Extraction, characterization and pharmacological evaluation of leaves and root bark of *Dalbergiella nyasae* (Baker f.)

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ABSTRACT

Introduction: *Dalbergiella nyasae (Baker f.)* belongs to the family Papilionaceae, small to medium sized herbal plant well known for its medicinal properties in the treatment of gastrointestinal infections including diarrhoea. This study aimed to determine the *in vitro* antimicrobial activity of crude extracts and fractions from leaves and root bark of *D. nyasae* against selected bacteria and yeast of gastrointestinal relevance. **Methods:** Fourteen extracts from leaves and root bark of *D. nyasae* have been screened for the antimicrobial activity against three bacteria species including Gram negative *E. coli* and *P. aeruginosa,* Gram positive *S. aureus* and one yeast species *C. albicans* using agar well diffusion, micro broth dilution and Total Activity (TA) analysis methods. Crude extracts have been qualitatively screened for the presence of phytoconstituents and HPLC fingerprint profiles determined. **Results:** Excellent antibacterial and antifungal activity against Gram positive *S. aureus* and one yeast species *C. albicans* respectively were observed in n-butanol fraction of leaves extract. In root bark, best antibacterial activity against *S. aureus* was observed in ethanol extract while against *P. aeruginosa* was observed in acetone extract. For *E. coli*, best antibacterial activity was recorded in ethanol extract. Phytochemical analysis demonstrated the presence of alkaloids, flavonoids, saponins and terpenoids. The HPLC fingerprint profiles of leaves extract recorded one major peak whereas root bark extract recorded two major peaks. **Conclusion:** This investigation established a good support for use of *D. nyasae* plant by traditional healers in Malawi as herbal medicine for gastrointestinal infections and a base for development of novel potent drugs and phytomedicine.

Keywords: Agar well diffusion, *In vitro* antimicrobial activity, micro broth dilution, phytomedicine, Total Activity (TA) analysis.

INTRODUCTION

Globally, over 10 million under five children die every year mostly from bacterial and fungal related infections (diarrhoea, pneumonia, meningitis, etc) and about 90% of these children come from the developing countries.[1] And the major cause of this morbidity and mortality in the developing countries includes among others poor availability of interventions and spread and emergency of antimicrobial resistance.

Multiple drug resistance has become a real problem in pharmacotherapeutics due to an increasing number of diseases exhibiting various levels of drug resistance.[2] Furthermore, the development of synthetic drugs has slowed down as a result of drug resistance.[3] Consequently, this has created a new renewed interest in the search for new drugs in order to combat resistance.

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Herbal medications and phytochemical screening of various plant species for medicinal leads are now receiving much attention. Some of the herbal medicines that are being considered as a source of new antibacterial drugs have been time-tested for thousands of years and are comparatively safe for both human use and the environment.[4] The plant genetic resource base in Africa has an enormous potential to provide diverse chemical, enzymes and genes that has remained unexploited at industrial scale for production and design of new pharmaceutical products.[5]

The plant *Dalbergiella nyasae* (vernacular name: mlembera or mkanganjovu), is native to Malawi Mozambique, Zambia, Zimbabwe and Tanzania, and belongs to the family of Papilionaceae. It is found in deciduous woodland and thickets, usually small to medium in size with leaves crowded near the ends of branches and inparipinnate with 6–9 pairs of oval leaflets and have a terminal leaflet.[6]

Despite the widespread use of *Dalbergiella nyasae* as traditional medicine in Malawi for treatment of infectious diseases, neither its phytochemistry nor pharmacological effects has been evaluated and reported on the efficacy of purported medicinal preparations. In Malawi, the predominant medicinal system in use is that of traditional medicine, especially in the rural areas where there is limited Government health services such as drug shortage, health personal and insufficient number of hospitals and up to 80% of the population relies on herbal plants as a source of primary health care.[7] However, most of the indigenous information on herbal remedies is passed on from generation to another as folk tales without documentation.[8] Therefore, the purpose of this validation study was to identify the active principles in the plant extracts and to investigate the *in vitro* antimicrobial effect of the crude extract and fractions of leaves and root bark of *Dalbergiella nyasae* used by traditional health practitioners in the treatment of gastrointestinal infections such as diarrhoea and dysentery.

MATERIAL AND METHODS

Plant material

The medicinal plant (*Dalbergiella nyasae*) was collected from Chingale area, in Zomba district (Southern Region of Malawi) and identified by Mr I.H. Patel at Malawi National Herbarium and Botanic Gardens with voucher specimen number 69951 (Salubeni, Tawakali and Mjojo 5840 (MAL) Chingale, Zomba 15°24'S 35°11'E). The root bark and leaves were separately shade dried, finely powdered using a blender and kept in airtight polyethylene bags at room temperature in the dark until used.

Microorganisms

Escherichia coli, Pseudomonas aeruginosa, *Staphylococcus aureus* and *Candida albicans* were obtained from the Department of Microbiology, University of Malawi. The test strains were maintained on nutrient agar slant at 4°C and subcultured on nutrient broth for 24 hrs prior to testing.

Direct extraction and solvent-solvent extraction of plant material

One gram of finely powdered sample was extracted three times with 10 ml of solvents (acetone, ethanol and distilled water) with vigorous shaking. The extracts were decanted after centrifuging at 5300 × g for 10 minutes and solvents removed at room temperature.[9]

For solvent-solvent extraction, 100 grams of finely powdered plant material was extracted with 1 litre of acetone (technical grade-Merck) in macerating bottle. The bottle was shaken for 1 hr, 6 hr and 24 hr on shaking machine and extracts decanted. Six grams of the extract collected was fractionated using solvents of varying polarities (dichloromethane, hexane, butanol, ethyl acetate, aqueous methanol and water). All the solvents were removed under reduced pressure using rotary evaporator at 45°C and dried under room temperature.

Determination of antimicrobial activity

The agar well diffusion method[10] was used to assay the extracts for antimicrobial activity. 0.2 ml of 1 in 100 dilutions of bacterial and fungal cultures (2.5×10^5 cfu ml^{-1}) was added to 20 ml of the melted and cooled Mueller Hinton Agar (MHA) and Sabourand Dextrose Agar (SDA) respectively. The contents were mixed by gentle swirling movements before being poured into sterile petri dishes. After solidification of agar, wells (6 mm) was bored in each plate. 100 µl of each extract dissolved in acetone was poured into appropriately labelled well.

Diameter of zones of inhibitions were determined as an indication of activity after incubating the plate at 37°C for 24 hrs for bacteria and at 25°C for 72 hrs for fungi. Acetone was included in each plate as negative control while chloromphenical and fluconazole were used as positive control for bacteria and fungi respectively. Activity index for each extract was calculated.