
RPCHLS	13.4±1.0	09.8±1.7	14.3±2.1	10.8±1.5	12.8±2.0	09.1±1.7	10.3±1.8	13.8±4.7
RPEALS	09.1±1.1	12.4±1.4	13.3±2.4	12.8±4.3	10.3±1.1	12.1±2.7	13.7±1.7	13.4±3.8
RPMELS	12.6±2.4	10.3±2.0	13.8±3.9	14.2±1.5	14.3±5.7	08.4±2.2	10.8±2.0	14.1±2.6
RPHEsB	11.3±2.1	10.8±1.9	13.7±2.1	12.2±1.8	11.4±1.7	08.9±2.8	12.6±2.1	12.0±2.4
RPCHsB	13.3±1.0	13.3±1.1	15.8±1.4	12.7±1.4	12.6±2.2	12.4±4.7	13.9±1.3	11.6±2.4
RPEAsB	12.9±2.0	11.5±1.6	11.5±1.0	09.3±1.6	10.4±3.2	12.7±3.5	14.3±2.5	12.3±1.8
RPMEsB	12.9±3.0	13.0±1.6	12.5±2.4	14.3±1.8	08.9±4.5	08.0±1.5	12.3±2.9	11.5±2.0
Positive Controls	19.5±3.5	22.5±2.1	31.5±0.7	22.5±0.7	22.5±2.1	09.0±0.0	-	25.8±1.8

RPHELs = *R. prinoides* hexane leaves extract; RPCHLS = *R. prinoides* chloroform leaves extract; RPEALS = *R. prinoides* ethyl acetate leaves extract; RPMELS = *R. prinoides* methanolic leaves extract; RPHEsB = *R. prinoides* hexane stem-bark extract; RPCHsB = *R. prinoides* chloroform stem-bark extract; RPEAsB = *R. prinoides* ethyl acetate stem-bark extract; RPMEsB = *R. prinoides* methanolic stem-bark extract; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*; *L. monocytogens* = *Listeria monocytogens*; *P. aeruginosa* = *Pseudomonas aeruginosa*, *S. marcescens* = *Serratia marcescens*; *P. digitatum* = *Penicillium digitatum*; *C. albicans* = *Candida albicans*. Tetracycline served as positive control for *E. coli* (wild), *E. coli* (H0157), *S. aureus* and *L. monocytogens*. Amoxicillin served as positive control for *P. aeruginosa* and *S. marcescens*. Miconazole nitrate served as positive control for *C. albicans*. DMSO served as negative control.

Table 2. The minimum inhibitory concentrations (MICs) of hexane, chloroform, ethyl acetate and methanolic extracts from leaves and stem-bark of *R. prinoides* on selected bacterial and fungal isolates.

Extracts	Minimum inhibition concentrations (MICs) (µg/mL)							
	<i>E. coli</i> (wild)	<i>E. coli</i> (H0157)	<i>S.</i> <i>aureus</i>	<i>L. mono</i> <i>cytogens</i>	<i>P. aeru</i> <i>ginosa</i>	<i>S. marc</i> <i>escens</i>	<i>P. digi</i> <i>tatum</i>	<i>C.</i> <i>albicans</i>
RPHELs	125	500	<31.25	<31.25	125	>1000	250	125
RPCHLS	<31.25	>1000	<31.25	500	62.5	500	500	<31.25
RPEALS	>1000	62.5	62.5	62.5	500	62.5	<31.25	62.5
RPMELS	62.5	500	62.5	<31.25	<31.25	>1000	500	<31.25
RPHEsB	250	500	<31.25	62.5	250	>1000	62.5	62.5
RPCHsB	<31.25	62.5	<31.25	62.5	<31.25	62.5	<31.25	125
RPEAsB	62.5	250	500	>1000	500	62.5	<31.25	62.5
RPMEsB	62.5	<31.25	62.5	<31.25	>1000	500	62.5	250

RPHELs = *R. prinoides* hexane leaves extract; RPCHLS = *R. prinoides* chloroform leaves extract; RPEALS = *R. prinoides* ethyl acetate leaves extract; RPMELS = *R. prinoides* methanolic leaves extract; RPHEsB = *R. prinoides* hexane stem-bark extract; RPCHsB = *R. prinoides* chloroform stem-bark extract; RPEAsB = *R. prinoides* ethyl acetate stem-bark extract; RPMEsB = *R. prinoides* methanolic stem-bark extract; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*; *L. monocytogens* = *Listeria monocytogens*; *P. aeruginosa* = *Pseudomonas aeruginosa*, *S. marcescens* = *Serratia marcescens*; *P. digitatum* = *Penicillium digitatum*; *C. albicans* = *Candida albicans*.