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Phytochemical and Antifungal Activities af Root Extract of *Barleria Prionitis* L.

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ABSTRACT: *Barleria prionitis*, a small perpetual, prickly shrub, belongs to the family Acanthaceae, normally called "porcupine flower" or "Vajradanti" with a lot of vernacular names. It is an indigenous herb of Southern Asia and some regions of Africa. The therapeutic use of leaf, flower, stem, seed, shoot, root and in certain cases complete herb of B. prionitis against numerous disorders including cough, fever, jaundice, asthma, severe pain, acne, cut wound are recognized by ayurvedic and other conventional medicinal systems. An exhaustive bibliographic research of this plant has been carried out by means of scientific engines and databases like Google Scholar, PubMed, and Science direct; as a result, it has been found that this herb possesses a rich phytochemical content and a wide range of pharmacological activities such as antimicrobial activity, anthelmintic activity, antidiarrheal activity, antioxidant activity, antifertility activity, anti-diabetic activity, anti-inflammatory activity, cytoprotective activity, antiarthritic activity, diuretic activity, hepatoprotective activity, enzyme inhibitory activity and antinociceptive activity without any toxic effects. The plant has been reported to have tannin, saponin, flavonoid, glycoside, alkaloid and phenolic compounds present in it. It is also a noteworthy origin of secondary metabolites such as β-sitosterol, lupeol, syringic acid, and vanillic acid. However, having a potential therapeutic importance, it is still underutilization.

KEYWORDS: Barleria prionitis, vajradanti, antimicrobial, phytochemical, antifungal, root extract, secondary metabolites

I.INTRODUCTION

Barleria prionitis is a shrub in the family Acanthaceae, native to Island and Mainland Southeast Asia, China, the Indian Subcontinent, the Arabian Peninsula and northeastern Africa. It is widely spread as an ornamental and weed, occurring in naturalised populations around the world. It used not only as an ornamental but also as a hedge and extensively as a component of folk medicines. As a weed it is regarded as problematic in many areas.[1,2,3] A much-branched shrub up to 1.8m tall, the lower leaf axils have spines 1–2 cm in length, one of the specific features of this *Barleria*.[1][2][3] The stem and branches are terete, smooth, lenticellate and glabrous. The leaves are elliptic to ovate (4-10.5 × 1.8-5.5 cm) with both surfaces pubescent when young but becoming glabrescent soon. The large golden-yellow flowers are clustered in the axils of the upper leaves and/or on bracts. [4,5]Apically spinose calyx lobes and a yellowish to orange corolla are used to distinguish this species from other *Barleria*.[2] In Zhōngguó/China, flowers appear October to December, while fruiting occurs from December to February, while in Pakistan there is all year flowering.[4] The Australian flowering and fruiting times are trimodal, from March to June, August to October and December.[5]

The subspecies *pubiflora* (see Taxonomy, below) is separated from the nominate subspecies by having longer anthers (5mm or more) and corolla (4.5–7 cm) and larger leaves (up to 17 x 5.5 cm).[3] Some of the habitats in which the shrub is found include roadsides, thickets, and dry places in evergreen broad-leaved forests.[1] It occurs up to 600 m. In Myanmar the shrub favours fields and pastures.[11]

A fast growing perennial plant widely commercialized as an ornamental, *B. prionitis* in many tropical areas has frequently escaped and grown as a weed in disturbed areas, forest edges, rocky outcrops, near streams, along roads, and in overgrazed pastures.[15] It able to grow in a wide range of climates and soil types and is adapted to grow in open, full sunny areas and in highly disturbed sites as well as understory of secondary forests.[6,7] It has great dispersal capability, spreading sexually by seeds and vegetatively by stem fragments. The shrub has potential to cause economic and environmental damage in that it formins dense thickets that displace native vegetation, prevent revegetation by native plants. Stock movement cam be impeded, waterway access restricted, and aesthetic values diminished. In Australia, the plant is on an alert list for environmental weeds, as it has the potential to seriously degrade ecosystems.[8,9]

Barleria prionitis is often the host to larvae of the *Phalanta phalantha* and *Junonia lemonias* butterflies.[16]

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II.MATERIALS AND METHODS

Qualitative Phytochemical Analysis

Preliminary phytochemical screening was carried out on the powdered leaf and stem material and chemically tested for the presence of various constituents using standard protocols [10,11]

Fluorescence Analysis

Fluorescent analysis of the dried powdered plant material plays an important role in the determining the quality and purity of the tested drug. A small quantity of the dry plant powder (leaves and stems) was placed separately onto clean microscope slides. Two drops of each prepared reagent were dispensed, mixed gently by slanting the slide and allowed to stand for 3 min for the thorough absorption of the solution by the plant powder. The slides were then viewed using a Nikon Eclipse microscope, using bright field light and UV-2A (excitation 320/380) illumination. The colours attained by the application of various reagents were recorded. Fluorescence analysis of the leaf and stem powder was carried out using the standard method [12,13]

Gas Chromatography-Mass Spectrometry (GC-MS)

This analysis is used to examine liquid, gaseous or solid samples and produce several different peaks in the gas chromatogram. Each peak generates a specific mass spectrum which is used for compound identification. Leaf and stem methanolic extracts were analysed using the GC-MS (QP-2010 Ultra SE, Shimadzu, Kyoto, Japan) instrument, with an Rx_5Sil Ms capillary column (0.25 µm internal diameter and 0.25 µm film thickness) from Restek (Bellefonte, PA, USA). The carrier gas, helium, had a flow rate of 0.96 mL/min, a total flow of 4.9 mL/min and a linear velocity of 36.7 cm/sec at a purge flow of 3.0 mL/min. The injection temperature was set at 250 °C. The oven temperature was set at 50 °C and held for 1 min, increased to 310 °C and held for a further 10 min. Chemical compounds (analytes) were identified by relating their retention times with those of the polychlorinated biphenyl (PCB) standards found in the National Institute of Standards and Technology (NIST) library. This analysis was conducted at the Department of Chemistry at the University of KwaZulu-Natal, Westville campus.

Antibacterial Bioassay

Crude (hexane, chloroform and methanol) leaf and stem extracts were transferred to Eppendorf centrifuge tubes, dissolved in 10% dimethyl sulfoxide (DMSO) at various concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 mg/mL and homogenised using a vortex. The prepared sample was stored at −4 °C until further use. The prepared crude extracts were subjected to antibacterial assays. Leaf and stem samples were tested against Gram-positive bacteria (*Bacillus subtillus* ATCC 6633, *methicillin*-resistant *Staphylococcus aureus* ATCC 43300 and *Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 25783 and *Escherichia coli* ATCC 35218). These bacterial strains were supplied by Professor Johnson Lin, School of Life Sciences (Microbiology Department), University of KwaZulu-Natal, and maintained in 75% glycerol at −80 °C before the experiment was conducted.[12,13]

In vitro antibacterial screening of the prepared extracts was conducted using the agar disc diffusion technique as per the Clinical and Laboratory Standards Institute (CLSI) guidelines . Both Gram-positive and Gram-negative bacteria from stock cultures were sub-cultured onto fresh agar plates and incubated overnight at 37 °C. Glass test tubes containing distilled water (15–20 mL) were autoclaved at 121 °C for 1 h. Colonies of bacteria from each Petri plate were harvested with a sterile loop and inoculated by transferring a loopful into glass test tubes containing 15 mL of sterile distilled water (0.5 McFarland scale). The absorbance of each bacterial culture was measured, adjusted and diluted to attain a viable cell count using the Cary 60 UV-Vis spectrophotometer.[14,15]

Each bacterial strain was separately smeared uniformly over the surface of the Mueller–Hinton agar plates with a sterile cotton swab. Sterile Whatman filter paper No. 1 discs (diameter 6 mm) were impregnated with 20 µL of the respective extract concentrations (3.125, 6.25, 12.5, 25, 50, 100 mg/mL) and dried at room temperature for 1 h before use [230]. The prepared sterile discs containing extracts were placed carefully onto the agar using sterile forceps. Petri plates were sealed and incubated for 24 h at 37 °C. Zones of inhibition evident around the filter paper were taken as positive results. The diameters of inhibition were measured and photographed within 18–24 h after incubation to

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determine if the extract exhibited any antibacterial activity. Filter paper discs loaded with streptomycin and gentamycin were used as positive controls and 10% DMSO as the negative control . The analyses were conducted in triplicate, and data were presented as mean ± standard deviation.[16,17]

Statistical Analysis

All experiments conducted for the antibacterial assay were carried out in triplicate. Values were expressed as mean ± standard deviation (significant at $p < 0.05$ level). Antibacterial data were statistically analysed using the one-way analysis of variance (ANOVA).

III.DISCUSSION

In Pakistan shrubs are grown as a hedge while its bitter quinine-like extract is used in traditional medicine to treat whooping cough and tuberculosis.[4]

It occurs widely as an ornamental plant.[13][15]

It is used in Indonesia as a component in traditional medicines (*obat*).[19][24] Tetum people in Belu, west Timor use the leaves to treat infected wounds.[17] On small Gili Iyang Island, to the northeast of Jawa, the plant is used to treat toothache.[18]

Parts of the plant are bitter, astringent in taste, and are regarded in Myanmar as highly beneficial for skin, blood and other diseases.[11] Often combined with sesame oil and fermented-rice washing-water, the whole plant, leaves (sometimes burnt to ash or crushed for juice), stems, branches, and roots are used together or separately.[18,19]

In India the root is placed on boils and glandular swellings; the bark is used for dropsy; and the leaf for toothache and rheumatism.[11]

It is used for various medicinal purposes in ayurvedic medicine. The juice of the leaves is applied to feet to prevent maceration and cracking in the monsoon season.[25]

Its leaves are known to contain 6-Hydroxyflavone, one of the chemical compounds that is a noncompetitive inhibitor of the protein cytochrome P450 2C9.[26]

The shrub has been intentionally introduced in many areas to be used as an ornamental, hedge plant, and as a medicinal herb.[15] In the West Indies, it was introduced around the 1900s and it appears in herbarium collections made in 1906 in Barbados and 1910 in Jamaica. In Australia, it was first recorded in the Northern Territory in 1963,[5] it was declared a noxious environmental weed in 2001.[20,21]

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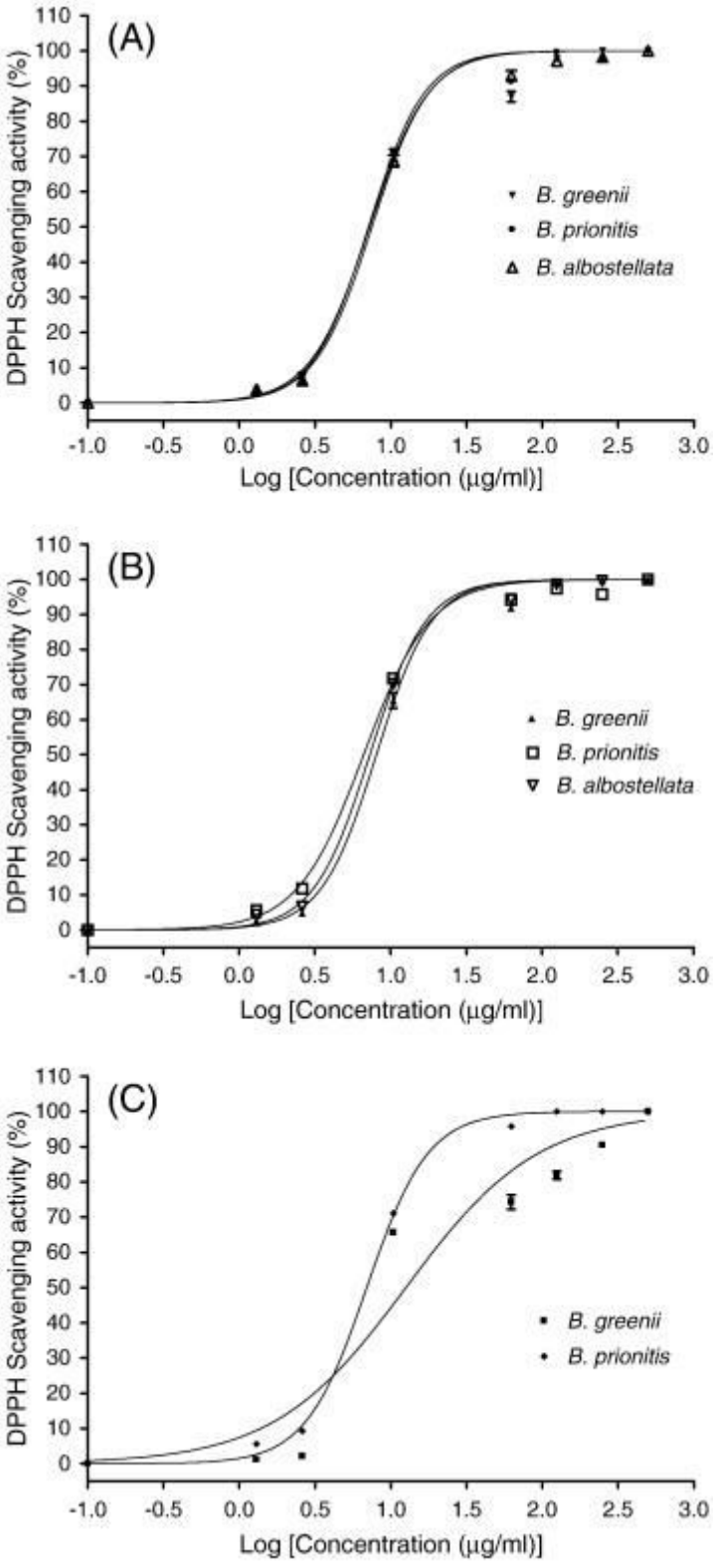
IV.RESULTS



Above Fig. 1. shows the dose–response radical scavenging activities observed in the MeOH extracts of different parts of *Barleria* species.

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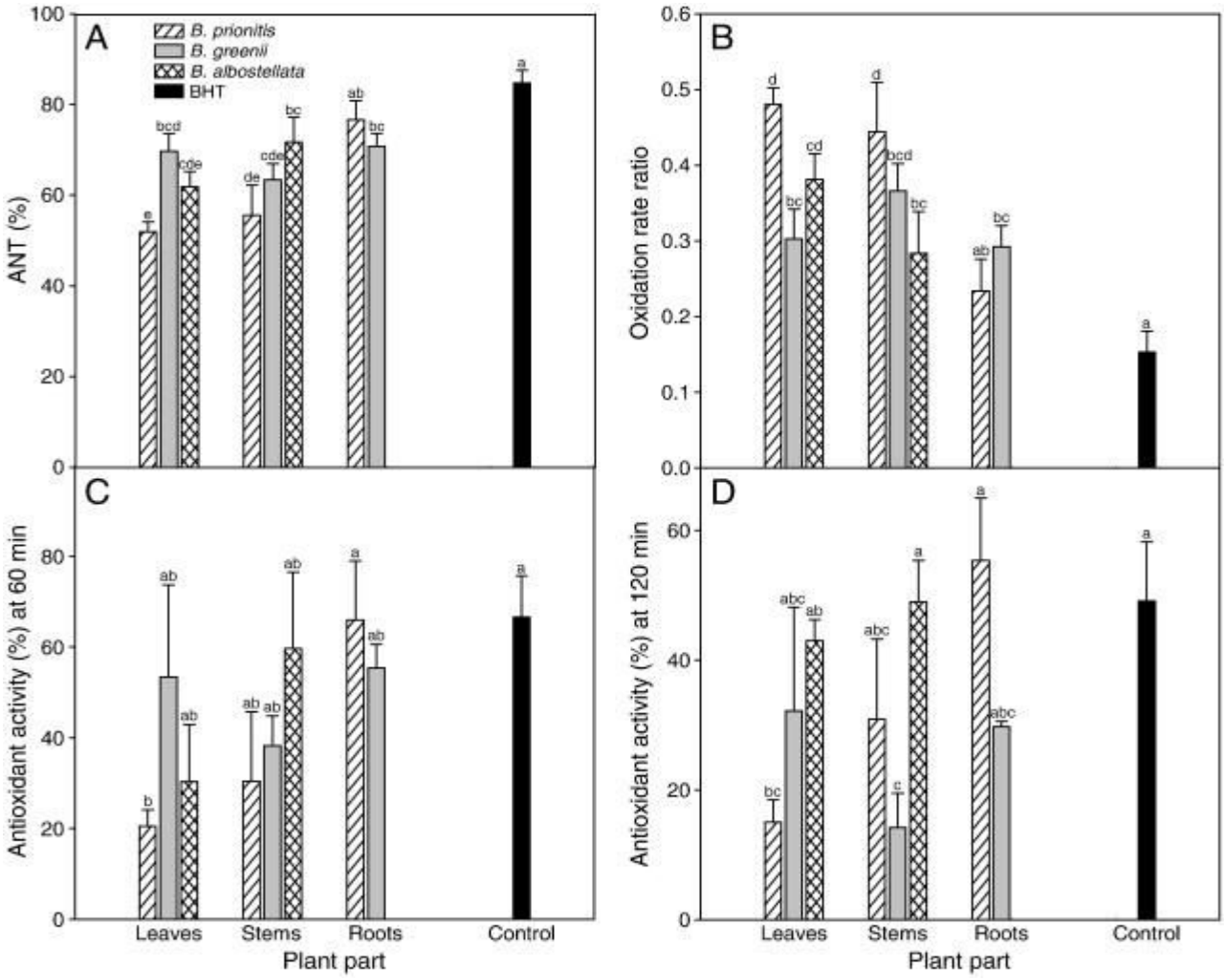

Fig. 2. Antioxidant activities of different parts of three *Barleria* species in *β*-carotene-linoleic acid model system. Bars bearing different letters in each graph are significantly different (*P* = 0.05) according to DMRT. (A) Antioxidant activity (ANT) based on the average *β*-carotene bleaching rate. (B) Oxidation rate ratio (ORR). (C) Antioxidant activity (AA) at *t* = 60 min. (D) Antioxidant activity (AA) at *t* = 120 min.

V.CONCLUSIONS

The observed activities might be due to the presence of flavonoids, iridoids and tannins in the different parts of these species. In some of the pharmacological assays, the leaves demonstrated activities higher than or equal to the other plant parts, [22,23]suggesting their potential in plant-part substitution. The harvesting of leaves or stems as a conservation strategy is certainly more sustainable than the destructive use of the roots of these plant species. However, the concept of substituting plant parts for sustainable exploitation appeared to be dependent on the species and/or biological activity evaluated. The isolation of specific bioactive compounds through bioassay-guided fractionation and their characterization as well as studies evaluating their safety may be necessary in the exploration of these species for potential new therapeutic drugs or drug leads.[24,25,26]

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