Pharm. Méd. Trad. Afr. 2004, Vol.13, pp.149-159

ANTI-CANDIDAL PROPERTY AND ACUTE TOXICITY OF *GLADIOLUS GREGASIUS* BAKER (*IRIDACEAE*)

J. C. Assob Nguedia[a], F.-X. Etoa[a], V. Penlap Beng[a], D. Lontsi[b], V. Kuete[a]; and R. Somo Moyou [c]

[a] *Laboratory of Applied Microbiology and Molecular Pharmacology, Faculty of Science, University of Yaoundé I. P.O. Box 812 , Yaoundé, Cameroon.*
[b] *Laboratory of Organic Chemistry, Faculty of Science, University of Yaoundé I. P.O. Box 812 , Yaoundé, Cameroon.*
[c] *Laboratory of Parasitology, Faculty of Medicine, University of Yaoundé I. P.O. Box 8445, Yaoundé, Cameroon.*

Corresponding author:
J. C. ASSOB NGUEDIA, Department of Biochemistry, Faculty of Science, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon. Fax: (237) 223 14 07 /tel. : 9629452; E-mail: juleclement@yahoo.fr.

ABSTRACT

Hydroethanolic 80% extract of the bulbs of *Gladiolus gregasius* Baker showed high degree of anti-candidal activities against *Candida albicans* and *Candida krusei*; the minimal inhibitory concentration on these strains were at equal concentration of 12.5 µg/ml compared to nystatine (3.2 µg/ml) the reference drug. However this extract has no inhibitiory activity against gram – and gram + bacterial strains: *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus* and *Streptococcus faecalis*. Phytochemical screening revealed an important saponins content which may explain this very high level anticandidal property of the extract. The evaluation of the acute toxicty on male (LD_{50}=10.5 g/kg) and female (LD_{50}=14.5 g/kg) rats revealed that the crude extract was not toxic. Some serum biochemical parameters were also evaluated to assess the liver (ALP, ASAT, ALAT, proteins) and kidney (urea and creatinin) functions; Significant differences ($p<0.05$) were noted between the control and test groups in male (4 g/kg) and female (8 g/kg body weight). These results suggest that *Gladiolus gregasius* can be used in the treatment of candidal infections.

Key words: Anticandidal activity; *Gladiolus gregasius* Baker; *Iridaceae;* Acute toxicity

1 INTRODUCTION

The growing importance of fungi in the medical field has attracted a lot of interest and has heightened awareness of the risks they pose as pathogens to humans. Yeast of the genus *Candida* is by far the most frequently isolated yeast from humans. It is found in the mouth, gastrointestinal tract and women genital tract as commensal of these anatomic sites. However, *C. albicans* is the most pathogenic of the opportunistic fungi and its transformation from a commensal to pathogen is elicited by local and systemic factors which render the human host susceptible to infections (Farell et *al.*, 1983; Ryley, 1986; Odds, 1988).

Medical records from the CPC (2001) has highlighted candidiasis as the most recurring causes of vaginal infection in women. Because current comercialised antibiotics are costly and have indeed considerable drawbacks in terms of serious side effects; Herbal drugs are playing important role in healthcare in Cameroon. The demand by most people in Cameroon for medicinal plants, as for the rest of Africa, have increased this last decade (Herbalgram, 2003). Some naturally occuring substances found in higher plants: terpenoid, flavonoids, alkaloids and saponins are an important group of molecules with antifungal properties (Cotoras et *al.*, 2001).

Gladiolus gregasius Baker belongs to the familly of *Iridaceae*. It is a bulbous slender herb with very narrow leaves, flowers are purple or white with purple throat. The plant is found in the West part of Cameroon and extend to the West African region (Hutchinson et Dalziel, 1968). It is used by traditional healers in the West Province of Cameroon to cure urogenital tract, skin and respiratory infections (Megne, 1997). But, up to now, nothing has been reported concerning the chemistry and biological properties of the plant. The aim of this paper is to present the phytochemical analysis of this plant and its antimicrobial properties as well as its acute toxicity on male and female albinos rats.

2. MATERIAL AND METHODS

Plant material: Bulbs of *G. gregasius* Baker (*Iridaceae*) were collected at Bafou (West Province of Cameroon) in 2002. The plant was authenticated at the Department of Vegetal Biology of the Yaoundé I University and at the National Herbarium of Yaoundé (Cameroon) where voucher specimens are deposited under the number 52405/HNC.

Preparation of extracts: Air-dried and ground powder (500 g) of *G. gregasius* bulbs was macerated at room temperature with 1 liter of 80% hydroethanolic solvent and the filtrate was evaporated to dryness using a ventilated oven at 40°C to produce 60 g of a brown extract. Before testing, the dry residue was dissolved in distilled and sterilized water to give a concentration to be used in the tests.

Phytochemical analysis: The phytochemical analysis were done on the extract to caracterise their biological properties using the common previously methods described by Harbone (1976).

Screening of antimicrobial activity: The microorganisms used in this work, provided by the Medical Bacteriology Laboratory of the "Centre Pasteur of Cameroon (Yaoundé)", included yeasts strains: *Candida albicans* (*C. albicans* (LMP0204U)), *Candida krusei* (*C. krusei* (LMP0304U)) and Gram + and Gram- bacteria strains: *Staphylococcus aureus* (*S. aureus* (LMP 0106U)), *Streptococcus pneumoniae ((S. pneumoniae)* (LMP 0210U)), *Escherichia coli* (*E. coli* (LMP 0201U)), *Proteus vulgaris* (*P. vulgaris* (LMP 0210U)), *Pseudomonas aeruginosa ((P. aeruginosa)* (LMP 0102U)), which were kept under the collection "LMP" in our Laboratory at 4°C. The tested microorganisms were activated on Nutrient agar (bacteria strains), or Sabouraud agar (yeasts strains) at 37°C for 24 hours. Two or three isolated colonies were diluted aseptically in sterile physiological water to yield density matching with the 0.5 Mac Farland turbidity standard (1.5 10^8 Colony Forming Units (CFU)/ml), and 100 fold dilution done to obtain a suspension with a final concentration of approximately 10^6 CFU/ml for the tests. Different methods were applied for the screening.

For *the hole-plate diffusion method* (Berghe et Vlietnick, 1991), petridishes were filled with the Agar Mueller Hinton (bacteria) medium or sabouraud dextrose agar (yeasts), and inoculated with the test microorganism. Wells (6 mm diameter) were then made and filled with 150 µl of the test sample: 80% hydroethanolic extract of *G. gregasius* extract, nystatine (yeasts) or gentamicine (bacteria) were included in every tests.

The Minimum inhibitory concentration (MIC), considered as the lowest concentration of the sample which inhibits the visible growth of microbe, was determined by the macrobroth dilution method (Carbonelle et *al.*,1987) in broth Mueller Hinton or Sabouraud medium supplemented with glucose 10% and phenol red. In this method, 0.4 ml of a freshly prepared cell suspension (10^6 CFU/ml) was added to 3.6 ml of susceptibility test broth containing serial twofold dilutions of each tested sample in glass tests tubes (13 by 100 mm) fitted with cotton

wood. These tubes were incubated in air at 37°C for 24 hours before being read. The MIC was considered as the lowest concentration of the sample that prevented visible growth.

Minimum Fungicidal Concentration (MFC) (the lowest concentration yielding negative sub cultures or only one colony) was also determined by subculturing 100 µl of each negative growth tube and positive growth control for 24 hours on fresh free from antibiotic agarised Mueller Hinton or sabouraud.

Animals : Adults Wistar albinos rats (120 – 140 g); were bred at the Departement of Biochemistry, University of Yaoundé I. They were all clinically healthy and maintained in standard environmental conditions of temperature (27.0±0.5 °C). They were fed a standard diet and tap water *ad libitum*. The bioassay was conducted in accordance with the internationally accepted principles (WHO, 1992) for laboratory use and care. Rats were deprived of food but not of water 12 h prior to administration of the test substance.

Acute toxicity: The animals were separated in six groups of four males and four females including one control (group1) and five treated. The remaining groups (2-6) received 4, 8, 12, 16, 20 g/kg of body weight each respectively of oral single doses of the 80% hydroethanolic extract. The control group received tap water at an equivalent volume. Observations were made and recorded systematically 1 and 2h after extract administration. The visual observations included changes in respiratory, motility and skin sensitivity, diarrhoeae, behavioral pattern, weight gain, food and water consumption. The number of survivors was noted after 48 hours and these were then maintained for a further 5 days, after which they were sacrificed by decapitation by making an incision on the jugular vein to collect blood. The medium letal dose (LD_{50}) was determined using the Behrens and Karber method (1983). Evaluation of the toxicity degree was based on previously described method (Delongeas et *al.*, 1983 ; Shorderet, 1989 ; Lu, 1992).

Biochemical estimation : Sera were assayed for Alkaline phosphatase (ALP) (Morgernstern et al., 1965), Aspartate aminotransferase (ASAT) (Reitman and Frankel, 1957), Alanine aminotransferase (ALAT) (Reitman and Frankel, 1957), creatinin (Cheesbrough, 1985), urea (Cheesbrough, 1985).

Statistical analysis: One way analysis of variance (ANOVA) was applied for determining the statatistical significance in various markers level between the control and the tested group. The level of significance was set at 0.05 and 0.01 (Wonnacott and Wonnacott, 1995).

3 RESULTS AND DISCUSSIONS

The phytochemical studies revealed the presence of an important content of saponins and glycosides and moderate level of polyphenol, phenols, triterpenes and steroids. Tannins, alkaloids and anthranoids were not noticed. The results of the solid diffusion method tests showed an important antifungal activity on *C. albicans* and *C. krusei* with inhibition zone of 21±01 and 22±02 mm respectively (table 1); however no inhibition zone was noticed on all the bacteria strains (*S. aureus, S. faecalis, E. coli, P. vulgaris and P. aeruginosa*). The inhibition pararameter of this extract on the two *Candida* strains presented in table 1 revealed an important inhibition parameter. In fact the minimal inhibitory concentration on these strains were at equal concentration of 12.5 µg/ml compared to nystatine (3.2 µg/ml) the reference drug. The minimal fungicidal concentration values also showed an important activity of the total extract (25 µg/ml) as regarded to that obtained with nystatine (6.4 µg/ml). This specific anticandidal activity of the hydroethanolic extract could be attributed to saponosides which have shown better inhibitory activity against fungi strains rather than against bacteria pathogens (Bruneton, 1999).

The same extract was tested for its acute toxicity on male and female albinos rats (Wistar).

Observations were made and recorded systematically 1 and 2h after extract administration. The visual observations which included changes in respiratory, motility and skin sensitivity, diarrhoeae, behavioral pattern, weight gain, food and water consumption significantly changed in group 3 (8 g/kg) in male and female rat as compared to the control. This implies that at high dose (≥8 g/kg) all the animals were physically affected and resulted to some death recorded. We registered total mortality at 12 and at 20 g/kg body weight respectively in male and female. The medium letal dose (LD_{50}) values were 10.5 g/kg and 14.5 g/kg of body weight on male and female rats respectively. It can be concluded that *G. gregasius* is not toxic as regard to the threshold of toxic substances (5 g/kg) stipulated by Delongeas et *al.* (1983) ; Shorderet, (1989) and Lu, (1992). On the other hand, these value of the LD_{50} showed that male rats were more susceptible to this extract than female. This difference in response to substance may be due to sex hormones which act on enzymes

involved in the metabolism of xenobiotics in mammalians; as this difference is always noticed after puberty (Hodgson et Guthrie, 1980).

Blood chemistry of male and female rats are shown in table 2 and 3 repectively. In male, it is observed that there is significant increased in ALP, ASAT, ALAT, urea ($p<0.05$) creatinin ($p<0.01$) values in the test groups (4 & 8 g/kg) when compared with the control. Significant increase ($p<0.01$) in ALP, ASAT and urea value appeared in group 3 (8 g/kg). ALAT and creatinine level sgnificantly ($p <0.01$) increased in group 4 (12g/kg) and 5 (16 g/kg) as compared to the control. Estimating the activities of serum marker enzymes like ASAT, ALAT and ALP can make assessment of liver function. When liver cell plasma membrane is damaged a variety of enzymes normally located in the cytosol are released in to the blood stream. Their estimations in the serum are useful quantitative marker of the extent and type of hepatocellular damage (Venukumar and Latha, 2002). The enhanced activities of these enzymes in all the treated male groups and in groups 3, 4 and 5 for female correspond to the liver damage induced by the extract. Creatinine is a nitrogenous waste product from the metabolism of creatine in the skeletal muscle. Creatinine diffuses freely throughout the body water. It is filtered from the blood, by the kidney and excreted in the urine. Urea is the main waste product of the protein breakdown. As the rate of production is generally fairly constant, raised plasma urea levels indicate decreased renal function. The estimation of creatinine and urea levels are therefore important tools for the investigation of the kidney damage (Cheesbrough, 1985). These biochemical parameters similarly increased in the all our tested group (male) and in groups 3, 4, 5 (female) indicated that the extract affected kidney function.

The high level anti-candidal and the non toxic properties of the 80 % hydroethanolic extract of *G. gregasius* ensure the safety of the consumers. Further chromagraphic studies of the fractions are needed in the search of new active molecules. The discovery of a potent herbal remedy that is safe is a big advantage in fungal infection therapy. It is vital in the treatment of systemic fungal infection that are usually frequent in immuno-compromised patients as toxicities induced by commercially antifungal drugs are often observed in these patients due to high dosage and prolonged therapy (Somchit et *al.*, 2003).

Acknowledgments

We wish to express our profound gratitude to Mr. Guimapi Charles Moodem, the traditional healer who provided the plant material, M. Koufani Analectus (National Herbarium of Yaoundé), the "Centre Pasteur du Cameroun (Yaoundé)" and to the Chinese Cooperation.

References

Berghe, V. A. ; Vlietinck, A. J. (1991) Screening Methods for Antibacterial and Antiviral Agents from Higher plants. Methods of plants Biochemistry. *Academic Press Limited.* Vol.6, 69 p.

Berhens, B. and Karber, G. (1983) "Mathematics for naturalists and agriculturalists". PWN, Warsaw. Pp. 218-219.

Bruneton, J. (1999) Pharmacognosie, Phytochimie, Plantes Médicinales. 3eme édition revue et augmentée. Edition TEC & DOC Paris. 1120 p

Carbonnelle, B. ; Denis, F. ; Marmonier, A. ; Pignon, G ; Vargues, R. (1987) Bactériologie médicale. Techniques usuelles. Ed. SIMEP. Paris. Pp.130-198.

Cheesbrough, M. (1985) Medical Laboratory Manual for tropical countries.

Cotoras, M. ; Garcia, C. ; Lagos, C. ; Folch, C. and Mendoza L. (2001) Antifungal activity on *Botritys cinera* of flavonoid and triterpenoid isolated from the surface of *Pseudogaphalium spp.* Bol. Soc. Chil. Quim. Vol. 46. no 4. pp. 1-4

CPC (Centre Pasteur du Cameroun) (2001) Rapport d'activité 2000-2001.

Delongeas, J. L. ; Burnel, D.; Netter, P.; Grignon, M., Mur, J. M.; ROYER, R. I.; Grignon, G. (1983) Toxicité et pharmacocinétique de l'oxychlorure de zirconium chez la souris et chez le rat. *J. Pharmcol.* 14 (4) : 437-447.

Ethnopharmacology 84 (1), 1-4.

Farell, S. M.; Hawkins, D. F. And Ryder, T. A. (1983) Scaning electron microscope study of *Candida albicans* invasion of cultured human cervical epithelial cells. Sabouraudia 21. 251-254

Harbone, J. B. (1976) Phytochemical methods. A guide to modern techniques of plant analyses. Chapman and hall, London. 150 p.

Herbalgram (2003) Studying markets to identify medicinal species for domestication: The case of *Annickia chlorantha* in Cameroon. *The Journal of the American Botanical Council*; 60:38-46.

Hodgson, E. and Guthrie, F. E. (1980) Introduction to biochemical toxicology. Blackwell Scientific Publication. USA. VOL. 1. pp 130-140.

Hutchinson, J. et Dalziel, J. M. (1968) Flora of West tropical Africa.

Lu , F. C. (1992). Toxicologie : Données générales, Procédures d'évaluation, organes cibles et évaluation du risque. Masson, Paris. Pp. 3-87.

Morgernstern, S.; Kessler, G.; Auerbach, J.; Flor, R.; Klein, B. (1965) An automated p-nitrylphosphate serum alcaline phosphatase procedure for the autoanalyser. Clin. Chem. 11, 876.

Odds, F. C. (1988) *Candida* and Candidosis. A review and bibliography. Second edition. Balliere Tindall.

Reitman, S. and Frankel S. (1957). A colrimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. Pp. 28-58. Réseau International des Instituts Pasteur et Instituts Associés. Yaoundé Cameroun. Pp. 27-32.

Ryley, J. F. (1986) Pathogenicity of *Candida albicans* with particular reference to vagina. Journal of medical and veterinary mycology. 24: 5-22.

Shorderet, M. (1989) Des concepts fondamentaux aux applications thérapeutiques. Pharmacologie. Edition Slatkine. Genève. Pp 30-43.

madasgacariensis leaf extracts. Phytotherapia. 16(2), 174-179.

Somchit, M. N.; Reezal I.; Elisha Nur and Mutalib, A. R. (2003) In vitro antimicrobial Activity of ethanol water extract of *Cassia alata*. Journal of Ethnopharmacology 84 (1), 1-4.

WHO (1992) Research guidelines for evaluating the safety and efficacy of herbal medecine. Manila, Phillipines, 38 p.

Wonnacott, T. H. and Wonnacott, R. J. (1995) Statistique: Economie- Gestion- Sciences- Médecine. 4ème Edition. Economia. Paris Pp 369, 370, 383.

Table 1: Antimicrobial parameters of the 80% hydroethanolic extract of *G. gregasius* and nystatine against the tested *Candida* species

souches	BGG 80%			Nystatine		
	Φ (250 µg/ml)	MIC (µg/ml)	MFC (µg/ml)	Φ (20µg/ml)	MIC (µg/ml)	MFC (µg/ml)
C. albicans	22±01	12.5	25	25±01	3.2	6.4
C. krusei	21±02	12.5	25	25±01	3.2	6.4

Φ: inhibition diameter

Table 2: Effect of the hydroethanolic extract of *Gladiolus gregasius* Baker on different biochemical parameters in the serum of male rats.

Serum biochemical parameters	Dose		
	N=4 0 g/kg	N=4 4 g/kg	N=1 8 g/kg
ALP (UI/l)	62,8±0,4	63,9±0,3*	67,4*
ASAT (UI/l)	40,8±0,6	43,1±0,5*	44,3*
ALAT (UI/l)	19,0±0,4	21,9±0,5*	22,8*
Urea (mg/l)	166,3±0,8	167,8±0,06*	173,2*
Creatinine (mg/l)	7,21±0,06	7,33±0,15**	7,61**

* Significantly different from control ($p<0,05$).

** Significantly different from control ($p<0,01$)

N : number of survivors

Table 3: Effect of the hydroethanolic extract of *Gladiolus gregasius* Baker on different biochemical parameters in the serum of female rats.

Serum biochemical parameters	Dose				
	0 g/kg N=4	4 g/kg N=4	8 g/kg N=2	12g/kg N=2	16g/kg N=2
ALP (UI/l)	63,4±0,2	62,6±0,3	61,3±0,5**	66,4±0,6**	67,2±0,5**
ASAT (UI/l)	40,7±0,3	41,9±1.4	44,5±0,3**	48,6±04**	50,1±0,2**
ALAT (UI/l)	18,9±0,7	18,6±0,6	19,4±0,4	21,0±0,3*	23,3±0,4*
Urea (mg/l)	167,2±0,8	168,4±1,1	168,9±0,6**	172,3±0,4**	175,6±0,8**
Creatinin (mg/l)	7,33±0,13	7,09±0,45	7,45±0,21	7,63±0,18*	8,21±0,22*

* Significantly different from control ($p<0,05$).

** Significantly different from control ($p<0,01$)

N : Number of survivors