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Antimicrobial and phytochemical properties of aqueous extracts of *Gladiolus* corm (family Iridaceae) from Benue State of Nigeria

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Abstract

The genus *Gladiolus*, embraces 260 species of a perennial herb belonging to the lily family: *Iridaceae*. In West Africa *Gladiolus* corm is used in food, and in ethnomedicines for treating gonorrhea and other infections. Often, the corm is used in combination with other plant materials, hence it is not known if the corm alone has antimicrobial effects. In this study aqueous extracts of the *Gladiolus* corms obtained in Benue State, Nigeria, were tested for their antimicrobial and phytochemicals properties. Antimicrobial effects were evaluated by measurement of inhibition zones using the bacteria, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Listeria monocytogenes;* and the fungi, *Candida albicans, Aspergillus niger* and *Trichophyton mentagrophyte.* The results showed that the extracts were active against *Pseudomonas aeruginosa* and A*spergillus niger,* but relatively inactive against the others; and contain alkaloids, tannins, saponins, cardiac glycosides, flavonoids and carbohydrate. Separation of the constituents by thin layer chromatography was attempted. The results confirm the basis for the traditional use of *Gladiolus* corm, and are discussed within the context of their economic and medical relevance. The presence of cardiac glycosides in corm calls for caution in its use. It also shows good prospects for its use as preservative for food and herbal medicines.

Keywords: Gladiolus, Corm, Antimicrobial, Phytochemical

Introduction

The genus *Gladiolus* includes about 260 species of a perennial plant belonging to the lily family, known botanically as *Iridaceae*, and commonly as sword lily. About 10 species are indigenous to Mediterranean Europe and Asia and 250 to sub-Saharan Africa, mostly southern Africa, where about 160 species are endemic (Goldblatt and Manning, 1998; Manning and Goldblatt, 2008). The genera *Oenostachys*, *Homoglossum*, *Anomalesia* and *Acidanthera*, previously considered independent entities, have since been reclassified as *Gladiolus* (Goldblatt and Devos, 1989). In most of Africa south of the Sahara *Gladiolus* thrives easily. The plants mostly have mottled flowers that may be white, pink, purple or

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orange. It appears however, that the more common species in tropical West Africa include: *G. primulinus, G. quartinianus, G. gregarius, G. delani* and G. *psittacinus* (Hutchinson and Dalziel, 1937). In Ghana, Nigeria, Cameroon and Botswana, *Gladiolus* corms are used in food and ethnomedicines for treating infections of the skin, gut, urogenital system, and upper respiratory tract (Nguedia *et al*., 2004). In Ghana and Igala land, Nigeria *G. quartinianus* is actually cultivated on a small scale. In South West Nigeria the corms called "baka" are used in treating gonorrhea, dysentery and other infectious conditions. For such purposes the corms are compounded with water, melon, and onions, to produce "agunmu" that is subsequently mixed with food or other herbal preparations. In Hausa land a preparation made from *Gladiolus* corms called "rumanan doki" is used to treat dysentery in humans and horses. In Ghana the corms are mixed with ginger as a potent evacuate for constipation and dysentery. In Idoma land, Benue State, Nigeria *Gladiolus* corms called "okpendu" or "okredu" are used in the preparation of "enyi" or "umu" – a non-alcoholic drink made from millet or sorghum. The corm is used either alone or in combination with other plant parts, and it is thought to exert some antimicrobial effects in such preparations. The corm is thought to retard fermentation, probably by suppressing microbial growth. Two key issues arise from the foregoing supposed actions of *Gladiolus* corms. First, do the corms, used alone, possess antimicrobial activity? Second, what are the chemical constituents of the corms?

In connection with the foregoing, Wang *et al* (2003) identified a new anthraquinone, 1,6,7-trihydroxy-3-methoxyanthraquinone, along with three known compounds methyl *trans-p*-hydroxycinnamate, eugenin and 1,3,6-trihydroxy-8-methylanthraquinone in the corm of *Gladiolus gandavensis* Van Houtt. Similarly, scientists in Cameroon and Botswana have identified 1,6,7-Trihydroxy-3-methoxy-8-methyl-anthraquinone and 1-Hydroxy-3,6,7-trimethoxy-8-methyl-anthraquinone in *Gladiolus psittacinus* Hook (Ngamga *et al*., 2007). In view of the foregoing, it is interesting that compounds similar to or the same as those just mentioned have been identified as antimicrobial constituents of *Mitracarpus scaber*, another herb used in tropical West Africa to treat infections. The said compounds have been reported in *M. scaber* by a host of workers in Nigeria and elsewhere: Tectoquinone Azaanthraquinone, Benzo-isoquinoline-5,10-dione and 2-Hydroxynaphthoquinone (Ogundaini, 1999; Okunade *et al*., 1999; Houghton *et al*., 2000; Ogundaini, 2005). These related findings and the issues raised earlier on the microbial activity of the phytochemical constituents of *Gladiolus* corm have prompted this present study. The results obtained are discussed within the context of their economic importance and medical relevance. Prospects for more advance work are also briefly discussed.

Experimental

Plant Material. Fresh samples of *Gladiolus* corms collected in Otukpo, Benue State, Nigeria and were authenticated by Professor S.W.H Hussaini, a plant taxonomist, of the Department of Botany, University of Jos; Mrs. Grace Ugbabe, a botanist; and Mallam Ibrahim Muazzam, an ethnobotanist, both of the Department of Medicinal Plant Research, NIPRD, Abuja. The corms were obtained by pulling or digging them out of the ground, and subsequently freed from dead tissues, sand and other foreign matters. The over ground parts were retained for purposes of authentication. Specimen vouchers were retained in the archives of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Jos, Jos.

Test Micro-organisms. These included two gram-negative bacteria *(Escherichia coli,*

AFC 1175 and *Pseudomonas aeruginosa*, AFCC 1045*)* two gram-positive bacteria *(Staphylococcus aureus* AFCC 9144 and *Listeria monocytogenes,* NCC 11994*)* and three fungal isolates *(Candida albicans, Aspergillus niger* and *Trichophyton mentagrophyte).*

Preparation of cold water extract: Approximately 10g of the corms were washed with water, cut into bits and placed in a beaker. 25mL of freshly distilled water was added and the mixture was covered with aluminum foil, and kept at room temperature (about 30^{o}C) for 48 hours at the end of which the extract was filtered-sterilised into sterile containers.

Preparation of soxhlet extracts. About 30g of washed corms were weighed, cut into bits, and packed into the chamber of a soxhlet extractor. 400mls of water was used as the solvent. A continuous 24-hour run was carried out, samples being withdrawn at six-hourly intervals and used for the screening. The work mostly utilized the 12-hour and the 18-hour soxhlet extracts.

Determination of anti-microbial activity by agar-plate diffusion assay techniques. Antibacterial Activity: Nutrient agar plates were seeded with standardized (0.5 McFarland standard) overnight broth culture of the different bacteria and labeled appropriately. In each of these plates, four wells were cut out using a sterile cork borer. The wells were then differently filled with 0.1ml the different antibacterial agents and labeled appropriately. The reference antibiotic was Gentamicin (280μg/ml) while the test agents were the cold water, 12-Hour and 18-Hour soxhlet extracts. They were allowed to diffuse into the medium at room temperature and were then incubated at 37^{o}C for 24hours. The diameters of zones of inhibition were measured using a transparent plastic rule. Each experiment was carried out five times and the mean of the diameter of the zone of inhibition was calculated.

Antifungal Activity: Sabouraud Dextrose Agar plates were seeded with standardised peptone water culture of *C. albicans*, and spore suspensions of *A. niger* and *T. mentagrophytes* and labeled appropriately. The same procedure was carried out as in the antibacterial screening above except that in this case, the reference antimicrobial agent was Clotrimazole (10μg/ml). The plates were incubated at room temperature (about 30^{o}C) for 48hours and the anti-fungal activity of the extracts determined by measuring the diameter of the zone of inhibition. The experiment was replicated four (4) times and the mean of the diameter of the zone of inhibition calculated.

Phytochemical Analysis. Both the cold water extract and the soxhlet extracts were subjected to various spot tests using appropriate reagents to investigate the presence of chemical constituents. Separation of the constituents was attempted by subjecting the cold water extract to thin layer chromatography (TLC) using the solvent systems - acetone: water: 25% ammonia (90:7:3); acetone: water: 25% ammonia (70:20:10); ethylacetate: methanol: water (100:13.5:10); and chloroform: Ethanol (9:1) The stationary phase was of silica gel. Spots were observed under daylight, UV at 254nm or by spraying with Dragendorff reagent. The Rf value for each spot was calculated as the distance moved by the spot over the distance moved by the solvent front, all from the origin – the point of application of the sample to the plate.

Results

The results of antimicrobial screening reveal that water extracts of the *Gladiolus* corms possess some anti-microbial activities as shown in Table 1.

Table 1: Antimicrobial activities of the aqueous extracts of *Gladiolus* corm

Microorganisms	Average diameter of zone of inhibition (mm)				
	Sterile water	12-h extract	18-h extract	Gentamicin	Clotrimazole
E. coli	0	0	0	21	-
P. aeruginosa	0	4	2	25	-
S. aureus	0	0	0	26	-
L. monocytogenes	0	0	0	22	-
A. niger	0	8	10	-	19
C. albicans	0	0	0	-	18
T. mentagrophyte	0	0	0	-	11

Water was used as the negative control. 12-Hour and 18-Hour indicate soxhlet extraction with water for 12 and 18 hours respectively. Gentamicin and clotrimazole were used at concentrations of 2.8mg/ml and 10ug/ml respectively.

Table 2: Phytochemical profile of aqueous extracts of *Gladiolus*

Phytochemical constituent	Cold water extract	12-h soxhlet extract	18-h soxhlet extract
Alkaloid	Present	Present	Present
Anthraquinone glycoside	Not detected	Not detected	Not detected
Carbohydrate	Present	Present	Present
Cardiac glycoside	Not detected	Not detected	Not detected
Cyanogenic glycoside	Not detected	Not detected	Not detected
Flavonoid	Present	Present	Present
Tannin	Present	Present	Present

The cold water extract was produced from 10 g of the corm infused in 25 mL of water at room temperature (~ 30^{o}C) for 48 hour. The 12-hour and 18-hour soxhlet extracts were prepared from 30g of corm and 400 mL of water

Table 3: TLC of the aqueous extracts of *Gladiolus* corm in different solvent systems

Method of spots detection	Solvent systems/ Number of separated spots/ Rf values			
	Ac:Wa:25%Am (90:7:3)	Ac:Wa:25%Am (70:20:10)	Ea:Me:Wa (100:13.5:10)	Ch:Et (9:1))
Day light	No separated spots	No separated spots	1 spot: 0.30	No separated spots
UV at 254nm	3 spots: 0.09; 0.61; 0.98	1 spot: 0.92	1 spot: 0.10	2 spots: 0.15; 0.38
Dragendorff	1 spot: 0.55	1 spot: 0.96	No separated spots	No separated spots

The cold water extract of Gladiolus corm was used for this test. Ac = acetone; Wa =:water; 25%Am = 25% ammonia; Ea = ethyl acetate; Me methanol; Ch = chloroform; Et = ethanol.

Slight activity was demonstrated against *Pseudomonas aeruginosa;* and a moderate activity against the fungus - *Aspergillus niger*, when compared with the reference drugs used. On the whole, it was observed that for the antifungal screening, the extract obtained after 18hours of soxhlet extraction was slightly more active (diameter of zone of inhibition: 20mm) than the 12-hour soxhlet extract (diameter of zone of inhibition of 18mm). However, for the antibacterial screening, it was observed that the 12-hour soxhet extract was slightly more active (diameter of zone of inhibition: 14mm) than the 18-hour extract (diameter of zone of inhibition: 12mm). These differences may not be significant.

The results of the phytochemical screening of the extracts shown in Table 2 indicate the presence of alkaloids, tannins, flavonoids, saponins, cardiac glycosides and carbohydrates. However, anthraquinone glycosides and cyanogenic glycosides were not detected.

The TLC profile of the cold water extract is shown in Table 3. The results show that better separation, as judged by UV detection, was obtained with acetone: water: 25% Ammonia (90:7:3) and chloroform: ethanol (9:1) than with the other systems, namely - acetone: water: 25% Ammonia (70:20:10) and ethylacetate: methanol: water (100:13:5:10). However, spraying with Dragendorff's spray reagent indicated better

alkaloid separation with acetone: water: 25% ammonia (70:20:10) than with Acetone: water: 25% Ammonia (90:7:3). The use of ethylacetate: methanol: water (100:13.5:10) and chloroform: Ethanol (9:1) solvent systems showed no alkaloid separation as judged by Dragendorff reagent.

Discussion

Bearing in mind the traditional use of *Gladiolus* corm in Benue State of Nigeria, it is of interest that the two microorganisms inhibited in this study are known to be implicated in spoilage processes (Baird 2004). The activity against *P. aeruginosa* is noteworthy considering that the organism has high intrinsic resistance against a number of common antimicrobial agents (Denyer and Russell 2004). On the whole the results show that the antimicrobial properties of *Gladiolus* corm can be exploited, in that there is a possibility that the corm can be developed as preservative for food and herbal medicines, the way the parabens are used in the preservation of medicinal syrups (Martindale, 1996) and n-octylgallate in the preservation of palm wine (Ameh, 1976; Uwaifo and Bassir, 1982; Okagbue, 1988); or as antimicrobial agent for medical and veterinary use. For such a purpose, more work is required to quantify and determine the nature of the activity as well as possible isolation of active constituents. The anti-microbial profile of the plant can be evaluated further, by increasing the number of test microorganisms. That there is no difference in the activity of the different extracts would imply that the active ingredient is stable to heat. Would a different solvent system give better extraction? A better TLC- separation may be achieved by removing tannins from the crude extract prior to separating and purifying other constituents, since high tannin contents tend to hinder the separation of other constituents. However, the presence of cardiac glycosides in *Gladiolus* corms calls for caution in their use.

The findings of this study seem to provide some justification for the traditional uses of this plant.

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