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Short Communication Report

INVESTIGATION ON THE ANTIBACTERIAL ACTIVITY OF THE AQUEOUS AND ETHANOLIC EXTRACTS OF THE LEAVES OF *Boerhavia diffusa* L.

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The use of medicinal plants in traditional medicine is well known in rural areas of many developing countries (Gupta *et al.*, 2005; Sandhu & Heinrich, 2005). According to World Health Organisation (WHO) more than 80% of the World's Population rely on traditional medicine for their primary health care needs (Ammara *et al.*, 2009). Human have always relied on plants for shelter, clothing, equipment, dental care, medicine, drinks and to nourish and sustain the body (Oladunmoye *et al.*, 2009). Medicines obtained from plants are relatively safer than synthetic alternative (Iwu *et al.*, 1999; Idu *et al.*, 2007).

Boerhavia diffusa L. belongs to the family Nyctaginaceae (Akobundu & Agyakwa, 1998; Odugbemi & Akinsulire, 2006). It is a hairless, semi-prostrate, perennial herb up to 60cm high with a thick, fleshy rootstock that reproduces from seeds. The stem is slender, jointed, more or less fleshy and woody below. It is greenish or sometimes purplish, low branching, glabrous and not sticky from glandular hairs. The leaves are opposite, somewhat fleshy, broadly-ovate, 2.5-4cm long and 2-4cm wide, with petioles 1-3cm long, blunt-tipped, entire and smooth (Akobundu & Agyakwa, 1998). The inflorescence is a many branched, elongated cyme formed in the leaf axils and at the terminals of the stems. The flowers are deep purple or crimson, about 2-5 flowers in a capitulum. The fruit is a one-seeded sticky capsule with five ribs and about 3mm long. It is a common weed of cultivated fields, waste areas, roadsides and lawns. It is widespread in West Africa (Akobundu & Agyakwa, 1998).

It is known that some plants are rich in secondary metabolites such as tannins, alkaloids, flavonoids, phenols, steroids, and volatile oils which are responsible for therapeutic activities (Cowan, 1999; Rabe & Vanstoden, 2000). Also the use of different plant parts, mostly their decoctions, infusions, oral administration and others have been used as popular medicine for various diseases. Some of these plant parts have been used as antimicrobial agents, since time past (Ikenebomeh & Metitiri, 1988; Okemo *et al.*, 2001). *B. diffusa* has been reported to cure many ailments including skin diseases, small pox, jaundice, gonorrhoea, asthma, cough, scabbies and yaws (Gill, 1992; Odugbemi & Akinsulire, 2006).

Plants have long since been deemed a valuable source of natural products for maintaining human health. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance to therapeutic treatments (Nagesh & Shanthamma, 2009).The study was carried out to determine the antimicrobial activity of the aqueous and ethanolic extracts from the leaves of *B. diffusa* on *E. coli*, *P. aeruginosa* and *S. aureus* and establish a basis for the therapeutic uses of the plant. The test bacteria used have been found to be resistant to the routinely used antibiotics in Nigeria. Resistance to newer antimicrobial agents such as Augmetntin, Amoxicillin, Gentamicin, Spectinonmycin and others have been reported (Murray, 1992, Beardsley, 1996, Udo & Grubb, 1996, Madunagu *et al., 2001*). Considering that micro-organism are continuously developing resistance to synthetic antibiotics, there is the need to identify compounds that would be added to or replace the current antimicrobial agents to which microbes have developed resistance.

Collection, Identification and processing of plant material: The leaves of *B. diffusa* were collected in August 2008 from Ekosodin village, Benin City, Edo State, Nigeria, identified by the second author, confirmed with appropriate literature (Akobundu & Agyakwa, 1998; Odugbemi & Akinsulire, 2006). Voucher specimen was deposited in the Herbarium of the University of Benin and sun-dried for six days. The dried leaves were grinded and made into a fine powder using mortar and pestle. The powder was kept in a sterile air tight container to avoid contamination.

Preparation of Aqueous extracts: Five grammes of the dried pulverized leaf powder was dissolved in 50ml of distilled water for 24 hrs and centrifuged at 3000rpm to enable proper diffusion of the active ingredients into the extraction medium. 10^{-1} and 10^{-2} dilution of the supernatant were obtained as concentrations at which antibacterial activity was carried out.

Preparation of Ethanolic extract: Five grammes of the dried pulverized leaf powder was dissolved in 50ml of alcohol (95% ethanol) for 24 hrs and centrifuged at 3000rpm to enable proper diffusion of the active ingredients into the extraction medium. 10^{-1} and 10^{-2} dilution of the supernatant were obtained as concentrations at which antibacterial activity was carried out.

Collection of Test Bacteria: The test bacteria were collected from the Department of Medical Microbiology, University of Benin Teaching Hospital, Benin City, Nigeria. Their identity was confirmed using cultural, morphological and biochemical test as previously described (Akinnibosun *et al.*, 2009). The bacteria isolates were maintained on nutrient agar slants at 4^{0}C.

Description of Research bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* have been previously described elsewhere (Prescott *et al.,* 2005; Akinnibosun *et al.*, 2008a, 2008b).

Determination of Antimicrobial activity: The zone of inhibition was measured by the Agar-well diffusion method (Stoke, 1975). Sterilized nutrient agar was poured into Petri-dishes to the level of obtaining a standard well and allowed to set. Nutrient broth inoculated with the test bacteria was poured into the already set Petri-dishes and uniform distribution was ensured. Sterile cork borer of 10mm diameter was used to punch holes in the agar. Each of the holes in a Petri-dish was filled with the extracts and incubated for 24 hrs at 37^{0}C. The active extracts had zones of inhibition which were measured to indicate the degree of sensitivity.

The antibacterial activity (as shown by zones of inhibition of the different concentrations) of the dried leaves extract of *B. diffusa* on the test bacteria are shown in Tables 1 and 2 while biochemical tests are shown in Table 3.

TABLE 1. ZONES OF INHIBITION OF AQUEOUS EXTRACT OF *B. diffusa* LEAVES ON TEST BACTERIA

Bacteria	Concentrations of extract	Zone of inhibition (mm)	
		Aqueous extract	Water (negative control)
Escherichia coli	10^{-1}	17.0	0.0
	10^{-2}	12.0	0.0
Staphylococcus aureus	10^{-1}	18.0	0.0
	10^{-2}	16.0	0.0
Pseudomonas aeruginosa	10^{-1}	19.0	0.0
	10^{-2}	17.0	0.0

TABLE 2. ZONES OF INHIBITION OF ETHANOLIC EXTRACT OF *B. diffusa* LEAVES ON TEST BACTERIA.

Bacteria	Concentrations of extract	Zone of inhibition (mm)	
		Aqueous extract	Water (negative control)
Escherichia coli	10^{-1}	23.0	0.0
	10^{-2}	14.0	0.0
Staphylococcus aureus	10^{-1}	19.0	0.0
	10^{-2}	14.0	0.0
Pseudomonas aeruginosa	10^{-1}	18.0	0.0
	10^{-2}	11.0	0.0

TABLE 3. BIOCHEMICAL CHARACTERISTICS OF TEST BACTERIA

Bacteria	Catalase	Coagulase	Methyl red	Motility	Oxidase
Escherichia coli	+	-	+	+	-
Staphylococcus aureus	+	+	+	-	-
Pseudomonas aeruginosa	+	-	-	+	+

The results of this work showed that the aqueous and ethanolic extracts of *B. diffusa* leaves had activity on *E. coli*, *S. aureus* and *P. aeruginosa*. This activity occurred at varying concentrations (Tables 1 and 2), indicating that the plant extracts contained active principle with broad antibacterial spectrum (Bankole, 1992). *E. coli* displayed the highest susceptibility in ethanolic extract (14.0mm – 23.0mm), followed by *S. aureus* (14.0mm – 19.0mm) and the least susceptible was *P. aeruginosa* (11.0 mm – 18.0mm). In aqueous extract, *P. aeruginosa* showed the highest susceptibility (17.0mm – 19.0mm), followed by *S. aureus* (16.0mm – 18.0mm) and *E. coli* exhibited the least susceptibility (12.0mm – 17.0mm).

It was observed that antimicrobial activity of the different extracts increased with increase in concentration. This is in agreement with the findings of Onwuliri & Dawang (2006), who found that increased concentrations of extracts of *Moringa oleifera* led to increased sensitivity in the organisms. The negative control (water) in this work did not show any zone of inhibition, confirming that the inhibitory agent(s) was (were) actually from the plant extracts.

These results provide evidence for the medicinal values of the tested plant. Thus its therapeutic utilization by patients with diseases or infections caused by the test bacteria should be further encouraged.

From the research above, it is seen that the results of this investigations support the ethnomedicinal use of this plant by local practitioners. Results from this study showed that the aqueous and ethanolic extracts of *B. diffusa* had antibacterial activity on *E. coli*, *S. aureus* and *P. aeruginosa*. *B. diffusa* being readily available in Nigeria, should therefore be recommended for local production of antibacterial drug in the country. However, more research has to be carried out so as to characterise the bioactive ingredients of the plants.

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