

Antimicrobial activities of extracts from leaves and stem-bark of *Buddleja salviifolia*

^{1,*}Pillai, M.K., ¹Matamane, R.P. and ²Mekbib, S.B.

¹Department of Chemistry and Chemical Technology, Faculty of Science and Technology, National University of Lesotho, Roma Campus, P. O. Roma 180, Kingdom of Lesotho, Southern Africa

²Department of Biology, Faculty of Science and Technology, National University of Lesotho, Roma Campus, P. O. Roma 180, Kingdom of Lesotho, Southern Africa

Article history:

Received: 12 May 2019

Received in revised form: 13 June 2019

Accepted: 16 June 2019

Available Online: 20 June 2019

Keywords:

Buddleja salviifolia,

Buddleja,

Scrophulariaceae,

Antibacterial activity,

Antifungal activity

DOI:

[https://doi.org/10.26656/fr.2017.3\(6\).195](https://doi.org/10.26656/fr.2017.3(6).195)

Abstract

Buddleja salviifolia has been used as a food ingredient for improving digestion, anti-emetic, to relieve coughs, colic and constipation. *B. salviifolia* has also been used in traditional medicine to treat varieties of diseases, which include eye infections, tuberculosis (TB), cervical cancer, syphilis, neurodegenerative conditions. A few articles on the antimicrobial activities of extracts from leaves of *B. salviifolia* have previously been reported. However, the antimicrobial activities of hexane, chloroform, ethyl acetate and methanolic extracts from the leaves and stem-bark of *B. salviifolia* have not been reported previously. Therefore, in the present study, we evaluated the antimicrobial activities of these extracts against six bacterial isolates viz. *Escherichia coli* (wild strain), *Escherichia coli* (O157:H7), *Listeria monocytogenes*, *Staphylococcus aureus*, *Serratia marcescens* and *Pseudomonas aeruginosa* and two fungal isolates viz. *Penicillium digitatum* and *Candida albicans*. Their inhibition zones were found to be in the range of 7.0±1.4 to 36.5±7.8 mm against bacterial isolates and 7.0±0.0 to 22.0±1.4 mm against fungal isolates. Additionally, the MIC values of these extracts were found to be in the range of <31.25 to >1000 µg/mL against both bacterial and fungal isolates. To conclude, extracts from *B. salviifolia* exhibited promising antimicrobial activities. Since *B. salviifolia* has wide varieties of applications as a food ingredient and in the traditional medicine, further studies on various parts of this plant will be useful to commercialize products.

1. Introduction

Buddleja salviifolia belongs to the Scrophulariaceae family of the *Buddleja* genus (Van Wyk and Van Wyk, 1997; Tank *et al.*, 2006; Van Laere *et al.*, 2011). *B. salviifolia* is also known by other names such as sagewood, wildsaile, saliehout, etc. (Van Wyk and Van Wyk, 1997). Approximately, 150 species are found in the genus *Buddleja* (Ream, 2006). *B. salviifolia* is widely distributed in Southern parts of Africa such as South Africa, Lesotho, Zimbabwe, Swaziland, etc. and grows mostly in rocky mountain slopes, dry hillsides, forest margins, coastal and along with the water courses (Palmer, 1977; Ream, 2006). *B. salviifolia* is a semi-evergreen plant with greyish appearance (Van Wyk and Van Wyk, 1997) and reaches up to 3 to 8-meter height with many stems at the base (Drummond, 1981). *B. salviifolia* can be characterized by its small, tubular flowers with enclosing stamens. It has a strong and sweet perfumed smell, especially in the early spring (Palmer,

1977). The leaves of *B. salviifolia* have been used as a food ingredient for improving digestion, anti-emetic, relieve nausea, coughs, colic and constipation (Kose *et al.*, 2015). Additionally, the leaves of *B. salviifolia* have been used in the traditional medicine to treat TB, syphilis, herpes, cervical cancer, complications associated with pregnancy, etc. (Kose *et al.*, 2015). The leaves of *B. salviifolia* have also been used to treat neurodegenerative conditions and eye infections (Pendota *et al.*, 2014). Our literature search showed that a 20% methanolic crude extract obtained from the leaves of *B. salviifolia* and its hexane, dichloromethane, ethyl acetate, and butanol fractions have previously been studied for their antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Candida albicans* (Pendota *et al.*, 2013). Additionally, three pure compounds viz. 4-hydroxyphenyl ethyl vanillate, acteoside, and quercetin have been isolated from the

*Corresponding author.

Email: kmharan@rediffmail.com

ethyl acetate fraction of the leaves extract (Pendota *et al.*, 2013) and these three pure compounds have also been evaluated for their antimicrobial activities (Pendota *et al.*, 2013). However, to the best of our knowledge, hexane, chloroform, ethyl acetate, and 100% methanolic crude extracts from the leaves and stem-bark of this plant have not been explored well for their antimicrobial studies. Therefore, the aim of the present study was to evaluate the antibacterial and antifungal activities of hexane, chloroform, ethyl acetate and methanol extracts from leaves and stem-bark of *B. salviifolia* against six bacterial isolates viz. *E. coli* (wild strain), *E. coli* O157: H7, *L. monocytogenes*, *S. aureus*, *Serratia marcescens* and *Pseudomonas aeruginosa* and two fungal isolates viz. *Penicillium digitatum* and *C. albicans*.

2. Materials and methods

2.1 Plant materials

Fresh leaves of *B. salviifolia* were collected in August 2018 inside the Roma Campus of National University of Lesotho, Lesotho, Southern Africa. Additionally, a piece of stem-bark was cut from the same plant. Mr. Moretloa Polaki, Lecturer, Department of Biology, Faculty of Science and Technology, National University of Lesotho, Roma Campus, Roma P.O. 180, Lesotho, Southern Africa identified the plant material. A voucher specimen viz. Matamane/BSLS/2018 and Matamane/BSSB/2018 leaves and stem-bark, respectively, 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, the Kingdom of Lesotho, Southern Africa.

2.2 Material processing

The leaves were allowed to air-dry at room temperature (about 18-24°C) for two weeks. The crushed leaves were ground into powder (523.86 g) using a dry laboratory blender (Waring Blender, Blender 80119, Model HGB2WT93, 240V AC, 3.5 AMPs, Laboratory and Analytical Supplies). The chopped stem-bark was allowed to air-dry at room temperature (about 18-24°C) for two weeks and then ground into powder (760.46 g) using the same blender.

2.3 Preparation of plant extracts

A 100.11 g of powdered leaves was extracted with hexane for three days at room temperature. The solution was filtered off using a vacuum filter (ATB, Model: 284065-H, Power: 230V 3.0A, 1320/min 50Hz). The solvent was removed in *vacuo*. The extract was transferred to pre-weighed clean and dry beaker. The

same procedure was repeated twice. Finally, the leaves were extracted with hexane at reflux condition for 10 hours. About 1.85 g of combined hexane extract was obtained after removal of solvent. The same procedure was repeated with chloroform, ethyl acetate and methanol separately. Approximately, 7.48, 8.13 and 14.61 g of chloroform, ethyl acetate and methanol leave extracts respectively, were obtained from 98.88 g, 100.23 g and 175.45 g of powdered leaves. Similarly, using the same procedure, 0.43, 1.07, 2.18 and 8.86 g of hexane, chloroform, ethyl acetate and methanol stem-bark extracts, respectively, were obtained from 200.09 g, 200.01 g, 202.52 g and 157.82 g powdered stem-bark.

2.4 Evaluation of antimicrobial activity

The antimicrobial activities of various extracts of *B. salviifolia* were evaluated by *in vitro* hole-plate diffusion method as described in the literature (Manilal *et al.*, 2009; Alghazeer *et al.*, 2012). 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 0.20 µm filter and then used for both antibacterial and antifungal activities. A volume of 0.1 mL of the broth culture of each bacterial isolate was spread on the NA (Nutrient Agar) plates separately. 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 35 µL aliquots of the extract. The plates were then incubated at 37°C for 24 hours. Tetracycline served as a positive control for *E. coli* (wild strain), *E. coli* (O157: H7), *S. aureus* and *L. monocytogenes*. Amoxicillin served as a positive control for *P. aeruginosa* and *S. marcescens*. DMSO served as a negative control. The Petri dishes containing 25 mL of Potato Dextrose Agar (PDA) were used for the antifungal assay. The agar plate was first spread with 0.1 mL of the fungi. Cylindrical cavities of size 4.00 mm height and 6.00 mm diameter were punched using a sterile cork-borer and filled with the 35 µL aliquots of the extract. The plates were incubated at 24°C for 48 hrs. Miconazole nitrate served as a positive control for *C. albicans*. However, we did not maintain positive control for *P. digitatum*. DMSO served as a negative control. The sensitivity of microorganisms to the various extracts of *B. salviifolia* was determined by measuring the diameter of inhibition zones around the holes of the agar surface. All experiments were performed in duplicate and the results were reported as the average of two experiments. A clear zone > 10 mm are considered as a positive result (Lima-Filho *et al.*, 2002). Inhibition zones of <12, 12-20 and ≥20 mm diameter is expressed as weak, moderate and strong activities respectively (Emmanuel *et al.*, 2012; Matela *et al.*, 2018).

2.5 Determination of minimum inhibitory concentrations (MICs)

The minimum concentration of the sample that is needed to inhibit the growth of the microorganisms is called MIC value (Alghazeer *et al.*, 2012; Alghazeer *et al.*, 2017). The MIC of $< 100 \mu\text{g/mL}$, 100 to $\leq 625 \mu\text{g/mL}$ and $> 625 \mu\text{g/mL}$ were considered as significantly active, moderately active and weakly active, respectively (Emmanuel *et al.*, 2012; Njimoh *et al.*, 2015; Alghazeer *et al.*, 2017). The MICs of various extracts of *B. salviifolia* were determined as described in the literature (Daud *et al.*, 2005; Alghazeer *et al.*, 2017). Briefly, a stock solution at a concentration of $1000 \mu\text{g/mL}$ of various extracts of *B. salviifolia* was prepared separately. Two-fold serial dilutions such as 1000, 500, 250, 125, 62.5 and $31.25 \mu\text{g/mL}$ were made from these stock solutions. A suspension of the microorganism is prepared at a concentration of one million to two million colony forming units (CFU)/mL by means growing the bacterial strain in nutrient broth in an incubator with continuous shaking (Matela *et al.*, 2018). Cylindrical cavities of size 4.00 mm height and 6.00 mm diameter were punched on the agar plate using a sterile cork-borer and then filled with the $35 \mu\text{L}$ aliquots of the extract. The plates were then incubated at 37°C for 24 hrs.

2.6 Microorganisms

A total of six bacterial isolates (four Gram-negative bacteria *viz.* *E. coli* (wild strain), *E. coli* O157: H7, *S. marcescens* and *P. aeruginosa*), (two Gram-positive bacterial isolates *viz.* *L. monocytogenes* and *S. aureus*) and two fungal isolates *viz.* *P. digitatum* and *C. albicans* were used in this study to evaluate the antimicrobial activities of the various extracts from the leaves and stem-bark of *B. salviifolia*. All these microorganisms are available at the Department of Biology, National University of Lesotho, Roma, Kingdom of Lesotho, Southern Africa.

2.7 Statistical analysis

Data analysis was performed using the SPSS 17.0 statistics program by means of two-way analysis of variance. The differences were considered statistically significant when $p \leq 0.05$.

3. Results and discussion

The antibacterial activity of various extracts of *B. salviifolia* is summarized in Table 1. BSHXLS, BSCHLS, BSEALS, BSMELS are *B. salviifolia* hexane leaves extract, chloroform leaves extract, ethyl acetate leaves extract and methanol leaves extract, respectively. BSHXSB, BSCHSB, BSEASB and BSMESB are *B. salviifolia* hexane stem-bark extract, chloroform stem-

bark extract, ethyl acetate stem-bark extract and methanol stem-bark extract, respectively. BSHXLS, BSCHLS, BSEALS, BSMELS, BSHXLS, BSCHSB, BSEALS and BSMESB exhibited inhibition zones of 10.0 ± 2.8 , 14.0 ± 1.4 , 15.0 ± 4.2 , 13.0 ± 1.4 , 13.5 ± 3.5 , 16.0 ± 0.7 , 10.5 ± 0.7 and 12.0 ± 3.5 mm respectively against *E. coli* (wild strain). This result showed that BSCHLS, BSEALS, BSMELS, BSHXSB, BSCHSB and BSMESB showed a moderate activity and the remaining extracts, BSHXLS and BSEASB, showed a weak activity against *E. coli* (wild strain). The positive control, tetracycline, showed an inhibition zone of 19.5 ± 3.5 mm. BSHXLS, BSCHLS, BSEALS, BSMELS, BSHXSB, BSCHSB and BSEASB exhibited inhibition zones of 14.0 ± 0.0 , 11.0 ± 1.4 , 13.0 ± 1.4 , 7.0 ± 1.4 , 8.0 ± 2.0 , 13.0 ± 4.2 and 11.0 ± 2.8 mm respectively, against *E. coli* (O157: H7). This result showed that BSHXSB, BSCHLS, BSEASB and BSMELS are weakly active. The BSHXLS, BSEALS and BSCHSB are moderately active. However, BSMESB did not exhibit any visible inhibition zone. The positive control, tetracycline, showed an inhibition zone of 22.5 ± 2.1 mm. BSHXLS, BSCHLS, BSEALS, BSMELS, BSHXSB, BSCHSB, BSEASB and BSMESB exhibited inhibition zones of 16.5 ± 0.7 , 18.5 ± 3.5 , 36.5 ± 7.8 , 13.0 ± 1.4 , 13.0 ± 3.5 , 13.5 ± 2.1 , 13.0 ± 0.0 and 15.5 ± 0.7 mm, respectively, against *L. monocytogenes*. This result showed that BSEALS is strongly active while the other extracts, BSHXLS, BSCHLS, BSMELS, BSHXSB, BSCHSB, BSEASB and BSMESB are moderately active. The positive control, tetracycline showed an inhibition zone of 22.5 ± 0.7 mm. BSHXLS, BSCHLS, BSEALS, BSHXSB, BSCHSB, BSEASB and BSMESB exhibited inhibition zones of 16.0 ± 2.8 , 14.0 ± 0.0 , 13.0 ± 1.4 , 16.0 ± 2.8 , 14.0 ± 1.4 and 15.5 ± 2.1 mm respectively, against *S. aureus*. Therefore, BSHXLS, BSCHLS, BSEALS, BSHXSB, BSCHSB, BSEASB and BSMESB are moderately active. BSMELS showed no visible zone of inhibition and therefore, it is inactive against *S. aureus*. The positive control, tetracycline, showed an inhibition zone of 31.5 ± 0.7 mm. BSHXLS, BSCHLS, BSEALS, BSMELS, BSHXSB, BSCHSB, BSEASB and BSMESB showed inhibition zones of 19.0 ± 1.4 , 11.0 ± 4.2 , 13.5 ± 0.7 , 14.0 ± 2.8 , 10.5 ± 2.1 , 11.0 ± 1.4 , 9.0 ± 1.4 and 7.5 ± 0.7 mm, respectively, against *S. marcescens*. This result showed that BSCHLS, BSMESB, BSCHSB, BSEASB and BSHXSB exhibited weak activity while the rest of the extracts, BSHXLS, BSEALS and BSMELS exhibited moderate activity. The positive control, amoxicillin, showed an inhibition zone of 9.0 ± 0.0 mm. BSHXLS, BSCHLS, BSEALS, BSMELS, BSHXSB, BSCHSB, BSEASB and BSMESB showed inhibition zones of 17.5 ± 4.9 , 20.0 ± 0.0 , 16.5 ± 0.7 , 12.5 ± 2.1 , 16.0 ± 2.8 , 14.5 ± 2.1 , 11.0 ± 4.2 and 16.0 ± 0.0 mm, respectively,

Table 1. Inhibitory effect of hexane, chloroform, ethyl acetate and methanolic extracts from leaves and stem-bark of *B. salviifolia* against bacterial and fungal isolates.

Extracts	Microorganisms/Inhibition zones (mm)							
	<i>E. coli</i> (wild strain)	<i>E. coli</i> (O157: H7)	<i>L. monocytogenes</i>	<i>S. aureus</i>	<i>S. marcescens</i>	<i>P. aeruginosa</i>	<i>P. digitatum</i>	<i>C. albicans</i>
BSHXLS	10.0±2.8	14.0±0.0	16.5±0.7	16.0±2.8	19.0±1.4	17.5±4.9	14.0±0.0	12.0±2.8
BSCHLS	14.0±1.4	11.0±1.4	18.5±3.5	14.0±0.0	11.0±4.2	20.0±0.0	12.5±0.7	12.5±2.1
BSEALS	15.0±4.2	13.0±1.4	36.5±7.8	13.0±1.4	13.5±0.7	16.5±0.7	11.5±2.1	13.5±2.1
BSMELS	13.0±1.4	7.0±1.4	13.0±1.4	-	14.0±2.8	12.5±2.1	9.0±1.4	18.0±5.7
BSHXSB	13.5±3.5	8.0±2.8	13.0±3.5	16.0±2.8	10.5±2.1	16.0±2.8	15.5±3.5	14.0±0.0
BSCHSB	16.0±2.8	13.0±4.2	13.5±2.1	12.0±2.8	11.0±1.4	14.5±2.1	12.5±4.5	9.5±3.5
BSEASB	10.5±0.7	11.0±2.8	13.0±0.0	14.0±1.4	9.0±1.4	11.0±4.2	14.0±2.8	12.0±4.2
BSMESB	12.0±3.5	-	15.5±0.7	15.5±2.1	7.5±0.7	16.0±0.0	7.0±0.0	12.0±5.6
Positive controls	19.5±3.5	22.5±2.1	31.5±0.7	22.5±0.7	22.5±2.1	9.0±0.0	N/A	25.8±1.8

BSHXLS = *B. salviifolia* hexane leaves extract, BSCHLS = *B. salviifolia* chloroform leaves extract, BSEALS = *B. salviifolia* ethyl acetate leaves extracts, BSMELS = *B. salviifolia* methanol leaves extract, BSHXSB = *B. salviifolia* hexane stem-bark extract, BSCHSB = *B. salviifolia* chloroform stem-bark extract, BSEASB = *B. salviifolia* ethyl acetate stem-bark extract, BSMESB = *B. salviifolia* methanol stem-bark extract. Tetracycline served as a positive control for *E. coli*, *E. coli* O157: H7, *L. monocytogenes* and *S. aureus*. Amoxicillin served as a positive control for *S. marcescens* and *P. aeruginosa*. Miconazole nitrate served as a positive control for *C. albicans*. DMSO served as a negative control. (-) = No visible inhibition zone is observed. N/A = Not applicable.

against *P. aeruginosa*. This result showed that BSEASB is weakly active and the other extracts, BSHXLS, BSCHLS, BSEALS, BSMELS, BSHXSB, BSCHSB and BSMESB are moderately active. The positive control, amoxicillin, showed an inhibition zone of 22.5±2.1 mm. The antifungal activity of various extracts of *B. salviifolia* is also summarized in Table 1. BSHXLS, BSCHLS, BSEALS, BSMELS, BSHXSB, BSCHSB, BSEASB and BSMESB exhibited inhibition zones of 14.0±0.0, 12.5±0.7, 11.5±0.0, 9.0±1.4, 15.5±3.5, 12.5±4.5, 14.0±2.8 and 7.0±0.0 mm, respectively, against *P. digitatum*. These results showed that BSMELS, BSEALS and BSMESB are weakly active while the rest, BSHXLS, BSCHLS, BSHXSB, BSCHSB and BSEASB, are moderately active against *P. digitatum*. BSHXLS, BSCHLS, BSEALS, BSMELS, BSHXSB, BSCHSB, BSEASB and BSMESB exhibited inhibition zones of 12.0±2.8, 12.5±2.1, 13.5±2.1, 18.0±5.7, 14.0±0.0, 9.5±3.5, 12.0±4.2 and 12.0±5.6 mm respectively, against *C. albicans*. This result indicated that BSCHSB exhibited weak activity while the other extracts, BSHXLS, BSCHLS, BSEALS, BSMELS, BSHXLS, BSEASB and BSMESB exhibited moderate activity. The positive control, miconazole nitrate showed an inhibition zone of 25.8±1.8 mm. The minimum inhibition concentrations (MICs) of various extracts of *B. salviifolia* are summarized in Table 2. The MIC value of BSMELS, BSHXSB and BSMESB was found to be with of 62.5µg/mL for each extract against *E. coli* (wild strain) while BSHXLS, BSCHLS, BSEALS, BSCHSB and BSEASB exhibited a moderate activity with MIC values of 500, 125, 125, 250 and 125 µg/mL, respectively, against the same bacterial isolates. The MIC value of BSMELS, BSCHSB and BSEASB was found to be 62.5

µg/mL for each extract against *E. coli* (O157: H7). However, the MIC values of BSHXLS, BSCHLS, BSEALS, BSHXSB and BSMESB were found to be 125, 500, 500, 125 and >1000 µg/mL, respectively, against *E. coli* H0157. The MIC value of BSHXLS and BSCHLS was determined to be >1000 µg/mL for each extract against *L. monocytogenes* while the MIC value of BSEALS, BSMELS, BSEASB and BSMESB was determined to be <31.25, 62.5 and 62.5 µg/mL, respectively, and the MIC value of BSHXSB and BSCHSB were found to be 125 and 250µg/mL respectively against the same bacterial isolates. The MIC value of BSCHLS and BSEALS was found to be 250 µg/mL for each, against *S. aureus*. The MIC value of BSMELS, BSHXSB, BSCHSB, BSEASB and BSMESB was found to be 500 µg/mL for each and the MIC value of BSHXLS was determined to be >1000 µg/mL against the same bacterial isolates. The MIC value of BSHXLS, BSCHLS, BSEALS and BSMELS was found to be 250µg/mL for each, against *S. marcescens*. BSMELS, BSHXSB and BSEASB showed MIC values of 125µg/mL for each and BSCHSB showed a MIC value of >1000 µg/mL against *S. marcescens*. The MIC value of BSCHLS, BSMELS, BSHXSB and BSEASB was found to be 62.5 µg/mL for each against *P. aeruginosa* while BSHXLS, BSEALS, BSCHSB and BSMESB exhibited MIC values of 250, 125, 125 and 250 µg/mL, respectively, against the same bacterial isolates. The MIC value of BSEALS, BSHXSB, BSCHSB and BSMESB was found to be 62.5, <31.25, <31.25 and 62.5 µg/mL, respectively against *P. digitatum* while the MIC value for BSHXLS, BSMELS and BSEASB were found to be 500, 500 and 250 µg/mL, respectively, and BSCHLS exhibited an MIC of >1000 µg/mL against this

Table 2. The minimum inhibitory concentrations (MICs) of hexane, chloroform, ethyl acetate and methanolic extracts of leaves and stem-barks of *B. salviifolia* extracts against bacterial and fungal isolates.

Extracts	Microorganisms/Minimum inhibitory concentrations ($\mu\text{g/mL}$)							
	<i>E. coli</i> (wild strain)	<i>E. coli</i> (O157: H7)	<i>L. monocytogenes</i>	<i>S. aureus</i>	<i>S. marcescens</i>	<i>P. aeruginosa</i>	<i>P. digitatum</i>	<i>C. albicans</i>
BSHXLS	500	125	>1000	>1000	250	250	500	125
BSCHLS	125	500	>1000	250	250	62.5	>1000	62.5
BSEALS	125	500	<31.25	250	250	125	62.5	62.5
BSMELS	62.5	62.5	62.5	500	125	62.5	500	62.5
BSHXSB	62.5	125	125	500	125	62.5	<31.25	62.5
BSCHSB	250	<31.25	250	500	>1000	125	<31.25	<31.25
BSEASB	125	62.5	62.5	500	125	62.5	250	125
BSMESB	62.5	>1000	<31.25	500	250	250	62.5	62.5

BSHXLS = *B. salviifolia* hexane leaves extract, BSCHLS = *B. salviifolia* chloroform leaves extract, BSEALS = *B. salviifolia* ethyl acetate leaves extracts, BSMELS = *B. salviifolia* methanol leaves extract, BSHXSB = *B. salviifolia* hexane stem-bark extract, BSCHSB = *B. salviifolia* chloroform stem-bark extract, BSEASB = *B. salviifolia* ethyl acetate stem-bark extract, BSMESB = *B. salviifolia* methanol stem-bark extract.

fungal isolates. The MIC value for BSCHLS, BSEALS, BSMELS, BSHXSB and BSMESB were found to be 62.5 $\mu\text{g/mL}$ for each against *C. albicans* while BSCHSB showed a MIC of 31.25 $\mu\text{g/mL}$. However, the MIC value for extract BSHXLS and BSEASB were found to be 125 $\mu\text{g/mL}$ for each against this fungal isolate.

Pendota et al. (2013) obtained a 20% methanolic crude extract from the leaves of *B. salviifolia*. Additionally, hexane, dichloromethane, ethyl acetate and butanol fractions were also obtained from this crude extract. The crude extract and these four fractions were screened for their MIC values against four bacterial isolates viz. *E. coli*, *S. aureus*, *B. subtilis* and *K. pneumoniae* and one fungal isolate, *C. albicans* (Pendota et al., 2013). They showed MIC values ranging from 390 -1560 $\mu\text{g/mL}$ against these microorganisms. The MIC values of crude extract and hexane, dichloromethane, ethyl acetate and butanol fractions were found to be 6250.0, 780.0, 1560.0, 1560.0 and 1560.0 $\mu\text{g/mL}$, respectively, against *E. coli*. The microorganisms *E. coli* is common to our study also. However, we found that the MIC value of methanolic leave extract as 62.5 $\mu\text{g/mL}$. The low MIC value in our study may be due to more extractive power of 100% methanol relative to 20% methanol. The MIC values of crude extract, hexane, dichloromethane, ethyl acetate and butanol fractions were found to be 3125.0, 1560.0, 780.0, 390.0 and 3125.0 $\mu\text{g/mL}$, respectively, against *S. aureus*. The microorganisms *S. aureus* also is common to our study. However, we found that the MIC value of methanolic leave extract as 500 $\mu\text{g/mL}$. This is again due to more extractive power of methanol relative to 20% methanol. The MIC value for crude extract, hexane and dichloromethane fractions were determined to be 3125.0 $\mu\text{g/mL}$ for each against *B. subtilis*. The ethyl acetate and butanol fractions showed MIC values of 780.0 and 1560.0 $\mu\text{g/mL}$, respectively, against *B. subtilis*. The MIC

values of crude extract and hexane, dichloromethane, ethyl acetate and butanol fractions were found to be 6250.0, 1560.0, 1560.0, 3125.0 and 3125.0 $\mu\text{g/mL}$ respectively, against *K. pneumoniae*.

Additionally, three pure compounds viz. 4-hydroxyphenyl ethyl vanillate, acteoside and quercetin have been isolated from the ethyl acetate fraction and were evaluated for their antibacterial and antifungal activities against the above microbes (Pendota et al., 2013). The MIC value of 4-hydroxyphenyl ethyl vanillate, acteoside and quercetin were found to be 125.0 $\mu\text{g/mL}$ for each, against viz. *E. coli*. The MIC values for 4-hydroxyphenyl ethyl vanillate, acteoside and quercetin were determined to be 250.0, 62.5 and 250.0 $\mu\text{g/mL}$, respectively, against *S. aureus* (Pendota et al., 2013). The MIC values of 4-hydroxyphenyl ethyl vanillate, acteoside and quercetin were found to be 125.0, 125.0 and 250.0 $\mu\text{g/mL}$, respectively, against *B. subtilis*. On the other hand, 4-hydroxyphenyl ethyl vanillate and acteoside exhibited a MIC value of 62.5 $\mu\text{g/mL}$ for each and quercetin showed a MIC value of 125.0 $\mu\text{g/mL}$ against *K. pneumoniae* (Pendota et al., 2013).

Against *C. albicans*, the MIC values of crude extract, hexane, dichloromethane, ethyl acetate and butanol fractions were reported to be 1560.0, 390.0, 390.0, 780.0 and 1560.0 $\mu\text{g/mL}$ respectively (Pendota et al., 2013). The MIC values of 4-hydroxyphenyl ethyl vanillate, acteoside and quercetin were found to be 250.0, 250.0 and 125.0 $\mu\text{g/mL}$, respectively, against the same fungal isolates, *C. albicans*. In our study, the MIC value of methanolic extract was found to be 62.5 $\mu\text{g/mL}$. The minimum fungicidal concentration (MFC) of crude extract, hexane, dichloromethane, ethyl acetate and butanol fractions have been reported to be 3125.0, 390.0, 390.0, 1560.0 and 1250.0 $\mu\text{g/mL}$, respectively, against *C. albicans*. The MFC of 4-hydroxyphenyl ethyl

vanillate, acteoside and quercetin were found to be 250.0 µg/mL for each, against *C. albicans*. (Pendota et al., 2013).

As such, the report from Pendota et al. (2013) has been limited only to MIC and MFC values of a 20% methanolic crude extract from the leaves of *B. salviifolia* and its hexane, dichloromethane, ethyl acetate and butanol fractions. They used four bacterial and one fungal isolate for their study. Additionally, the MIC and MFC values of three pure compounds viz. 4-hydroxyphenyl ethyl vanillate, acteoside and quercetin were also evaluated against these four bacterial and one fungal isolate (Pendota et al., 2013). However, in our study, we discussed not only MIC values but also the exact inhibitory effects of eight extracts (four extracts from leaves and the other four from stem-bark) against six bacterial and two fungal isolates.

It has been reported that *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, *C. albicans* etc. are responsible for some common ocular infections (Pendota et al., 2013). For example, *E. coli* is responsible for ocular inflammations such as keratitis, blepharitis and conjunctivitis (Bruiser and Burd, 1996). Some *Bacillus* spp. are responsible for a severe eye interior inflammation with 70% vision loss after trauma and/or surgery infection (David et al., 1994). In South Africa, leaves extract of *B. salviifolia* has been used to treat eye infections (Pendota et al., 2014). This may be due to the fact that *B. salviifolia* might have contained antibacterial metabolites against *E. coli*, *Bacillus* spp. etc. El-Ganiny et al. (2017) reported that extracts from *B. salviifolia* exhibited a significant biofilm inhibition and also eradicated preformed biofilm. Additionally, *B. salviifolia* have been showing a variety of other bioactivities such as anti-hypersensitive, analgesic, immunosuppressive, antitumor activities etc. (Herbert et al., 1991).

4. Conclusion

We evaluated antibacterial and antifungal activities of hexane, chloroform, ethyl acetate, methanol extracts from leaves and stem-bark of *B. salviifolia*. Gram-negative bacteria such as *E. coli* (wild strain), *E. coli* O157: H7, *S. marcescens* and *P. aeruginosa*, Gram-positive bacteria such as *L. monocytogenes* and *S. aureus* and two fungal isolates viz. *P. digitatum* and *C. albicans* were used in this study. All extracts exhibited antimicrobial activity with inhibition zones ranging from 7.0±1.4 to 36.5±7.8 mm against bacterial and 7.0±0.0 to 22.0±1.4 mm against fungal isolates. Additionally, all these extracts were also evaluated for their minimum inhibitory concentrations (MICs) and were found to be in the range of <31.25 µg/mL to >1000 µg/mL against both

bacterial and fungal isolates. To conclude, *B. salviifolia* exhibited promising antimicrobial activities. *B. salviifolia* has been used as a food ingredient. In traditional medicine, the leaves of *B. salviifolia* have been used to treat eye infections and neurodegenerative conditions. Therefore, further studies on various parts of this plant will be useful to commercialize products.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank the National University of Lesotho for its support.

References

- Alghazeer, R., El-Saltanil, H., Saleh, N., Al-Najjar, A. and Hebail, F. (2012). Antioxidant and antimicrobial properties of five medicinal Libyan plants extracts. *Natural Science*, 4, 324-335. <https://doi.org/10.4236/ns.2012.45045>
- Alghazeer, R., Elmansori, A., Sidati, M. and Gammoudi, F. (2017). In vitro antibacterial of flavonoid extracts of two selected Libyan Algae against multi-drug resistant bacteria isolated from food products. *Journal of Biosciences and Medicine*, 5, 26-48. <https://doi.org/10.4236/jbm.2017.51003>
- Bruiser, J.H. and Burd, E.M. (1996). Principles of Diagnostic Ocular Microbiology. Infections of the eye. 2nd Ed., p. 67-77. Boston, USA: Little Brown Company.
- David, D.B., Kirkby, G. B. and Noble, B.A. (1994). *Bacillus cereus* endophthalmitis. *British Journal of Ophthalmology*, 78, 577-580. <https://doi.org/10.1136/bjo.78.7.577>
- Daud, A., Gallo, A. and Sanchez, A. (2005). Antibacterial properties of *Phrygilanthus acutifolius*. *Journal of Ethnopharmacology*, 99(2), 193-195. <https://doi.org/10.1016/j.jep.2005.01.043>
- Drummond, R.B. (1981). Trees of Southern Africa., p. 778 –779. Cape Town: Tien Wah Press (Pty) Ltd.
- El-Ganiny, A.M., Shaker, G.H., Aboelazm, A.A. and El-Dash, H.A. (2017). Prevention of bacterial biofilm formation on soft contact lenses using natural compounds. *Journal of Ophthalmic Inflammation and Infection*, 7, 1-7. <https://doi.org/10.1186/s12348-017-0129-0>
- Emmanuel, M.T., Anatole, C.P. and Veronique, P.B. (2012). Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastrointestinal relevance.

- Journal of Ethnopharmacology*, 142(1), 265-273. <https://doi.org/10.1016/j.jep.2012.05.005>
- Herbert, J.M., Maffrand, J.P., Taoubi, K., Augerean, J.M., Fouraste, I. and Gleye, J. (1991). Verbascoside isolated from *Lantana camara*, an inhibitor of protein kinase C. *Journal of Natural Products*, 54, 1595-1600. <https://doi.org/10.1021/np50078a016>
- Kose, L.S., Moteete, A. and Van Vuuren, S. (2015). Ethnobotanical survey of medicinal plants used in the Maseru district of Lesotho. *Journal of Ethnopharmacology*, 170, 184-200. <https://doi.org/10.1016/j.jep.2015.04.047>
- Lima-Filho, M., Carvalho, U. and Freitas, M. (2002). Antibacterial activity of extracts of six Macroalgae from the Northeastern Brazilian Coast. *Brazilian Journal of Microbiology*, 33, 311-313. <https://doi.org/10.1590/S1517-83822002000400006>
- Manilal, A., Sujith, S., Kiran, G.S., Seivin, J., Shakir, C., Gandhimathi, R. and Panokkar, M.V.N. (2009). Biopotentials of seaweeds collected from southwest coast of India. *Journal of Marine Science and Technology*, 17, 67-73.
- Matela, K.S., Mekbib, S.B. and Pillai, M.K. (2018). Antimicrobial activities of extracts from *Gleditsia triacanthos* L. and *Schinus molle* L. *Pharmacology Online*, 2, 85-92.
- Njimoh, D.L., Assob, J.C.N., Mokake, S.E., Nyhalah, D.J., Yinda, C.K. and Sandjon, B. (2015). Antimicrobial activities of a plethora of medicinal plant extracts and hydrolates against human pathogens and their potential to reverse antibiotic resistance. *International Journal of Microbiology*, 2015, 1-15. <https://doi.org/10.1155/2015/547156>
- Palmer, E. (1977). A Field Guide to The Trees of Southern Africa. p. 274-275. Great Britain, Glasgow, UK: William Collins Sons and Co Ltd
- Pendota, S.C., Aderogba, M.A., Ndlhala, A.R. and Van Staden, J. (2013). Antimicrobial and acetylcholinesterase inhibitory activities of *Buddleja salviifolia* (L.) Lam. leaf extracts and isolated compounds. *Journal of Ethnopharmacology*, 148(2), 515-520. <https://doi.org/10.1016/j.jep.2013.04.047>
- Pendota, S.C., Ndlhala, A.R., Aremu, A.O., Aderogba, M.A. and Van Staden, J. (2014). Anti-inflammatory, antioxidant and in silico (i) studies of *Buddleja salviifolia* (L.) Lam leaf constituents. *Journal of Ethnopharmacology*, 93, 79-85. <https://doi.org/10.1016/j.sajb.2014.03.012>
- Ream, J. (2006). Production and invasion of Butterfly Bush (*Buddleja davidii*) in Oregon., p. 6. Oregon, USA: Oregon State University.
- Tank, D.C., Beardsley, P.M. and Kelcher, S.A. (2006). Olmsted RG. Review of the systematics of Scrophulariaceae S.J. and their current disposition. *Australian Systematic Botany*, 19(4), 29-307. <https://doi.org/10.1071/SB05009>
- Van Laere, K., Van Huylbroeck, J. and Van Bockstaele, E. (2011). Introgression of yellow flower colour in *Buddleja davidii* by means of polyploidisation and interspecific hybridization. *Horticulture Science*, 38(3), 96-103. <https://doi.org/10.17221/61/2011-HORTSCI>
- Van Wyk, B. and Van Wyk, P. (1997) Field guide to Tress of Southern Africa featuring more than 1000 species., p. 258-259. Cape Town: Struik Publishers.