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Full Length Research Paper

Potentials of *Gladiolus* corms as an antimicrobial agent in food processing and traditional medicine

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The genus *Gladiolus* (Family: Iridaceae) has 260 species of a perennial herb. In West Africa the corms of *Gladiolus* species are used in food and Traditional Medicine, often in combination with other plant materials. This study aimed at verifying the basis for the use of these corms in both instances. Aqueous extracts of the corms obtained in Benue State, Nigeria, were tested for antimicrobial effects; and screened for key phytochemicals. Antimicrobial effects were evaluated by measuring the diameters of inhibition zones on agar plates, using clinical isolates of 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, at concentrations of 75 – 400 mg of the plant material per mL of water, were active against *Pseudomonas aeruginosa* and *Aspergillus niger*, but relatively inactive against the others. The extracts contained alkaloids, tannins, saponins, cardiac glycosides, flavonoids and carbohydrates, but attempts at TLC separation were only marginally successful. The somewhat selective antimicrobial effects of the extracts do however; suggest the basis for the use of these corms. But the presence of cardiac glycosides - a cardiotoxin, calls for caution in their use.

Key words: *Gladiolus,* corm, iridaceae, antimicrobial, food processing, traditional medicine, phytochemicals, Benue State.

INTRODUCTION

The plant genus *Gladiolus,* commonly called sword lily, includes 260 species of a perennial herb belonging to the lily family - Iridaceae. Ten species are native to Eurasia and 250 to sub-Saharan Africa, where 160 species are endemic (Goldblatt and Manning, 1998; Manning and Goldblatt, 2008). The genera *Oenostachys*, *Homoglossum*, *Anomalesia* and *Acidanthera*, previously grouped as such, are now reclassified as *Gladiolus* (Goldblatt and Devos, 1989). Generally, *Gladiolus* thrives well in sub-Saharan Africa, and mostly with mottled flowers that may be white, pink, purple or orange. But it appears that the more common species in tropical West Africa include: *G. primulinus*, *G. quartinianus, G. gregarius*, *G. delani* and G. *psittacinus* (Hutchinson and Dalziel, 1968; Manning and Goldblatt, 2008). In Ghana, Nigeria, Cameroon and Botswana, *Gladiolus* corms are used in food, and in ethnomedicines for treating infections (Nguedia *et al.,* 2004). In Ghana and Igalaland, 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 Hausaland a preparation made from *Gladiolus* corms called “rumanan doki” is used to treat dysentery in humans and horses. In Ghana the

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corms are mixed with ginger as a potent evacuant for constipation and dysentery (Hutchinson and Dalziel, 1968). In Idomaland, 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, sorghum or maize.

It is of note that in both food and medicine, the corms are used either alone or in combination with other plant materials, but the scientific rationale for using them in each case remains unknown. The use of the corms in treating infections however, points to antimicrobial activities of some sort. In this connection therefore, it is of note that Wang et al. (2003) have identified a new trihydroxy-3-methoxyanthraquinone, along with Methyl-hydroxycinnamate, Eugenin and 1,3,6-Trihydroxy-8-methylanthraquinone in the corms of *Gladiolus gandavensis.* 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* (Ngamga *et al*., 2007). In view of the foregoing, it is of interest that compounds similar to those just mentioned have been identified as the antimicrobial constituents of *Mitracarpus scaber*, another herb used in West Africa to treat infections (Hutchinson and Dalziel, 1968). The said constituents of *M. scaber* reported by workers in Nigeria and elsewhere, include: Tectoquinone Azaanthraquinone, Benzo-isoquinoline-5, 10-dione and 2-Hydroxynaphthoquinone (Ogundaini, 1999; Okunade et al., 1999; Houghton et al., 2002; ../Gladiolus PAPERS/science.htm - aff2 Ogundaini, 2005). These apparently related findings and the pointer to antimicrobial activities in *Gladiolus* corms have prompted this present study. The results, which showed that the extracts contain alkaloids, tannins, saponins, cardiac glycosides, flavonoids and carbohydrate; and are active against *P. aeruginosa* and *A. niger*, but relatively inactive against 6 other microbes, are discussed within the context of their economic importance and medical relevance.

MATERIALS AND METHODS

Plant material

Fresh samples of *Gladiolus* corms collected in 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 sample retained at the department of Medicinal Chemistry and Quality Control, NIPRD, is coded: Gladiolus-Benue-NAO-Nov-09). The corms were obtained by digging them out the ground, and subsequently freed from scales and dead tissues, sand and other foreign matter. The aerial parts were retained for purposes of authentication.

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).*

Equipment and other items

Weighing balance (OHAUS Dial-o-Gram, capacity: 310 g), analytical balance, various calibrated glass wares, heating mantle, Soxhlet extractor, glass petri dishes, cork borer, autoclave, incubator, nutrient broth, nutrient agar, Sabouraud Dextrose agar, peptone water, thin layer chromatography plates (5 x 20 cm), TLC plate spreader, silica gel G. Chemicals included 1% HCl, 5% ferric chloride, 20% potassium hydroxide, 10% ammonia solution, lead sub-acetate solution, Dragendorff’s reagent, Mayer’s reagent, Molisch’s reagent, Fehling’s solution, A and B and Chloroform.

Preparation of cold water extract

Approximately 10 g of the corms were washed with water, cut into bits and placed in a beaker. 25 mL of water was added and the mixture was covered with sterilized aluminum foil, and kept at room temperature (about 30°C) for 48 h at the end of which the extract was filtered, and the filtrate transferred into sterile containers.

Preparation of Soxhlet extracts

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

Determination of anti-microbial activity by agar-plate diffusion assay techniques

Antibacterial activity

Nutrient agar plates were seeded with the overnight broth culture of the different bacteria and labeled appropriately. In each of these plates, four wells (a central well surrounded by three other wells) 2 mm deep were cut out using a sterile cork borer. Each of these wells was then labeled appropriately. The central well containing the antibacterial drug, gentamicin (2.8 mg/ml) is the ‘positive control’. The remaining 3 wells were marked ‘negative control’ (water), 12 h Soxhlet test extract and 18 h Soxhlet test extract. Using sterilized pipettes, 0.1 ml each of the test (ranging in concentrations of 75 – 400 mg/ml) and control samples were added into the appropriate wells and allowed to diffuse at room temperature for 10 min. The plates were then incubated at 37°C for 24 h, and the antibacterial activity evaluated by measuring the diameter of zone of inhibition using a transparent plastic ruler. 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 peptone water culture of the different fungi and labeled appropriately. The same procedure was carried out as in the antibacterial screening above except that in this case the control was clotrimazole (10 $\propto$g/ml). The plates were incubated at room temperature (about 30°C) for 48 h

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

Microorganisms	Average diameter of zone of inhibition (mm)				
	Sterile water (-control)	12 h extract	18 h Extract	Gentamicin (+ control)	Clotrimazole (+ control)
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 and 18 h indicate Soxhlet extraction of the finely cut corm with water for 12 and 18 h respectively. Gentamicin and clotrimazole were used at concentrations of 2.8 and 10 ug/ml respectively.

and the anti-fungal activity of the extracts evaluated by measuring the diameter of the zone of inhibition. The experiment was carried out four (4) times and the mean of the diameter of the zone of inhibition was 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 254 nm or by spraying with Dragendorff reagent. The Retention 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 antimicrobial activities as shown in Table 1.

The extracts showed slight activity against the gram-negative bacterium - *Pseudomonas aeruginosa;* and a moderate activity against the fungus - *Aspergillus niger,* when compared with the standard drugs used as controls. On the whole, it was observed that for the antifungal screening, the extract obtained after 18 h of Soxhlet extraction was more active (diameter of zone of inhibition: 10 mm) than the extract obtained after 12 h of Soxhlet extraction (diameter of zone of inhibition of 8 mm). However, for the antibacterial screening, it was observed that the extract obtained after 12 h of Soxhlet extraction was more active (diameter of zone of inhibition: 4 mm) than that obtained after 18 h of Soxhlet extraction (diameter of zone of inhibition: 2 mm). These differences may however be considered insignificant, or as an experimental fluke.

The results of 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 thin layer chromatography 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

Some aspects of pharmaceutical research are offshoots of work in nutrition and dietetics; and often, the very property for which a plant is used in food, underlies its use also in medicine. The use of *Gladiolus* corm in West African Traditional Medicine appears to fall in this category. In Benue State *Gladiolus* corms are used in the production of "Enyi" or "Umu" – a pleasantly sweet and very slightly sour, non-alcoholic beverage made from cereals. The pleasantly sour taste of the beverage suggests the presence of such acids as lactic acid, produced by microbes (Ameh, 1976). On the other hand, the absence of ethanol and of sharply sour-tasting acids, such as acetic citric acids, suggests that the microbes known to produce such molecules are probably

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	Present	Present	Present
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 corms infused in 25 mL of water at room temperature (~ 30^{o}C) for 48 hour. The 12 and 18 h Soxhlet extracts were prepared from 30g of corms and 400 mL of water at room temperature, and run continuously for 12 or 18 h.

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

	Solvent systems/ Number of separated spots/ Rf values			
Method of spots detection	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:Wa: 25%Am is acetone:water: 25% ammonia. Ea:Me:Wa is ethyacetate:methanol:water. Ch:Et is chloroform:ethanol. The rations in which the solvents are mixed are indicated in parenthesis

suppressed. It would thus seem that the role of the corm in the production of the beverage is to selectively inhibit some types of fermentation, which the beverage is prone to undergo because of the ready availability of atmospheric yeast and bacteria. In this connection, it is well known for instance that atmospheric contamination of palm sap by yeast and bacteria is the basis for the production of palm wine from palm sap (Ameh, 1976).

This explains why palm sap (which is sweet, straw-colored and free of ethanol) and fresh palm wine (sweet, milky white and only slightly alcoholic) lose their sweetness as the glucose in them ferments to ethanol (due to atmospheric yeast – *Saccharomyces*) and lactic acid (due to atmospheric *Lactobacillus*). The souring of cassava paste in the production of gari (granulated or grated cassava paste) is also known to be caused by atmospheric *Lactobacillus* (Okagbue, 1988). It is therefore of interest that the two microbes most inhibited in this study are potentially among the most harmful.

Firstly, *Pseudomonas aeruginosa* is a notorious pathogen in humans and animals; hence *Gladiolus* corm can be used to control infections caused by that organism or related bacteria. Secondly, the activity against *Aspergillus niger* is important from the point of view of agricultural production and storage, in that, even though the mould is used in industrial production of citric acid, it is nevertheless a spoilage organism for many types of food stuff. Although *Aspergillus flavus* was not included in this present study, it is likely that the activity of this notorious mould could be controlled by *Gladiolus* corm, and that capacity suppress the production of aflatoxins– a major cause of food poisoning especially in Africa. Indeed, the World Health Organization requires that food and medicinal plant materials be routinely tested for these carcinogenic mycotoxins (WHO, 1998). Apart from the foregoing economic implications, the antimicrobial properties of *Gladiolus* corm are consistent with the possibility that the material can be used a preservative in herbal medicines, in the same way that the parabens are used in preserving pharmaceutical syrups (Martindale, 1996) and n-octylgallate in palm wine (Ameh, 1976; Uwaifo and Bassir, 1982).

This study therefore, draws attention to the medical, agricultural and veterinary importance of this little known herb; and to the fact the plant is becoming rather rare, which calls for concerted effort towards its conservation. While effort is being made to conserve this key resource, we suggest that work be commenced in the direction of isolation and biochemical characterization of the phytochemical constituents of *Gladiolus* corm so as to advance its promises in agriculture, industry and medicine, including animal health. More immediate work should include the determination of the antimicrobial spectrum, by including more test organisms, such as

Aspergillus flavus; and the determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of extracts in various solvents against sensitive microbes. However, the fact that *Gladiolus* corms contain cardiac glycosides must be taken into account in any formulation utilizing the material.

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