Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2017.08.009

Antioxidant, antibacterial and phytochemical properties of two medicinal plants against the wound infecting bacteria

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ARTICLE INFO

Article history:
Received 18 Jul 2017
Received in revised form 8 Aug 2017
Accepted 14 Aug 2017
Available online 18 Aug 2017

Keywords:
Diabetes mellitus
Antioxidant
Phytochemical
Brachylaena elliptica
Brachylaena ilicifolia
Wound

ABSTRACT

Objective: To investigate the antioxidant, antibacterial and phytochemical properties of ethanol extracts of *Brachylaena elliptica* and *Brachylaena ilicifolia* against wound infecting bacteria normally found in diabetic patients.

Methods: The *in vitro* antioxidant activity of the two plants extracts were investigated spectrophotometrically using 1,1-diphenyl-2-picrylhydrazyl, nitric oxide, azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt, hydrogen peroxide (H_2O_2) and ferric reducing power. The antibacterial assay and minimum inhibitory concentration (MIC) was determined using the agar dilution method against five bacteria strains using amoxycillin and ciprofloxacin as positive control. The phytochemical analyses (tannins, total phenol, flavonoids, flavonols, proanthocyanidin, alkaloids and saponins) were assessed using standard methods.

Results: The ethanol extract of both plants exhibited strong antioxidant activities in some cases when compared to the standards (vitamin C and BHT). The antibacterial activity of both plants showed an appreciable broad spectrum activity against these wound pathogens with MIC value ranges between 0.3 mg/mL and 5 mg/mL. Tannins, phenols, flavanols, proanthocyanidins and alkaloids content of *B. ilicifolia* were significantly higher than those in *B. elliptica*. However, there were no significant differences in the flavanoid content of both plants extracts.

Conclusions: These results indicated that the ethanol leaf extracts of these plants have antioxidant and antibacterial activity against the tested bacteria possibly due to the presence of bioactive compounds and therefore could be used as alternative therapy against wound infection caused by these bacteria in diabetic patients.

1. Introduction

Diabetes mellitus is a chronic disorder that affects the metabolism of carbohydrates, fats, protein and electrolytes in the body due to an absolute or relative deficiency of insulin. It is also described by an abnormal increase in blood sugar level that causes glycation of the body proteins, which leads to complications such as diabetic nephropathy, neuropathy, atherosclerosis, coronary heart disease and development of wounds [1]. Other than organ complications, patients with diabetes also suffer from various infectious diseases, such as foot and skin infection compared to patients without diabetes [2]. However, studies have shown that poor management of diabetes adds to the development of microbial infection in diabetic patients [3].

Infection of a wound occurs due to physical injuries that result in an opening or breaking of the skin thereby causing the invasion of tissues by one or more species of pathogenic microorganism. Wounds allow bacteria, such as *Staphylococcus* spp., *Clostridium* spp., gain access to the internal tissue and cause the establishment of infections [4]. Studies have indicated that there are many bacterial species responsible for wound infections [5]. Bacteria such as *Pseudomonas aeruginosa (P. aeruginosa)*, characterized by the formation of a green

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Foundation project: This research was supported by a grant from Govan Mbeki Research and Development Centre, University of Fort Hare (Grant No. C228).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

pigment, which later develops to a black lesion, reported to play a significant role in wound infection [6]. Podei *et al*. [7] reported the isolation of *Proteus mirabilis (P. mirabilis)* from the wound. *Staphylococcus areas* (*S. aureus*) have also been identified and isolated in the wound from people with diabetes [8]. Bacteria being one of the most important factors responsible for wound infections and delayed wound healing in diabetic patients. They generally have the genetic ability to acquire multiple routes of resistance toward antibiotics. Due to the widespread usage of antibiotics drugs employed in the treatment of infectious diseases, some antibiotics, while effective, reported to associate with undesirable side effects [9]. For example, the uses of some antibiotics have been reported to cause allergic and immunosuppression. Due to these reasons, medicinal plants with antimicrobial agents are needed to treat infectious diseases with little or no adverse effects.

Since the beginning of time, humans have been dependent on plants for medicinal purposes. Medicinal plants have been used to treat a variety of diseases in Southern Africa and the introduction of western drugs has not changed this in a traditional setting [10]. Current estimation indicates that about 80 million people worldwide still depend on plants for their health needs. In South Africa, about 60% of the population use plants in conjunction with pharmaceuticals [11]. In developed and underdeveloped countries, rural people still depend upon herbal medicines for the treatment of various diseases, as they are cheaper, and are believed to have fewer side effects [12,13]. Several line of studies have also reported that medicinal plants contain a wide variety of free radical scavenging molecules such as phenols, anthocyanins tannins, alkaloid and saponins, which act against wound infecting bacteria [11]. Agyaye *et al*. [14] revealed that medicinal plants are very good sourcing of antioxidants and reported to play a major role in the treatment of wound infections. The identification and isolation of secondary metabolites from plants origin have recently become a major interest to most researchers [15]. However, previous studies have showed that some phenolics compounds such as coumarin and quercetin found in most medicinal plants reported to possess antibacterial activity against some pathogenic bacteria strains [16]. In addition, compounds such as butulinic acid and 2-alpha hydroxyurosolic acid isolated from *Curtisia dentate* have also found to possess antimicrobial properties [15]. The study evaluated the antioxidant and phytochemical properties of *Brachylaena elliptica (B. elliptica)* and *Brachylaena ilicifolia (B. ilicifolia)* ethanol leaf extracts and their effects on bacteria that commonly infect wounds of diabetic patients.

2. Material and methods

2.1. Chemicals

Aluminum chloride ($AlCl_3$), butylatedhydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethybenzthiazoline-6-sufonic acid) (ABTS), Folin-Ciocalteu's phenol reagent, gallic acid, iron III chloride ($FeCl_3$), potassium ferricyanide [$K_3Fe(CN)$], hydrogen peroxide (H_2O_2), nitric oxide (NO), quercetin, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), trichloroacetic tannic acid and vitamin C, were all purchased from Merck (South Africa). All chemicals and solvents used in this experiment were of analytical grade.

2.2. Microorganisms

P. aeruginosa (ATCC 19582), *Proteus vulgaris (P. vulgaris)* (KZN) and *P. mirabilis* (ATCC 7002) *S. aureus* (ATCC 2593), *Streptococcus pyogenes (S. pyogenes)* (Laboratory strain), were obtained from the AEMREG (Applied and Environmental Microbiology Research Group), Department of Biochemistry and Microbiology, University of Fort Hare, South Africa. These bacteria strains were chosen for their pathological effects on wounds in diabetic patients. Amoxicillin and ciprofloxacin antibiotic drugs were used as control.

2.3. Collection of plant materials

The leaves of *B. elliptica* were collected from a thick forest in the Amathole district (Eastern Cape, South Africa) while *B. ilicifolia* leaves were collected from brush land near Grahamstown (Eastern Cape Province of South Africa). Both plants were identified and authenticated at the Giffen Herbarium, University of Fort Hare, South Africa, where Voucher specimens with their corresponding numbers [BRA (47) 8936 for *B. elliptica* and BRA (47) 1512 for *B. ilicifolia*] were kept. The leaves of *B. elliptica* and *B. ilicifolia* were separated from the rest of the plant, washed with clean tap water to remove debris and then oven-dried to a constant weight at 40 °C. The dried plant materials were pulverized into fine powder using an electric blender (Commercial Blender type GB27, Hamilton Beach Brands, Inc. China).

2.4. Preparation of extracts

Approximately 60 g of the powdered samples were extracted with ethanol, maintained on a mechanical shaker [Labcon laboratory service (Pty), South Africa] for 24 h, after which the extract was decanted, filtered through whatman No. 1 filter papers in a Buchner funnel, and the filtrate was concentrated to dryness using a rotary evaporator (Heidolph Laborata 4000, Heidolph instruments, GmbH & Co, Germany) to give the extracts needed for this study [17].

2.5. Antioxidant assays

2.5.1. Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity of the plant extracts was determined according to the method described by Liyana-Pathiranan and Shahidi [18]. One milliliter (1 mL) of the extract or standards (vitamin C and BHT) at different concentrations (0.2–1.0 mg/mL) prepared in triplicate was mixed with 1 mL of DPPH (0.135 mM) prepared in methanol. Thereafter, the mixtures were vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was then measured spectrophotometrically at 517 nm. The percentage DPPH scavenging activity of the extract or standard was calculated using the formula: DPPH radical scavenging activity (%) = $[(A_C - A_S)/A_C] \times 100$, where A_C is the absorbance of the control and A_S is the absorbance of the test sample (extract or standard).

2.5.2. Reducing power assay

The reducing power of the plant extract was determined as described by Aiyegoro and Okoh [19]. The extract or standard

(vitamin C and BHT) was prepared initially in distilled water in increasing concentrations from 0.2 to 1.0 mg/mL. One milliliter (1 mL) of the extract or standard was mixed individually with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1% w/v). The mixture was later incubated for 20 min at 50 °C. This was then followed by the addition of 2.5 mL of trichloracetic (10% w/v), and centrifuged at 3 000 rpm for 10 min (Labline centrifuge model). Thereafter, 2.5 mL of the upper layer solution (supernatant) was withdrawn and mixed with 2.5 mL of distilled water and 0.5 mL of ferrous chloride (0.1%, w/v). The absorbance was measured at 700 nm against a blank sample containing the mixture without the extract.

2.5.3. Determination of ABTS radical scavenging activity

The ABTS radical scavenging activity assay was performed using the method described by Adedapo et al. [20]. The radical was prepared by mixing equal amounts of 7 mM ABTS and 2.4 mM potassium persulphate. These were later allowed to react for 12 h in the dark at room temperature. The resulting solution (1 mL) was further mixed with 60 mL of methanol to obtain an absorbance of (0.706 ± 0.001) units at 734 nm by addition of drops of the original ABTS/potassium persulphate. Thereafter, one milliliter (1 mL) of extract or standard prepared in methanol at different concentrations (0.2–1.0 mg/mL) was later mixed with 1 mL of the ABTS/methanol solution. The absorbance of the resulting solution was then measured spectrophotometrically at 734 nm after about 7 min. The percentage ABTS scavenging activity of the extract or standard was calculated using the formula: ABTS radical scavenging activity (%) = $[(A_C - A_S)/A_C] \times 100$, where A_C is the absorbance of the control ($ABTS^+$ methanol) and A_S is the absorbance of the test sample (extract or standard).

2.5.4. Determination of nitric oxide (NO) radical scavenging activity

Nitric oxide scavenging activity was determined by the adapted method described by Oyedemi *et al*. [21]. Briefly, 0.5 mL of the extract or standard (vitamin C and BHT) at different concentrations (0.2–1.0 mg/mL) was mixed with sodium nitroprusside (2 mL, 10 mM) prepared in 0.5 mM phosphate buffer saline (pH 7.4). The mixture was incubated for 2.5 h at 25 °C. After incubation period, 0.5 mL of incubation solution was withdrawn and mixed with 0.5 mL of Griess reagent [prepared by mixing 1 mL sulphanilic acid (0.33% in 20% glacial acetic acid) with 1 mL of naphthylenediamine dihydrochloride (0.1% w/v)]. The mixture was incubated for 30 min at room temperature and the absorbance was then measured at 540 nm. The percentage of nitric oxide scavenging ability of the plant extracts or standard compounds was calculated using the formula: NO radical scavenging activity (%) = $[(A_C - A_S)/A_C] \times 100$; where A_C is the absorbance of the control reaction (NO radical + methanol) and A_S is the absorbance of the test samples (extract or standard).

2.5.5. Determination of hydrogen peroxide (H_2O_2) radical scavenging assay

Hydrogen peroxide scavenging activity of the plant extract was determined using the adapted method described by Sarma *et al*. [22]. Briefly, one milliliter (1 mL) of the extract or standard (vitamin C and BHT) prepared in distilled water at different concentrations (0.2–1.0 mg/mL) was mixed with 0.6 mL of 4 mM Hydrogen peroxide solution (prepared in 0.1 mM phosphate buffer (pH 7.4). This was later left to react for 10 min, after which the absorbance was measured at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging ability of the plant extracts and standard compounds was calculated using the formula: H_2O_2 radical scavenging activity (%) = $[(A_C - A_S)/A_C] \times 100$; where A_C is the absorbance of the control reaction (Phosphate buffer + H_2O_2) and A_S is the absorbance of the test samples (extract or standard).

2.6. Antibacterial assay

2.6.1. Bacterial strains

Antibacterial activity of the leaf extract of *B. elliptica* and *B. ilicifolia* was determined using the agar dilution method [23]. Five bacterial strains (two gram positive and three gram positive) namely, *S. aureus* (ATCC 2593), *S. pyogenes* (Laboratory strain), *P. aeruginosa* (ATCC 19582), *P. vulgaris* (KZN) and *P. mirabilis* (ATCC 7002) were investigated; amoxicillin and ciprofloxacin antibiotic drugs were used as control. The bacteria strains were sub-cultured on nutrient agar plates and incubated overnight for 24 h at 37 °C. The identical colonies from the culture were suspended in sterile saline and adjusted to give an optical density of 0.1 at 600 nm The adjusted inoculum was then diluted 1:100 in broth to give an approximate inoculum of (5×10^5) CFU/mL as compared with Mcfarland standard.

2.6.2. Minimum inhibitory concentration (MIC)

The antibacterial activity of the extracts was determined using the standard method of the Clinical Laboratory Standard Institute [24]. The nutrient agar (Biolab) was prepared based on the manufacturer's instructions and then placed in a water bath at 50 °C. Thereafter, a stock solution of the extracts was prepared in DMSO (Sigma) and further diluted in agar (Biolab) at 50 °C to give a final concentrations ranging from 0.3 to 5.0 mg/mL. One milliliter (1 mL) from each dilution of the extract was then mixed with molten sensitivity test agar (19 mL) at 50 °C and later poured into sterile petri dishes to allow the agar to cool. The plates containing only nutrient agar, and another set containing nutrient agar and solvent of extraction were served as negative controls, while the plates containing amoxicillin (standard drug) and ciprofloxacin (standard drug) were used as positive controls. Thereafter, the surface of the agar was allowed to dry before it was streaked with standardized overnight broth cultures of the test bacteria. The plates were then incubated for 24 h at 37 °C under aerobic conditions. Each test was performed in triplicate. The MIC was defined as the lowest concentration of the extract or standards (amoxicillin and ciprofloxacin) that completely inhibited the visible growth of the organism.

2.7. Phytochemical analysis

2.7.1. Determination of tannin content

The tannin content was determined according to the method described by Mbaebia *et al*. [25] with some modification. Briefly,

0.2 g of each extract in triplicate was dissolved in 20 mL of 50% methanol. The mixture was shaken vigorously on a mechanical shaker and then placed in a water bath at 80 °C for 1 h to ensure thorough mixing. After which the mixture was filtered into a 100 mL volumetric flask. To the standard concentrations of tannic acid as well as the filtrates from the extracts, 20 mL of distilled water containing 2.5 mL of Folin-Ciocalteu reagent and 10 mL of 17% sodium carbonate (Na_2CO_3) were added to the filtrate and mixed together properly. All mixtures in triplicate were then made up to 100 mL with distilled water and were allowed to stand for 20 min. A bluish-green color developed at the end of the reaction and the absorbance of the mixtures was read at 760 nm using UV–VIS spectrophotometer (AJ-C03). Total tannins content was expressed as tannin standard equivalent (mg/g) using the following equation from the calibration curve $Y = 0.059\ 3x - 0.048\ 5$, $R^2 = 0.982\ 6$, where y was the absorbance and x was the tannic acid equivalent (mg/mL). Total tannin content (T) in milligram tannic acid equivalent per gram of dry extract (mg/g) was calculated using the formula: $T = C \times V/m$, where V is the volume of the extract (mL) used in the assay, C is the tannic acid equivalent (mg/mL) and m is the weight (g) of the pure plant extract used in the assay.

2.7.2. Determination of phenol content

The total phenols content was determined spectrophotometrically with Folin Ciocalteau's phenol reagent using the modified method of Wolfe *et al*. [26]. Briefly, 0.5 mL of the extract solution was mixed with 0.5 mL of Folin-Ciocalteu reagent (previously prepared as 10% v/v dilution in distilled water). Thereafter, 4 mL of anhydrous sodium carbonate (75%) was added, producing a blue colored solution. The resulting mixtures were vortexed and incubated at 40 °C for 30 min. The absorbance was then measured at 765 nm using UV–VIS spectrophotometer (AJ-C03). Total phenolic content was then expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve $Y = 0.121\ 6x$, $R^2 = 0.936\ 5$, where y was the absorbance and x was the tannic acid equivalent (mg/mL). The total phenolic content was calculated using the formula: $T = C \times V/m$, where T is the total phenolic content; V is the volume of the extract (mL) used in the assay, C is the tannic acid equivalent established from the calibration curve in (mg/mL) and m is the weight of the pure plant extract used in the assay.

2.7.3. Determination of flavanoid content

The flavonoid content was determined according to the method described by Ordonez *et al*. [27]. Briefly, 0.5 mL of the extract (prepared at a concentration of 0.1 mg/mL) was mixed with 0.5 mL of 2% aluminum chloride (prepared in ethanol). The mixture was incubated for 1 h at room temperature, after which the absorbance was read at 420 nm. A yellow color indicating the presence of flavonoid. Total flavonoid content was calculated as mg/g of quercetin equivalent using the calibration curve obtained $Y = 0.025\ 5$, $R^2 = 0.981\ 2$, where y was the absorbance and x was the quercetin equivalent concentration (mg/mL). The total flavonoid content was calculated using the formula: $T = C \times V/m$, where T is the total flavonol content; V is the volume of the extract (mL) used in the assay, C is the quercetin equivalent (mg/mL) and m is the weight (g) of the pure plant extract used in the assay. Values were expressed as quercetin equivalent per gram of dry plant extract (mg Qe/g).

2.7.4. Determination of flavanol content

Total flavonol content in the plant extract was determined by adopting the procedure described by Kumaran and Karunakaran [28]. Briefly, 2 mL of the extract was mixed with 3 mL of ethanol (95%), 0.2 mL of aluminum chloride (10% w/v) and 0.2 mL of sodium acetate solution (50 g/L). The resulting mixture was made up to 10 mL with distilled water, vortexed thoroughly and allowed to stand for 2.5 h at room temperature, after which absorbance was read at 440 nm. Total flavonol content was calculated as mg/g of quercetin equivalent using the calibration curve obtained $Y = 0.025\ 5$, $R^2 = 0.981\ 2$, where y was the absorbance x was the quercetin equivalent concentration (mg/mL). The total flavonol content was calculated using the formula: $T = C \times V/m$, where T is the total flavonol content; V is the volume of the extract (mL) used in the assay; C is the quercetin equivalent (mg/mL) and m is the weight (g) of the pure plant extract used in the assay. Values were expressed as quercetin equivalent per gram of dry plant extract (mg Qe/g).

2.7.5. Determination of proanthocyanidins content

Total proanthocyanidin contents were determined by an adapted method described by Oyedemi *et al*. [21]. The extract was prepared at a final concentration of 1 mg/mL. To 0.5 mL of the extract, in triplicate, 3 mL of 4% vanillin-methanol solution and 1.5 mL of hydrochloric acid were added and vortexed. The mixtures were thoroughly mixed and allowed to stand for 15 min at room temperature. The absorbance was then measured at 500 nm. Total proanthocyanidins content was calculated as mg/g of catechin equivalent using calibration curve $Y = 0.582\ 5x$, $R^2 = 0.927\ 7$, where y was the absorbance x was the catechin equivalent (mg/mL). The amount of proanthocyanidin in the extracts in mg/g was calculated with the formula: $T = C \times V/m$, where V is the volume of the extract (mL) used in the assay, C is the catechin equivalent (mg/mL) and m is the weight (g) of the pure plant extract used in the assay.

2.7.6. Estimation of alkaloid content

The alkaloid content in plant extracts was quantitatively determined following the method described by Harborne [29]. Five grams (5 g) of the powdered plant extract were dissolved in 20 mL of 20% acetic acid prepared in ethanol (v/v). The mixture was allowed to stand for 4 h and then filtered. The filtrate was collected and placed in a water bath for about 30 min at boiling temperature. Thereafter, ammonium hydroxide (concentrated) was added drop wise into the extract which later produced some precipitation. The collected precipitate was washed with dilute ammonium hydroxide (NH_3OH) and then filtered. The residues left in the filter paper were allowed to dry in an oven and the resulting dried papers with residue were weighed. The alkaloid content was then calculated using the equation:

$$\%\text{Alkaloids} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100\%.$$

2.7.7. Estimation of saponin content

The saponin content in plant extracts was determined following the method described by Obadoni and Ochuko [30]. Briefly, 50 mL of 20% ethanol (prepared in distilled water) was added to 10 g of the plant sample and kept in a shaker for 30 min. The mixture was heated over a water bath for 4 h at 55 °C. The resulting mixture

was then filtered and the residue was re-extracted with 50 mL of 20% ethanol (prepared in distilled water) which was later reduced to 40 mL in a water bath at 90 °C. Thereafter, the concentrate was then transferred into a 250 mL separatory funnel and then extracted twice with diethyl ether (20 mL). Thereafter, the ether layer was discarded while the purification process was repeated. Sixty milliliter (60 mL) of *n*-butanol was added and later washed twice with 10 mL of 5% aqueous sodium chloride (Nacl). The remaining solution was then heated over a water bath and evaporated to dryness to a constant weight at 40 °C. The saponins content was calculated using the equation:

$$\text{Saponin contents } (\text{mg/g}) = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100\%.$$

2.8. Statistical analysis

All the experiments were performed in triplicate and, where applicable, the data obtained were analyzed statistically using one-way analysis of variance (ANOVA), and the difference between samples were determined by Duncan's multiple range test using the minitab program (version 12 for Windows). The data were expressed as the mean ± standard deviation and values were considered significant at $P < 0.05$.

3. Results

3.1. DPPH radical scavenging activity

In this study, the effect of ethanol leaf extracts of *B. elliptica* and *B. ilicifolia* against the DPPH radical was evaluated (Table 1). The results showed that both plants scavenge the radical in a concentration-dependent manner. In addition, comparable scavenging activities of both plant extracts were observed with those of the standard compounds. However, *B. elliptica* showed higher DPPH scavenging activity compared to *B. ilicifolia* extract within the concentration range of 0.6–1.0 mg/mL. It was also observed that the concentration that can scavenge 50% of the radical (IC_{50}) values of *B. elliptica*, *B. ilicifolia* and that of vitamin C and BHT were 0.65, 0.64, 0.61 and 0.61 mg/mL, respectively.

3.1.1. Reducing power

The ferric reducing antioxidant ability of the extracts was estimated to determine the potential of the extracts to reduce the Fe^{3+}-TPTZ (ferric tripyridyl triazine) by electron donation, to (Fe^{2+}) ferrous which has an intense blue color [31].

In this study, the Vitamin C produced a much higher antioxidant activity compared to both plants extracts and BHT at all the tested concentration, in a concentration dependent manner. In comparison, extracts of *B. ilicifolia* possessed better ferric reducing abilities than the *B. elliptica* at all the tested concentration investigated in this study (Table 2).

3.2. ABTS radical scavenging activity

The antioxidant activity of ethanol leaf extract of *B. elliptica* and *B. ilicifolia* against ABTS radical is shown in Table 3. The extracts of both plants showed strong scavenging ability of ABTS at all the concentrations tested, but the scavenging effect was lower than the standard compounds (vitamin C and BHT). However, the IC_{50} value of *B. ilicifolia* extract (0.34 mg/mL) was found to be lower than *B. elliptica* (0.55 mg/mL) and the standard compounds [vitamin C (0.54 mg/mL) and BHT 0.61 (mg/mL)]. On the other hand, since a low IC_{50} value indicated a high antioxidant ability of a compound, *B. ilicifolia* extract having lower IC_{50} value indicated a better scavenging activity of ABTS radical when compared to *B. elliptica*. Therefore, the high percentage of ABTS scavenging activities observed in this study suggested the high degree of antioxidant potential of both plant extracts.

3.2.1. Nitric oxide radical scavenging activity

In this study, both plant extracts displayed a significant nitric oxide scavenging activity at all the tested concentrations (Table 4). In comparison, *B. ilicifolia* extract displayed a higher NO radical scavenging activity when compared with that of the *B. elliptica*, Vitamin C and BHT at a concentration of 0.4 mg/mL. It was also observed that the IC_{50} values of *B. elliptica*, *B. ilicifolia*, vitamin C and BHT were noted to be 0.50, 0.66, 0.59 and 0.70 mg/mL respectively.

3.2.2. Hydrogen peroxide radical scavenging activity

In this study, the results showed that both plants had a strong potential in eradicating hydrogen peroxide at all the tested concentrations, but lower when compared to the standard compounds (Table 5). The scavenging activities of both plant extracts and the standard compounds were concentration-dependently decreased in hydrogen peroxide activity, with *B. ilicifolia* displaying the most effective antioxidant activity, having the lowest IC_{50} value of 0.57 mg/mL when compared with *B. elliptica* (0.60 mg/mL), BHT (0.63 mg/mL) and vitamin C (0.64 mg/mL).

3.2.3. Antibacterial activity

Table 6 shows the result of the antibacterial activity of the *B. elliptica* and *B. ilicifolia* leaves tested against five bacterial strains that are linked with wound infections in diabetic patients. The MIC results show that the ethanol extract of both plants had

Table 1
DPPH radical scavenging activity of the ethanol extract of *B. elliptica* and *B. ilicifolia*.

S/N	Concentrations (mg/mL)	DPPH radical scavenging activity (%)			
		B. elliptica	*B. ilicifolia*	Vitamin C	BHT
1	0.2	91.54 ± 0.02[a]	92.49 ± 0.06[a]	97.42 ± 0.06[b]	97.19 ± 0.08[b]
2	0.4	91.36 ± 0.11[a]	90.65 ± 0.12[a]	96.79 ± 0.09[b]	96.50 ± 0.28[b]
3	0.6	89.04 ± 0.18	88.29 ± 0.25	96.08 ± 0.09[a]	96.02 ± 0.13[a]
4	0.8	87.76 ± 0.20	86.09 ± 1.49	95.46 ± 0.02[a]	95.58 ± 0.14[a]
5	1.0	87.09 ± 0.31	82.67 ± 1.82	94.62 ± 0.16[a]	94.35 ± 0.32[a]

Each value represents mean ± SD ($n = 3$). Values with the same letter superscript within the same concentration do not differ significantly ($P < 0.05$).

Table 2
Reducing power of the ethanol extract of *B. elliptica* and *B. ilicifolia* (Absorbance@700 nm).

S/N	Concentrations (mg/mL)	*B. elliptica*	*B. ilicifolia*	Vitamin C	BHT
1	0.2	0.337 ± 0.01[a]	0.366 ± 0.06[a]	1.331 ± 0.08	0.615 ± 0.08
2	0.4	0.475 ± 0.00	0.576 ± 0.10	2.168 ± 0.08	0.786 ± 0.00
3	0.6	0.572 ± 0.02	0.841 ± 0.05[a]	2.169 ± 0.07	0.870 ± 0.02[a]
4	0.8	0.690 ± 0.05	1.071 ± 0.05	2.283 ± 0.01	1.219 ± 0.08
5	1.0	0.763 ± 0.04	1.243 ± 0.11	2.288 ± 0.01	1.315 ± 0.08

Each value represents mean ± SD (n = 3). Values with the same letter superscript within the same concentration do not differ significantly ($P < 0.05$).

Table 3
ABTS radical scavenging activity of the ethanol extract of *B. elliptica* and *B. ilicifolia* (%).

S/N	Concentrations (mg/mL)	*B. elliptica*	*B. ilicifolia*	Vitamin C	BHT
1	0.2	98.09 ± 0.02[a]	98.54 ± 0.07[a]	99.84 ± 0.09[b]	99.97 ± 0.03[b]
2	0.4	97.60 ± 0.03[a]	98.11 ± 0.02[a]	99.57 ± 0.03[b]	99.92 ± 0.02[b]
3	0.6	96.82 ± 0.01	97.87 ± 0.06	99.45 ± 0.04[a]	99.86 ± 0.03[a]
4	0.8	96.77 ± 0.00[a]	96.89 ± 0.03[a]	99.37 ± 0.09[b]	99.68 ± 0.03[b]
5	1.0	96.37 ± 0.03	92.43 ± 0.38	99.24 ± 0.01[a]	99.53 ± 0.08[a]

Each value represents mean ± SD (n = 3). Values with the same letter superscript within the same concentration do not differ significantly ($P < 0.05$).

Table 4
Nitric oxide radical scavenging activity of the ethanol extract of *B. elliptica* and *B. ilicifolia* (%).

S/N	Concentrations (mg/mL)	*B. elliptica*	*B. ilicifolia*	Vitamin C	BHT
1	0.2	84.29 ± 0.03	85.67 ± 0.06	83.69 ± 0.02	82.21 ± 0.03
2	0.4	80.44 ± 0.03	84.81 ± 0.06	83.66 ± 0.02	82.06 ± 0.07
3	0.6	77.95 ± 0.05	81.25 ± 0.04[a]	71.87 ± 0.01	81.39 ± 0.06[a]
4	0.8	77.01 ± 0.07[a]	76.45 ± 0.07[a]	70.56 ± 0.03	79.46 ± 0.08
5	1.0	75.95 ± 0.11	72.77 ± 0.12	68.00 ± 0.08	77.7 ± 0.01

Each value represents mean ± SD (n = 3). Values with the same letter superscript within the same concentration do not differ significantly ($P < 0.05$).

Table 5
H_2O_2 radical scavenging activity of the ethanol extract of *B. elliptica* and *B. ilicifolia* (%).

S/N	Concentrations (mg/mL)	*B. elliptica*	*B. ilicifolia*	Vitamin C	BHT
1	0.2	94.49 ± 0.29	91.13 ± 0.87	99.78 ± 0.01[a]	99.72 ± 0.07[a]
2	0.4	92.59 ± 0.77	85.10 ± 2.46	99.62 ± 0.03[a]	98.55 ± 0.23[a]
3	0.6	86.10 ± 0.98	77.87 ± 0.73	99.44 ± 0.03	96.98 ± 0.43
4	0.8	81.65 ± 0.14	75.85 ± 4.27	99.37 ± 0.44	95.79 ± 0.44
5	1.0	79.32 ± 1.29	70.43 ± 0.50	98.78 ± 0.79	93.67 ± 2.62

Each value represents mean ± SD (n = 3). Values with the same letter superscript within the same concentration do not differ significantly ($P < 0.05$).

Table 6
MIC (mg/mL) of *B. elliptica* and *B. ilicifolia* ethanol leaf extracts.

Bacteria	Gram (+/−)	*B. elliptica*	*B. ilicifolia*	Amoxicillin	Ciprofloxacin
S. aureus	+	na	5	0.6	0.3
S. pyogenes	+	2.5	2.5	0.6	0.3
P. aeroginosa	–	2.5	2.5	0.6	0.3
P. vulgaris	–	5	na	0.6	0.3
P. mirabilis	–	5	5	0.6	0.3

na = not active.

a broad spectrum and was able to inhibit the growth of the tested bacteria strains between the ranges of 0.3–5.0 mg/mL.

The extracts of *B. elliptica* and *B. ilicifolia* displayed a strong antibacterial activity against *P. aeruginosa* and *S. pyogenes*, having MIC values of 2.5 and 2.5 mg/mL respectively. It was also observed that both plant extracts exhibited effective antibacterial activity against *P. mirabilis*, with MIC values of 5 mg/mL. *B. elliptica* leaf extract was found to be inactive in *S. aureus* but activity was found with *B. ilicifolia* when tested with a MIC value of 5 mg/mL. The results also indicated no significant antibacterial activity against *P. vulgaris* when tested with *B. ilicifolia* extract,

but activity was presented in *B. elliptica* with MIC value of 5 mg/mL. Nevertheless, none of the extracts of both plants showed more potent than that of the standard drugs (amoxicillin and ciprofloxacin) with MIC values of 0.6 and 0.3 mg/mL respectively.

3.2.4. Phytochemical analysis

The phytochemical analysis of the ethanol leaf extracts of *B. elliptica* and *B. ilicifolia* revealed the presence of phenols, flavanols, tannins, flavanoids, alkaloids, proanthocyanidins, and saponins (Table 7). Many plants are now used for the traditional treatment of diseases, possibly due to the presence of these phytochemicals, which have been reported to possess high medicinal value. The results show that *B. ilicifolia* possesses a high content of proanthocyanidin (417 mg/g catechin equivalent), tannin (211 mg/g tannic acid equivalent), total phenol (98.6 mg/g tannic acid equivalent), and total flavonols (48.8 mg/g quercetin equivalent), when compared with those of *B. elliptica* (Table 7). It was also observed that there were no significant differences in the flavonoid contents of the leaf extracts of *B. elliptica* and *B. ilicifolia* ($P > 0.05$). The alkaloid content in the extracts of *B. ilicifolia* (38.3 mg/g) was significantly higher when compared to that of *B. elliptica* (24.3 mg/g), while saponin content in *B. ilicifolia* was significantly lower compared to that of *B. elliptica* extract.

Table 7
Phytochemical analysis of the ethanol extract of *B. elliptica* and *B. ilicifolia*.

Compounds	Amount of phytochemical compounds in mg/g	
	B. elliptica	*B. ilicifolia*
Saponins	34.3 ± 0.57	13.3 ± 0.58
Alkaloids	24.3 ± 0.58	38.3 ± 0.57
Proanthocyanidins[3]	156 ± 25.3	417 ± 32.9
Flavanoids[2]	11.5 ± 5.05[a]	8.86 ± 2.25[a]
Flavonols[2]	44.1 ± 0.09	48.8 ± 0.06
Phenols[1]	75.4 ± 0.02	98.6 ± 0.04
Tanins[1]	143 ± 0.06	211 ± 1.06

Each value represents mean ± SD ($n = 3$). Values with the same letter superscript within the same concentration do not differ significantly ($P < 0.05$). The subscript indicates: [1]Expressed as mg/g of the extracts as tannic acid equivalent; [2]Expressed as mg/g of the extracts as quercetin equivalent; [3]Expressed as mg/g of the extracts as catechin equivalent.

4. Discussion

Studies have indicated that medicinal plants are very good sources of antioxidant and reported to play a significant role in the treatment of diseases globally [32]. Many of these plants have been indicated to possess high antioxidant properties such as the reduction of DPPH radicals, ABTS, NO_2 and ferric ion reducing power due to the presence of bioactive secondary metabolites which are rich in antioxidant and free radical scavenging properties [33]. Therefore, we considered using DPPH, ferric reducing power, ABTS, NO_2 and H_2O_2 radicals as a model for antioxidant capacity in this study.

The DPPH radical scavenging activity results showed that both plants scavenge the radical in a concentration-dependent manner. The scavenging ability of both plant extracts that occurs in varying degrees depending on their antioxidant capacities might be due to the presence of polyphenol compounds, most especially phenols that have the ability to donate the hydrogen atoms in their hydroxyl groups [34]. This might have played a significant role in eradicating the radical.

The ferric reducing antioxidant ability of the extracts was estimated to determine the potential of the extracts to reduce the Fe^{3+} to Fe^{2+} and is associated with the presence of a redundant molecule or complex that serves as the electron donor and/or free radical scavengers. In this study, we observed a concentration-dependent increase in the absorbance of reaction mixture for both plant extract and the standard drugs (vitamin C and BHT), indicating that the extracts have the ability to reduce oxidative stress. However, studies have also suggested that plant secondary metabolites such as phenol and flavanol are largely involved in the antioxidant activities of the extracts [35–37]. Therefore, This result supports the findings of El-Hashasa *et al.,* [38] who reported that the reducing power of a plant correlates with its phenolic content.

The ABTS scavenging method measures the antioxidant properties of the plant extracts using a blue chromophore. In this study, both plants extracts scavenged ABTS radical in a concentration dependent-manner at all the tested concentration investigated. The high percentage of ABTS scavenging activities observed in this study could be attributed to a high content of bioactive components which is similar to the report in previous studies [39].

NO radical is produced from sodium nitroprusside in aqueous solution, which reacts with oxygen to form nitrile. However, over production of this radical has been implicated in the pathogenesis of various diseases such as diabetes, carcinomas and arthritis [40]. The results of nitric oxide radical scavenging activity showed that ethanol extracts of both plants inhibited the formation of nitric oxide. This may be due to the antioxidant principle in the extracts, which competed with oxygen to react with nitric oxide and thus inhibited generation of nitrile [41]. Our data, therefore, supported the findings of previous studies who reported strong NO radical scavenging activity of aqueous extracts of *B. elliptica* and *B. ilicifolia* [42]. The activity of ethanol leaf extracts of these plants may possibly help to stop the chain reactions instigated by excessive production of nitric oxide and may play a significant role in preventing inflammatory signaling processes involving nitric oxide.

H_2O_2 is an important reactive oxygen species based on its ability to cause oxidative degradation of cell membrane lipids to give rise to the occurrence of mutagenesis and cytotoxicity. It is rapidly broken down into water and oxygen, thereby producing hydroxyl radical that can initiate lipid peroxidation and cause DNA damage in the body [43]. Therefore, eradication of hydrogen peroxide radical in order to prevent the body system from this radical is very important. In this study, our results indicated that both plants exhibited a strong potential in eradicating hydrogen peroxide at all the tested concentrations investigated, but lower when compared to the standard compounds. The higher activity observed against hydrogen peroxide in this study may be attributed to the high polyphenols content. Therefore, It can be deduced from this study that both extracts converted the hydrogen peroxide to water and oxygen, causing a decrease in hydrogen peroxide concentration in assay mixtures as the concentration of the extracts increases.

Several lines of studies have indicated that gram-negative bacteria are more resistant toward anti-microbial agents than gram-positive bacteria, due to the presence of multilayered structure of gram-negative which are not present in gram-positive bacteria [44]. In this study, our results showed that the extracts of both plants inhibited growth of two out of three

gram negative bacteria used in this study. Our finding on *P. aeruginosa* inhibited by the extract of *B. ilicifolia* supported the report of Sagbo *et al.* [42] who reported the antibacterial properties of an aqueous extract of *B. ilicifolia*, but contradicted the MIC value obtained on *P. aeruginosa*. Several lines of studies have also reported that flavonoids, tannins, alkaloids and polyphenols compounds possess antibacterial properties [21]. It can therefore be indicated that the observed antibacterial activity of the ethanol leaf extracts of *B. elliptica* and *B. ilicifolia* against some bacteria strains associated with wounds in patients suffering from diabetes could be due to the presence of bio-active compounds. Therefore, the results of this present study suggest that ethanol leaf extracts of both plants could be an effective herbal remedy for treatment of wound infections caused by these bacteria in patient suffering from diabetes.

The phytochemical analysis of ethanol extract of *B. elliptica* and *B. ilicifolia* revealed the presence of phenols, saponins, alkaloids, flavonols and flavonoids. It has been reported that medicinal plants play a significant role in the treatment of various diseases due to the presence of these phytochemicals, which possess high medicinal value. Tannin has been linked to antibacterial properties, inhibition of lipid oxidation and treatment of cancer [45,46]. The presence of this phytochemical in the ethanol leaf extract of both plants could possibly play a significant role in the antioxidative and antibacterial properties observed in this present study. Alkaloids are one of the bioactive components in plant with the potential to protect cells against foreign invading agents due to that toxic nature. The presence of alkaloids in the extracts of both plants may justify their ethno-medicinal usage for the treatment for various diseases. The saponins are among one of the largest secondary metabolites, serving as a potential source of antibacterial and anticancer agents. The presence of saponin in the ethanol leaf extracts of *B. elliptica* and *B. ilicifolia* could underline the antibacterial activities investigated in this study.

Phenols, flavonols, proanthocyanidins and flavonoids are very important plant bioactive component reported to possess strong antioxidant, antibacterial and numerous biological activities due to their molecules structures reported to contain a hydroxyl group or phenolics ring [16,47]. Several studies have indicated that these compounds are very effective in scavenging free radicals due to their redox properties, thereby having the capacity to link with proteins and bacterial membranes to form complexes [48].

The ethanol leaves extract of *B. elliptica* and *B. ilicifolia* exhibited strong antioxidant and free radical scavenging properties, which may be due to the presence of flavanoids, tannin, alkaloid and polyphenols compounds. The antibacterial activity of the extract against tested bacteria shows that it has the potential to be used for the treatment of wound infections caused by these bacteria in diabetic patients.

Conflict of interest statement

The authors declare that they have no competing interests.

Acknowledgments

This research was supported by a grant from Govan Mbeki Research and Development Centre, University of Fort Hare (Grant No. C228).

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