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Evaluation of *Securidaca longipenduculata* leaf and root extracts for antimicrobial activities

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The aqueous, ethanol and methanol extracts of the roots and leaves of *Securidaca longipenduculata* were studied for their antimicrobial activity against *Escherichia coli*, *Shigella sp.* and *Salmonella typhi*, using the agar gel diffusion method. The phytochemicals present, as well as the minimum inhibition concentration (MIC) and minimum bacteriocidal concentration (MBC) of the extracts were also determined using standard methods. Results obtained indicated that the aqueous root extract was the most potent and had MIC and MBC of 50 and 200 mg/ml, respectively, against *Shigella spp* and *S.typhi*. Leaf extracts and methanolic root extracts showed no activity. Differences in the phytochemical composition in the various extracts could be responsible for the different antimicrobial activities. The high antimicrobial activity observed particularly from the aqueous root extract verified the ethno-medical claim of the plant in the treatment of diarrhoea, and could be a source for antimicrobials in the face of increasing drug resistance.

Key words: Evaluation, antimicrobial activity, *Securidaca longipenduculata*.

INTRODUCTION

In Africa, traditional medicine is practiced and plants have been exploited for the treatment of many infections and diseases. Plants readily synthesize substances for defense against attack by insects, herbivores and micro-organisms (Majorie, 1999). The antimicrobial nature of these substances has been well documented (Majorie, 1999). The extract of some food spices and a water-soluble arrowroot tea extract have been reported to inhibit some intestinal pathogens including *E. coli* 0157:H7 (Aboaba and Efuwape, 2001; Kim and Fung, 2004).

Many plant extracts owe their potency to the presence of substances such as tannins and phenolic compounds. These substances are usually found in various parts of the plants like roots, leaves, shoots and bark. Many plants have therefore become sources of important drugs and the pharmaceutical industries have exploited traditional medicine as a source of bioactive agents that can be used in the preparation of synthetic medicine. In recent times, emphasis has been placed on the use of natural materials in the control and treatment of various infections and diseases as some chemically synthesized drugs have undesirable side-effects (Aboaba et al., 2006).

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuing search for new and more potent antibiotics (Raghavendra et al., 2005). This type of resistance to antimicrobial agents is an increasing problem in many areas of the world, especially in developing countries (Shears, 2000).

Securidaca longipenduculata Fres (Polygalaceae) is a medicinal herb commonly used in parts of Africa. The plant is a savannah shrub with twisted bole or slender erect branches and grows up to 30 ft high (Iwu, 1986). The plant has many different medicinal uses (Matthews, 1994). It occurs in various parts of western, northern and astern Nigeria (Iwu, 1986), and in Malaysia, Guinea, Cuba and several Asian countries (Anonymous, 2000).

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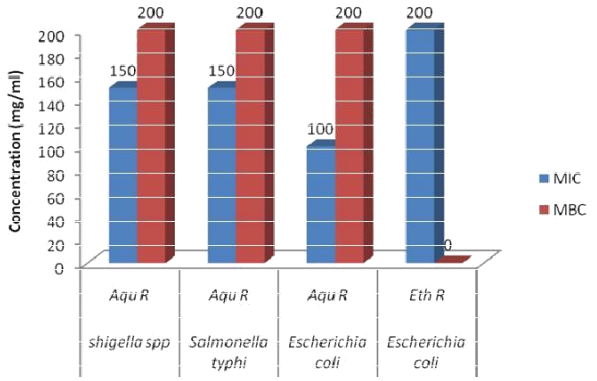

Figure 1. MIC and MBC of *S. longipenduculata* extracts on susceptible test isolates. Abbreviations: Aqu = Aqueous; Eth = Ethanol; R = Root; L = Leave.

With the recent surge in multi-drug resistant organisms, there is an urgent need to extend the search for anti-microbials towards medicinal plants. *S. longipenduculata*, which has long been used in traditional medicines by the locals, can serve as a source of new pharmaceuticals in curbing susceptible microorganisms and thus the development of traditional medicines.

MATERIALS AND METHODS

Collection and identification of plant materials

S. longipenduculata was collected within Jos by the help of the locals. The plant was subsequently identified and confirmed by Professor Hussein of the Botany Department of the University of Jos, where the voucher specimen has been deposited.

Extraction of plant materials

The roots and leaves were shade-dried and pulverized into fine powder using a pestle and mortar. Aqueous (cold) and Soxhlet extractions using methanol and ethanol, as described by AOAC (1980), were prepared. Twenty grams (20 g) of each sample were weighed into 100 ml of the solvent (water, methanol and ethanol). For aqueous extraction the samples and solvent were stirred every 30 min for 3 h and allowed to stand for 24 h. The extracts were concentrated using a rotary evaporator and dried in a hot air oven set at 40°C.

Phytochemical screening

Phytochemical screening of the different extracts was carried out to determine the presence of tannins, saponins, cardiac glycosides, steroidal rings, alkaloids, flavonoids and anthraquinones in the leaf and root extracts, as previously described by Sofowora (1982) and Trease and Evans (1989).

Source of bacterial isolates

The bacterial isolates *Escherichia coli, Salmonella typhi* and *Shigella* sp. were obtained from the stock cultures of the Federal College of Veterinary and Medical Laboratory Sciences, National Veterinary Research Institute, Vom.

Preparation of the test bacteria

A stock culture of each bacterium was sub-cultured onto appropriate selective media and their identity confirmed by biochemical and serological tests. A colony of the pure bacterial cultures was cultured in 10 ml of nutrient broth in sterile McCartney bottles and incubated at 37°C overnight. The overnight broth culture was adjusted to a suspension density equal to 0.5 McFarland turbidity standards (Andrews, 2001), which has an approximate cell density of 1.5×10^6 colony forming units (cfu)/ml.

Sensitivity test

Nutrient agar plates were prepared and dried in the incubator at 37°C for 24 h. The plates were then flooded with 1 ml of the standardized bacterial inoculum and swirled to allow the inoculum to spread over the entire surface of the agar. After allowing absorption of the inoculum onto the media, the agar was allowed to dry in an incubator (Andrews, 2001; Aboaba et al., 2006). Wells of 7 mm were then punched into the agar with a sterile cork borer. Each well was filled with 0.1 ml of each plant extract at the reconstituted concentrations of 200, 150, 100, 50, 25 and 12.5 mg/ml. The same volumes of sterile distilled water and 10 µg/ml gentamicin served as negative and positive controls, respectively (Andrews, 2001; Aboaba et al., 2006). The resulting zones of inhibition were measured in millimetres (mm) using a transparent ruler after 18 to 20 h of incubation (Andrews, 2001). The transparent cleared zones showed bacteriocidal activity, while the clear zones containing microcolonies showed bacteriostatic activity (Aboaba et al., 2006).

Determination of minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC)

The extracts that exhibited antibacterial activity were diluted two-fold with nutrient broth in a series of nine test tubes. An aliquot of 1 ml of each bacterial suspension (1.5×10^6 cfu/ml) was inoculated into each tube. All tubes were incubated at 37°C for 18 - 20 h. The lowest concentration that did not permit any visible growth when compared with the control was considered as the MIC (Andrews, 2001; Aboaba et al., 2006). The contents of the tubes that did not show visible growth were inoculated onto nutrient agar and incubated at 37°C for 24 h. The MBC was considered as the lowest concentration that could not produce a single bacterial colony (Andrews, 2001; Aboaba et al., 2006).

RESULTS

The aqueous root extract showed a relatively wide zone of inhibition on the three enteric organisms and has both bacteriocidal and bacteriostatic action on the isolates at different concentrations. The ethanol root extract only showed bacteriostatic activity, without bactericidal activity, on *E. coli* but with no activity on other isolates used in the study. There was no activity observed against any isolates with leaf extracts (Table 1 and Figure 1).

Phytochemical screening of the extracts indicated that tannin, cardiac glycosides and steroidal rings were pre-

Table 1. Antimicrobial activity of *S. longipenduculata* extracts.

Extract	(200 mg/ml)	(200 mg/ml)	150 mg/ml	100 mg/ml	50 mg/ml	Negative control	Positive control	Organism
Aqu R	14	14	3	-	-	-	19	Sp
Aqu L	-	-	-	-	-	-	21	Sp
Eth R	-	-	-	-	-	-	19	Sp
Eth L	-	-	-	-	-	-	20	Sp
Aqu R	19	19	6	-	-	-	25	St
Aqu L	-	-	-	-	-	-	20	St
Eth L	-	-	-	-	-	-	25	St
Eth L	-	-	-	-	-	-	22	St
Aqu R	21	21	9	4	-	-	25	Ec
Aqu L	-	-	-	-	-	-	24	Ec
Eth R	13	13	-	-	-	-	25	Ec
Eth L	-	-	-	-	-	-	23	Ec

Key: Aqu = Aqueous; Eth = Ethanol; R = Root; L = Leave; - = No zone of inhibition; Sp = *Shigella* sp.; St = *Salmonella typhi*; Ec = *Escherichia coli*.

Table 2. Phytochemical composition of the *S. longipenduculata* extracts.

Plant extracts (*S. longipenduculata)*	Tannins	Saponins	Cardiac glycosides	Steroidal ring	Alkaloids	Flavinoids	Anthraquinone
Aqueous (root)	**++**	**+++**	**+**	**+**	**+++**	**+++**	**-**
Aqueous (leave)	+	+	+	+	-	-	-
Ethanol (root)	+	++	+	+	++	-	-
Ethanol (leave)	+	-	+	+	-	-	-
Methanol (root)	+	-	+	+	-	-	-
Methanol (leave)	+	+	+	+	+	-	-

Key: +++ = Highly present, ++ = moderately present, + = faintly present, - = not present

present in all the extracts, while anthraquinone was absent. In addition, the aqueous extract of the root showed the presence of saponins, flavonoids and alkaloids, while the ethanol extract of the root also showed the presence of saponins and alkaloids (Table 2).

DISCUSSION

We observed from this study that an aqueous root extract of *S. longipenduculata* has potential in the management of microbial infections. The extract seemed to posses both bacteriostatic and bacteriocidal effects against *S. typhi* and *Shigella* sp., suggesting that a therapeutic concentration could be attained in a living host. The high antimicrobial activity of the aqueous root extract observed is similar to the findings of Ajali and Chukwurah (2004), which attributed these activities to the high content of flavonoid. In addition, Abubakar et al. (2005) and Okoli et al. (2006) reported that *S. longipenduculata* possess anti-trypanosomal and anti-inflammatory activities, respectively.

Although, the leave extracts showed the presence of essential phytochemicals, none had any antimicrobial activity. This could be attributed to the phytochemicals being present perhaps in lower concentrations or may be less polar than in the root of the plant. The antimicrobial activity observed, particularly from the aqueous root extract, could be due to varied phytochemicals present. These phytochemical components have been documented as major biological active components, which also exhibits their effects on physiological activity (Sofowora, 1993). Many phytomedicines exert their effects through the additive or synergistic action of several compounds acting at a single or multiple target sites associated with physiological process (Tyler, 1999).

Conclusion

The aqueous root extract of *S. longipenduculata* had the highest antimicrobial activity, followed by the ethanol root extract. However, none of the leaf extracts had any acti-

activity. Consequently, the aqueous root extract of the plant is a promising herbal antimicrobial alternative in the face of increasing resistance to synthetic antibiotics. Based these findings and the medicinal potential of this plant, we suggest that further work be done on this plant in order to determine its major active principles.

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