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Short Communication

In vitro antimicrobial activity of leaf extract of *Berlina grandiflora* Hutch. and Dalz.

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Antimicrobial properties of *Berlina grandiflora* Hutch. and Dalz. leaf extract were investigated against both clinical and laboratory isolates. Susceptibility of these isolates to the extract was determined using disc diffusion method. The antimicrobial screening had wide range of activity on *Escherichia coli, Stapylococcus aureus* and *Streptococcus* spp. The diameter of zone of inhibition by the extract was 7, 8.7 and 9 mm respectively. The minimum inhibitory concentrations (MIC) were 32.81, 19.38 and 11.72 µg/ml for *E. coli, S. aureus* and *Streptococcus* spp. Based on the current findings, it can be concluded that *B. grandiflora* has antimicrobial activity against certain microorganisms.

Key words: *Berlina grandiflora*, antimicrobial agent, minimum inhibition concentration, *in vitro.*

INTRODUCTION

There is an increasing demand for medicinal plants and plant products as alternative to orthodox medicines especially in developing countries (Murray, 1998). The use of plants and their natural products in Nigeria is a widespread practice in the treatment and management of diseases (Iwu, 1982). This plant based traditional medicine system continues to play essential role in health care, with about 80% of individuals from developed countries relying mainly on traditional medicines for their primary health care (Nascimento et al., 2000; Owolabi et al., 2007). The plants are usually ingested as decoctions and teas or used as spices in the preparation of local delicacies (Okafor, 1975). The investigation of certain plants for their antimicrobial properties may yield useful results. Researchers are increasingly becoming involved in the screening of such plants with the aim of establishing their potential antimicrobial effects and identifying the compounds responsible for the antimicrobial properties (Abinu et al., 2007; Ndukwe et al., 2007).

Berlina grandiflora Hutch. and Dalz. which belong to the family *Leguminasae* is a very popular plant in Nigerian Traditional Medicine. The plant is found all over West Africa as well as in Central and Southern Africa (Dalziel, 1937*). B. grandiflora* is one of the plants whose leaves, stem and bark are used in herbal medical practice. Different parts of this plant are used for the treatment of many ailments.

In Congo Brazaville, the bark is used as a purgative and the leaves are used for treating intestinal problems and cure for malaria (Akuodor et al., 2010). The bark and fruits are used to stupefy fish in South Africa (Asuzu et al., 1993), whereas in Ghana, the stem is used as a chewing stick and in the preparation of enemas against constipation. This work was undertaken to investigate the antimicrobial activity of leaf extracts of *B. grandiflora* against three clinical bacterial isolates. The study will

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make for more economic and optimal use of the plant in alternative medicine.

MATERIALS AND METHODS

Plants collection

The leaves of *B. grandiflora* were collected from Suleja, Niger State, Nigeria. The plant was identified and authenticated by Mallam Ibrahim Muazzam and Mrs. Grace Ugbabe of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria where Voucher specimen (No. 6400) was deposited at the herbarium unit of the Institute for future reference.

Plant preparation and extraction

The leaves were air-dried at room temperature and ground into powder using mortar and pestle. The powdered material (200 g) was macerated with 1.5 L of 70% methanol in water for 24 h with constant shaking. The resultant mixture was filtered using Whatman (No. 1) filter paper and the filtrate concentrated to dryness in vacuum at 4°C using rotary evaporator.

Phytochemical test

Phytochemical screening was carried out using standard method (Trease and Evans, 1989) for detecting the presence of secondary metabolites: alkaloids, tannins, saponins, terpenes, flavonoids, steroids and carbohydrates.

Test organisms

Three clinical bacterial isolates of *Streptococcus* species, *Escherichia coli* and *Staphyloccus aureus* obtained from the Department of Microbiology Laboratory of Federal Medical Centre Owerri, Nigeria using modified Collins et al. (1995) method, a loop full of each of the microorganisms was suspended in 10 ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37°C for 24 h. The organism were then stored at 4°C until needed.

Evaluation of antimicrobial activity

The antimicrobial activity was determined by the paper disc diffusion method (Ayandele and Adebiyi, 2007) agar plates (MHA, oxoid) of Broth cultures of the test isolates at a concentration of 10^6 Cfu/ml of organism was introduced into a sterile Petri dish and 15 ml of molten Mueller Hinton agar were added. The content was thoroughly mixed and allowed to solidify. Sterilized paper discs (6 mm in diameter), soaked in equal volumes of the plant extract (2000 µg/mc per disc) were applied over each of the culture plates. The experiment was performed in triplicate. The plates were allowed to stand for 1 h for prediffusion of the extracts to occur (Esimone et al., 1998) and incubated at 37°C for 24 h.

Determination of minimum inhibitory concentration (MIC)

Nutrient agar method as described by (Ndukwe et al., 2007) was used to determine the minimum inhibitory concentration (MIC) of the extract. The nutrient agar solution was prepared according to the manufacturer's standard. 3 ml of the dilution was mixed with molten nutrient agar into Petri-dishes and allowed to set. Extracts concentration of 250, 200, 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml were prepared. The agar was streaked with an overnight broth culture of the bacterial isolates and incubated for 24 h at 37°C after which they were observed for growth or death of the test organisms. The lowest concentration inhibiting growth was taken as the MIC.

Table 1. Antimicrobial activity of methanolic extract of *B. grandiflora* leaf compare to control by disc diffusion method.

Test organisms	Zone of inhibition (mm)
Streptococcus spp.	9.0 ± 0.58
Escherichia coli	7.0 ± 0.00
Staphylococcus aureus	8.7 ± 0.33

Values are expressed as mean ± S.D.

RESULTS

The ethanolic extract of *B. grandiflora* revealed the presence of flavonoids, tannins, saponins, terpenes, flavonoids, steroids and carbohydrates.

Antimicrobial activity of the extract

The results of the antimicrobial activity of the ethanolic extract against the test organisms, *E. coli, S. aureus* and *Streptococcus* spp. are shown in Table 1. The zone of inhibition of the growth of the isolates is a function of the antimicrobial activity of the extract. *B. grandiflora* showed antimicrobial activity against the tested organism, while distilled water used as control was inactive against the organisms.

Minimum inhibitory concentration of the extract

Result of the minimum inhibitory concentration is shown in Table 2. Higher concentration of the extract inhibited the growth of *E. coli* compared to *S. aureus* and *Streptococcus* spp. The MIC of the methanol extract of *B. grandiflora* was 100, 50 and 25 for *E. coli, S. aureus* and *Streptococcus* spp. respectively.

DISCUSSION

The results obtained showed that methanolic extract of *B. grandiflora* have concentration dependent inhibitory effect on the test organisms *E. coli, S. aureus* and *Streptococcus* spp. Phytochemical studies revealed the presence of flavonoids, tannins, saponins, terpenes, flavonoids, steroids and carbohydrates. These bioactive

Table 2. Minimum inhibitory concentration (MIC) of methanoli extract of *Berlina grandiflora* by dilution method.

Test organisms	MIC µg/ml
Streptococcus spp.	11.72 ± 4.84
Escherichia coli	32.81 ± 15.12
Staphylococcus aureus	19.38 ± 8.51

Values are expressed as Mean ± S.D.

components are known to be bactericidal, pesticidal or fungicidal in nature thus conferring the antimicrobial property to plants (Lutterodt et al., 2005).

The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *E. coli* causes septicemias and can infect the gall bladder, meninges wounds, skin lesions and lungs (Black, 1996), and also a number of food related diseases that manifest themselves in the form of diarrhoea (Adams and Moss, 1999). *S. aureus* causes boil, ulcers, food poisoning, toxic shock and pneumonia while S*treptococcus* causes sore throat, otitis media, brouchitis etc.

Flavonoids, and tannins present in the extract have been associated with antimicrobial effects in various studies using plant extracts (Abo et al., 1999). Flavonoids are known to be inhibitory to *S. aureus* and it has been used in treatment of inflamed tissues (Ali et al., 1996). The finding corroborates the reports of Esimone et al. (1998), Osadebe and Ukwueze (2004) who independently found that various plant extracts inhibit the growth of some hospital bacteria isolates.

The antimicrobial effect of *B. grandiflora* which is evident from this study explains the long history of the use of this plant in traditional medicine for the treatment of different microbial infection. This plant extract has demonstrated a broad spectrum of activity against both Gram-positive and Gram- negative bacteria. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial infections.

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