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

Idowu Jonas Sagbo[1,2], Anthony Jide Afolayan[1], Graeme Bradley[2*]

[1]*Department of Botany, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa*

[2]*Department of Biochemistry and Microbiology, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa*

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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

*Corresponding author: Graeme Bradley, Department of Biochemistry and Microbiology, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa.
Tel/Fax: +27 768343754.
E-mail: GBradley@ufh.ac.za (G. Bradley).

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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