IJPAS ONLINE
ISSN:1597-9261
FACULTY OF SCIENCE
BAYERO UNIVERSITY,
KANO - NIGERIA
INTERNATIONAL JOURNAL OF PURE AND APPLIED SCIENCES

FULL LENGTH RESEARCH PAPER

Preliminary phytochemical and antibacterial screening of *Scadoxus multiflorus*

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Received: July 2008 *Accepted: August 2008*

ABSTRACT

Phytochemical and antibacterial properties of methanol, acetone and aqueous extracts obtained from subterranean part of Scadoxus multiflorus *were investigated in an attempt to evaluate its medicinal potential. The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides with a very high content in the aqueous extract. The concentration of the phytoconstituents were in the order of aqueous > methanol > acetone. Growth inhibition was determined using agar well diffusion method against five selected bacteria species. Aqueous extract showed broad spectrum activity against the tested organisms. Methanol and acetone extracts only inhibited the growth of Staphylococcus aureus and* Escherichia coli. *The result of this study validates the use of aqueous extract of this species in ethanomedicine and could provide a lead in the isolation of antibacterial agents from aqueous extract of* Scadoxus multiflorus.

Keywords: Scadoxus multiflorus, *antibacterial, phytochemical, ethnomedicine*

INTRODUCTION

In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infections. This situation, coupled with the undesirable side-effects of certain antibiotics and the emergence of previously uncommon infections is a serious medical problem (Marchese and Shito, 2001; Poole, 2001). This situation has forced scientists to search for new antimicrobial substances from various plants (Karaman *et al*., 2003). According to Mathekga and Mayer (1998), *in-vitro* screening methods have provided the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations.

*Scadoxus multiflorus (*Martyn) Rafinesque belongs to the family Amaryllidaceae, widely distributed in tropical and Southern African regions particularly in lowland to mountain forest, secondary forest, forest margins, Savannah woodland, open grassland and in the shade of trees at river banks (Germishuizen, 1997). The genus *Scadoxus* contains alkaloid which is reported to be toxic and many species are used in Cameroon, Gabon, Angola and Central African Republic as arrow and fishing

poison (Neuwinger, 2004). In Guinea and northern Nigeria, the bulbs are used to make a fishing poison. The bulb is also used to treat dropsy, scabies and poorly healing wounds. In South Africa, *Scadoxus* species are commonly used to treat coughs and gastro-intestinal problems and forms part of a medicine taken during pregnancy to ensure a safe delivery (Van Wyk *et al*., 1997). Ethnomedical information obtained from the indigenous pastoralist inhabitants of Jangeme village in Zamfara State revealed that, bulb extracts are used for the treatment of wounds.

As far as our literature search could ascertain, information on the phytochemical and antibacterial properties of *S. multiflorus* growing in this part of the world has not been reported. This type of information is vital in the current search of antimicrobial agents from higher plants. This study aimed at investigating the phytochemical and antibacterial properties of *S. multiflorus* by preliminary bioassay screening.

MATERIALS AND METHODS

Collection and extraction of plant materials

Bulbs of *S. multiflorus* were collected from Jangeme Village along Gusau- Magami Road in Zamfara State, Nigeria (Latitude: 12° 10' 0 N, Longitude: 6° 15' 0 E). The plant was authenticated in the Department of Biological Sciences of Usmanu Danfodiyo University, Sokoto where voucher specimen was deposited both at the herbarium and the Biological garden. The leaves of the plant were cut off and the bulbs were sliced into pieces, air dried and pulverized. A portion (50 g) of the powdered plant material was separately extracted in acetone, methanol and water for 24 hrs. The extracts were filtered using filter paper and concentrated to dryness under reduced pressure at 40^0C and kept in a refrigerator at 4^0C before use.

Antimicrobial assay

Five laboratory isolates of bacteria were collected from the Department of Microbiology of Usmanu Danfodiyo University, Sokoto. The isolates were: *Staphylococcus aureus*, *Salmonella paratyphi*, *Bacillus metagarium*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Before use, each bacterial culture was standardized with fresh sterile nutrient broth corresponding to standard 4 of McFarland. The extracts were tested for their antibacterial properties using well agar diffusion method (Pelczar *et al*., 1993). The nutrient agar (Antec Diagnostic Products, UK) plates were prepared and seeded with the test organisms. Five wells of 6.0 mm diameter each were made in the plates with a sterile cork borer and filled with 20, 40, 60, 80 and 100 mg/ml extracts respectively. The inoculated plates were allowed to congeal for 30 min to allow pre-diffusion time and then incubated at 37^0C for 24 hrs. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells (Cheesbrough, 2000). The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

Phytochemical screening

Adopting the methods of Trease and Evans (1989) and Sofowora (1982), the acetone, aqueous and methanol extracts of *S. multiflorus* bulb were tested for the presence of flavonoids, alkaloids, tannins, glycosides and saponins.

RESULTS AND DISCUSSION

The result of phytochemical screening of acetone, methanol and aqueous extracts of *S. multiflorus* bulbs revealed the presence of alkaloids, flavonoids, tannins, saponin and cardiac glycosides (Table 1). The concentration of the various classes of secondary metabolites varies amongst the extracts evaluated. In all the extracts evaluated, the concentration of the constituents are in the order of aqueous > methanol > acetone. The presence of these components in this species is an indication that it may perhaps have some medicinal potential. This is probably due to the fact that each of the components identified has record of one therapeutic usage or another. For instance, plants rich in saponins have immune boosting and anti-inflammatory properties (Kenner and Requena, 1996). Similarly, tannins have been reported to have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane (Elmarie and Johan, 2001). The antibacterial activities of alkaloids and flavonoids have been reported by a number of

authors (Adesina *et al.*, 2000; Ajoku *et al.*, 2005; Dboh and Abudu 1997; Onwuliri and Wonany, 2005).

The aqueous extract exhibited antibacterial activity against Gram-positive and Gram-negative bacteria (Table 2). Generally, *Staphylococcus aureus* was observed to be the most susceptible organism, while *Pseudomonas aeruginosa* and *Bacillus metagarium* were the least susceptible. The susceptibility of *Staphylococcus aureus* to the aqueous extract of this species is an indication of the potential of the extract as a drug that can be used against this organism. Methanol and acetone extracts did not show any appreciable activity on the tested organisms except on *Staphylococcus aureus* and *Escherichia coli* at a concentration of 80 mg/ml. The activity of this plant extracts against the Gram-negative bacteria is noteworthy as high resistance of this group of bacteria has been reported (Rabe and Van Staden, 1997; Afolayan, 2003). It is possible that the antibacterial activity exhibited by the extracts of this plant species may be attributed to the presence of alkaloids and flavonoids in substantial amounts as observed in the phytochemical screening. The result of this study justifies the use of aqueous extract of *S. multiflorus* in ethanomedicine for the treatment of infectious diseases caused by bacteria.

Acknowledgment
The authors are grateful to the Department of Microbiology of Usmanu Danfodiyo University, Sokoto for consent to use bacterial collection.

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Table 1: Phytochemical constituents of *S. multiflorus*

Constituents	Extracts		
	Methanol	Acetone	Aqueous
Alkaloids	++	+	+++
Flavonoids	+	-	++
Tannins	-	-	+
Saponins	-	-	+
Cardiac glycosides			
i. Salkowski	++	+	+++
ii. Lieberman B.	++	+	++
iii. Keller Kiliani	++	+	++

Key:
+++ = Present in high concentration, ++ = Moderately Present, + = Trace, - = Absent.

Table 2: Antibacterial activity of *S. multiflorus*

		Diameter of inhibition zone (mm)				
Extractant	Conc. mg/ml	1	2	3	4	5
Aqueous	20	15.0	14.0	-	-	-[a]
	40	17.0	15.5	13.5	-	-
	60	17.3	16.0	14.3	-	-
	80	19.7	16.5	14.7	14.0	14.0
	100	21.3	17.3	17.3	15.3	16.5
Methanol	20	-	-	-	-	-
	40	-	-	-	-	-
	60	15.0	-	14.3	-	-
	80	16.0	-	14.3	-	-
	100	16.0	-	16.0	-	-
Acetone	20	-	-	-	-	-
	40	-	-	-	-	-
	60	-	-	-	-	-
	80	14.0	-	-	-	-
	100	14.3	-	-	-	-

Values are means of three replicate
Key: -[a] = < 8.0mm

1 *Staphylococcus aureus*
2 *Salmonella paratyphi*
3 *Escherichia coli*
4 *Bacillus metagerium*
5 *Pseudomonas aeruginosa*