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EVALUATION OF ANTIMICROBIAL ACTIVITY OF THE STEM BARK OF *CYLICODISCUS GABUNENSIS* (MIMOSACEAE)

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Abstract

Ethyl acetate(EA) extract of the stem bark of *Cylicodiscus gabunensis* (CG) was analysed phytochemically and evaluated for its antimicrobial activity against 17 pathogenic species isolated from patient: *Escherichia coli, Klebsiella pneumoniae, Shigella dysenteriae, Shigella flexneri, Morganella morganii, Proteus vulgaris, Proteus mirabilis , Salmonella typhi , Citrobacter freundii , Enterobacter cloacae , Enterobacter agglomerans , Staphylococcus aureus , Streptococcus feacalis , Pseudomonas aeruginosa , Bacillus cereus T, Candida albicans* and *Candida glabrata .* Flavonoids, saponins, tannins, polyphenols, coumarins, triterpenes and/or sterols and reducing sugars were detected in the (EA) extract of CG. The best MIC and MBC values for the microorganisms sensitive to the extract were 0.00078 and 0.00315 mg/ml respectively. The greater and remarkable antimicrobial activity of the (EA) extract of CG was recorded with *Staphylococcus aureus, Proteus vulgaris* and *Bacillus cereus T.* These results provide a rationalization for the traditional use of this plant for the treatment of infections diseases.

Key words: Antimicrobial activity, *Cylicodiscus gabunensis*

Introduction

In recent years multiple drug/chemical resistance in both human and plant pathogenic microorganism have been developed due to indiscrimate use of commercial antimicrobial drug/chemical commonly used in the treatment of infection diseases (Davis, 1994; Loper, 1991 and Service, 1995). This situation provided the impetus to the search of new antimicrobial substances from various sources like medicinal plants (Clark, 1996; Cordell, 2000).

Cylicodiscus gabunensis (Mimosaceae) commonly called Denya (Ghana), Edum (Gabon), Adoum, Bokoka (Cameroon), Bouemon (Ivory Coast) (Chudnoff *et al*., 1984) is a large tree with a cylindrical trunk. The stem is more or less pyramidal in shape with widespread branches. The bark has a strong odour. The leaves are imparipinnate subsessile, alternate and are slightly asymmetrical. The inflorescence is on the branches. The flowers are small, 2-5 mm long and 2-3 mm wide. The pods are long, hanging up to 1 m long and 4 cm broad, acute at the base and acuminate at the apex (Adjanohoun *et al*., 1996). *Cylicodiscus gabunensis* (CG) is found in the dense, humid forest and it is widespread from Cameroon to Cabinda (Adjanohoun *et al*., 1996).

Cylicodyscus gabunensis is traditionally known for its medicinal use. The barks of the stem of CG are used to prepare remedies for headache, filariasis, rheumatism and gastrointestinal disorders (Adjanohoun et al, 1996). Four saponins were isolated from the alcoholic extract of the bark of CG (Tchivounda *et al*., 1991). However, so far there have been no attempts to study the potential of CG antimicrobial activity against a wide range of pathogenic species isolated from patient such as bacteria and yeasts species.

The present study was conducted to investigate antimicrobial property of ethyl acetate (EA) extract of CG stem bark against 17 pathogenic species isolated from patient which have not been evaluated in previous studies and to determine the phytochemical constituents of the extract.

Materials and Methods

Plant Material

The stem bark of CG was collected in the morning on Mount Eloumdem, Yaoundé- Cameroon in January. Identification of the plant was confirmed in the National Herbarium Yaoundé (reference number of the plant: N° 21574/SRF/CAM). The plant material (stem bark of CG) was then air dried at room temperature. The dried plant material was ground into a fine powder.

Preparation of extract

This was carried out by soaking the dried powdered plant (250 g) in bottle with 3.5 l of ethyl acetate (EA) and kept for 72 hours. The plant- (EA) mixture was then sieved. The filtrate (extract) was concentrated by evaporating under vacuum (EA) using a rotary evaporator. The extract was further concentrated by allowing it to stand overnight in an oven at 30°C. The yield of the extraction was calculated by dividing the weight of the extract obtained by the weight of the dried material plant extracted.

Phytochemical screening

The freshly prepared extract was chemically tested qualitatively for the presence of chemical constituents such as alkaloids, leucoanthocyanins, flavonoids, saponins, tannins, anthraquinones, polyphenols, coumarins, sterols and/or triterpenes, anthocyanins, cardiac glycosides, reducing sugars, glycosides, anthranoids and steroids. They were identified using characteristic colour changes using standard procedures previously described (Farnsworth, 1966; Harbone, 1973; Odebiyi and Sofowora, 1978). Each test was qualitatively expressed as negative (-) or positive (+); the intensity of the characteristic colour was expressed as (++) or (+++).

Microorganisms

The test microorganisms used for the antimicrobial activity screening were: *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Shigella dysenteriae (S. dysenteriae), Shigella flexneri (S. flexneri), Morganella morganii (M. morganii), Proteus vulgaris (P. vulgaris), Proteus mirabilis (P. mirabilis), Salmonella typhi (S. typhi), Citrobacter freundii (C. freundii), Enterobacter cloacae (E. cloacae), Enterobacter agglomerans (E. agglomerans), Staphylococcus aureus (S. aureus), Streptococcus feacalis (S. feacalis), Pseudomonas aeruginosa (P. aeruginosa), Bacillus cereus T (B. cereus T), Candida albicans (C. albicans), Candida glabrata (C. glabrata)*. These microorganisms were obtained from Bacteriology and Mycology Laboratories of Centre Pasteur of Yaoundé and Bacillus cereus T was obtained from the A.F.R.C. Reading Laboratory of Great Britain.

Disc diffusion assay

The dried plant-extract was dissolved in the same solvent of extraction, ethyl acetate (EA) to a final concentration of 250 mg/ml. Sterile paper discs (6 mm of diameter) prepared from Whatman number one filter paper were impregnated with 10 µl of the crude extract of the plant as described by Edward (1980) from a stock of 250 mg/ml. Each disc contains 2.5 mg of the extract. These paper discs were kept in an incubator at 37°C for 24 hours to evaporate the solvent. Antimicrobial tests were then carried out by disc diffusion method (Murray et al, 1995).

100 µl of the suspension of the tested microorganism (0.5 Mc Farland standard turbidity) containing 10^8 CFU/ml of bacterial, 10^6 CFU/ml of yeast prepared from an overnight Mueller Hinton agar culture for bacterial and Sabouraud with chloramphenicol agar for yeast was used to seed each prepared and dried Mueller Hinton agar plate for bacteria and Sabouraud with chloramphenicol agar for yeast. The discs were arranged and firmly pressed on the agar surface of each seeded plate. These plates, after staying at 4°C for 2 hours were incubated aerobically at 37°C for 24 hours for bacteria and at 25°C for 24 hours for yeast. Similarly, paper discs containing standard concentration of

antibiotics (gentamicin and nystatin) as described by Matsen (1979) were prepared and used for the susceptibility test. Negative control was also prepared using the same solvent employed to dissolve the plant extract. Antimicrobial activity was evaluated by measured the zone of inhibition against the tested microorganism and were expressed by – (negative), + (zone of inhibition ≤ 8mm in diameter), ++ (zone of inhibition > 8 mm and ≤ 20 mm in diameter) and +++ (zone of inhibition > 20 mm in diameter). The result recorded for each bioassay was the average of 3 tests.

Determination of MIC and MBC

The MIC and MBC values were also evaluated for the microorganisms that were determined as sensitive to (EA) extract of CG in disc diffusion assay, according to liquid dilution method (Vanden berghe et al, 1991). A broth macrodilution susceptible assay was used as reported by Delarras (1998) for the determination of MIC. All the tests were performed in peptone water with red phenol supplemented with glucose 1% (w/v) (PPG1%). Red phenol is a colour indicator .It is red in a basic or neutral milieu and yellow in an acidic milieu .The growth of microorganisms is associated with the oxidation or the fermentation of the glucose of the milieu and the liberation of acid metabolic which change the colour of the red phenol from red to yellow.

Bacterial strains were cultured overnight at 37°C in Muller Hinton and yeast stains at 25°C in Sabouraud with chloramphenicol agar. Test strains were suspended in normal saline (NaCl 9‰), adjusted to 0.5 Mc Farland standard turbidity and suspended in PPG1% to give a final density of 5x105 CFU/ml.

For the susceptibility testing; in the first step, 4 ml of PPG1% were distributed from the second to the 17th 10-ml test tubes of each 10-ml sterile test tube lines. Each line of 10-ml test tubes is used to one test microorganism. Dry extract was initially dissolved in Tween 80 (100 µl) and then in the PPG1% to reach the highest concentration 16 mg/ml to be tested. 8 ml of these suspensions were added in the 1st test tube of each line of the 10-ml test tube and then 4 ml of scalar dilution were transferred from the 2nd to the 16th test tube. The 17th test tube was considered as growth control since no extract solution was added. Then, 1 ml of microbial suspension was added to each test tube. The final volume of each 10-ml test tube was 5 ml. The final concentration of extract adopted to evaluate the antimicrobial activities was included from 12.8 mg/ml (1st test tube) to 0.00039 mg/ml (16th test tube). Gentamicin for bacteria strains and Nystatin for yeast strains at the concentration range of 0.32 to 0.000019 mg/ml was prepared in (PPG1%) and used as standard drugs for the positive control. The content of each test tube was mixed and then incubated under normal atmospheric condition at 37°C for 24 hours (for bacterial strains) and at 25°C for 24 hours(for yeasts strains). The bacterial and yeasts growth was indicated by the colour change of the test tube content from the red to yellow. The MIC was defined as the lowest concentration of the extract to inhibit the growth of microorganims (1st red test tube content of each line) and confirmed by platting 5 µl sample from that red test tube on Mueller Hinton agar for bacteria strains and on Sabouraud with chloramphenicol agar for yeast strains. The MBC were determined by platting 5 µl sample from red test tubes on Mueller Hinton agar or on Sabouraud with chloramphenicol agar without extract. The MBC was the concentration at which there was no microbial growth. The extract testing in this study was screened three times against each microorganism.

Results and Discussion

The yield of the extraction was 10.02% w/w. The antimicrobial activities of the stem bark of CG extract against microorganisms examined in the present study and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zone and zone diameter, MIC and MBC values. The results were given in the Tables 1and 2, and Figure1.

The EA extract of CG had antimicrobial activity against any of the bacterial and fungal isolates tested (Table 1). Figure1 illustrate the zone of lysis by disc-diffusion method using crude extract of CG against 17 pathogenic species isolated from patient on comparison with Gentamicin and Nystatin. The antimicrobial effect of this extract was found to be comparable to that of the conventional antibacterial and antifungal drug .Maximal inhibition zone values for the microorganisms sensitive to the (EA) extract of CG was 19.83 mm against K.pneumoniae (Figure 1).

Table1: Antimicrobial activity of the (EA) extract of CG (stem bark) against microorganisms tested.

Drugs / Pathogenic microorganisms	(EA) extract CG stem bark	Gentamicin	Nystatin
M.morganii	++	++	NT
E.coli	++	++	NT
S.aureus	++	+++	NT
P.vulgaris	++	+++	NT
C.freundii	++	++	NT
S.flexneri	++	++	NT
S.dysenteriae	++	++	NT
E.cloacae	++	+++	NT
E.agglomerans	++	++	NT
S.feacalis	++	++	NT
P.aeruginosa	++	+++	NT
P.mirabilis	++	+++	NT
S.typhi	++	++	NT
K.pneumoniae	++	++	NT
B.cereus	++	++	NT
C.albicans	++	NT	++
C.glabrata	++	NT	++

(–): Negative.
(+): Zone of inhibition ≤ 8mm in diameter.
(++): Zone of inhibition >8mm and ≤ 20mm in diameter.
(+++): Zone of inhibition >20mm in diameter.
NT: Not tested.

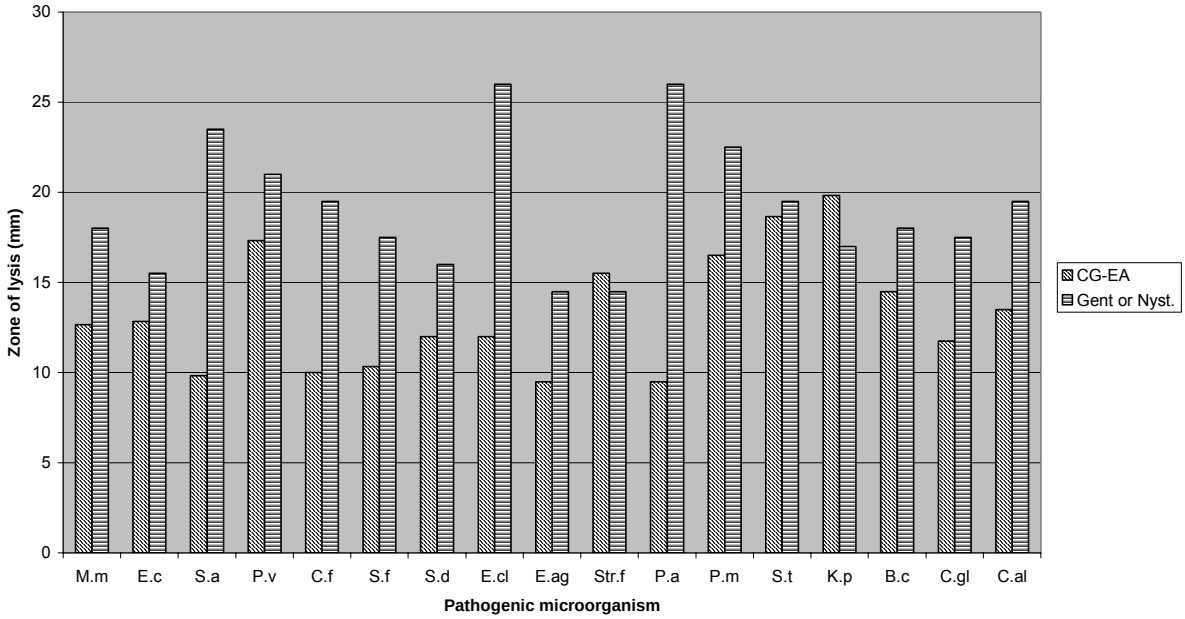
Considering that in this study crude extract was employed, the MIC values of 8 mg/ml or below against microorganism tested was considered as active. The best MIC and MBC values for the microorganisms sensitive to the (EA) extract of CG were 0.00078 and 0.00315 mg/ml respectively (Table 2). The sensibility towards different pathogens by the EA extract of CG was in the following order *B.cereus, S.aureus, P.vulgaris, P.mirabilis, C.freundii, S.typhi, E.coli, M.morganii, E.agglomerans, C.albicans, C.glabrata ,S.feacalis, P.aeruginosa, S.dysenteriae, E.cloacae, S.flexneri* and *K.pneumoniae* (Table 2). A higher antimicrobial activity of the (EA) extract of CG was recorded with *Staphylococcus aureus, Proteus vulgaris* and *Bacillus cereus T.* These results are interesting since some of these microorganisms are commonly involved in acute diarrhoea diseases which are one of the principal causes of death in infants (Syder et al, 1982; Lutterrodt et al, 1989).

From Table 3, it is seen that flavonoids, saponins, tannins, polyphenols, coumarins, triterpenes and/or sterols and reducing sugars are the major compounds in the CG-EA extract. The presence of flavonoids, saponins, tannins in this extract may explain some of its antimicrobial actions since antimicrobial actions of most of these phytochemical substances have been documented (Lewis, 1977; Plasuntherum, 1982; Palacious, 1983; Ahmad, 1986). Coumarins show a better activity against plant microbial pathogens rather than against human pathogens (Ojala et al, 2000). The antimicrobial activity of the (EA) extract of CG against *E.agglomerans* which is much plant microbial pathogen rather than human pathogen (http://membres.lycos.fr/microbio/systematique/Eb.html) may be due to the presence of coumarins in this extract. However, the antimicrobial activities demonstrated by the extract could be due to the presence of other antimicrobial substances not covered by the screening.

Conclusion

The obtained results shown that (EA) extract of CG stem bark had antimicrobial activity against a wide range of pathogenic species isolated from patient such as bacteria and yeasts species.

Figure 1: Zones of lysis (mm) of plant extracts (CG-EA) and conventional antibiotics (Gentamycin and Nystatin) against 17 pathogenic microorganisms as detemined by disc-diffusion



Key
M.m: Morganella morganii; E.c: Escherichia coli; S.a: Staphylococcus aureus; P.v: Proteus vulgaris; C.f:Citrobacter freundii; S.f: Shigella flexneri; S.d: Shigella dysenteriae; E.cl: Enterobacter cloacae; E.ag: Enterobacter agglomerans; Str.f: Streptococcus faecalis; P.a: Pseudomonas aeruginosa; P.m: Proteus mirabilis; S.t: Salmonella typhi; K.p: Klebsiella pneumoniae; B.c: Bacillus cereus; C.gl: Candida glabrata; C.al: Candida albicans

Table 2: The MIC and MBC values of the (EA) extract of CG (stem bark) against the microorganisms tested in macrodilution assay.

Inhibitionparameter(EA)extract Of CG / Pathogenic Microorganisms	MIC (mg/ml)	MBC(mg/ml)
P.aeriginosa	0.10000	3.2000
S.feacalis	0.05000	1.6000
E.cloacae	0.40000	3.2000
K.pneumoniae	3.20000	12.800
S.dysenteriae	0.10000	0.8000
S.flexneri	3.20000	6.4000
P.mirabilis	0.00156	0.0125
E.coli	0.00312	0.2500
C.freundii	0.00156	0 0125
B.cereus T	0.00078	0.0062
S.aureus	0.00078	0.0031
E.agglomerans	0.01250	0.2000
S.typhi	0.00156	0.0125
P.vulgaris	0.00078	0.0250
M.morganii	0.00312	0.2000
C.glabrata	0.02500	0.4000
C.albicans	0.02500	0.2000

MIC: minimal inhibition concentration (mg/ml). MBC: minimal bactericidal concentration (mg/ml).

Table 3: Chemical composition of (EA) extract of CG (stem bark).

Chemical compounds	Observations
Alkaloids	–
Leucoanthocyanins	+
Saponins	++
Tannins	+++
Antraquinones	–
Polyphenols	++
Coumarins	++
Sterols and/or Triterpenes	+++
Cardiac Glycoside	+
Reducing sugars	+++
Glycosides	+
Anthranoid	–
Steroids	+
Flavonoids	+++
Anthocyanin	–

(–): Absence of chemical compound
(+): Presence of chemical compound.
(+)< (++) < (+++): Base of the intensity of characteristic colour.

The presence of flavonoids, saponins, tannins, polyphenols, coumarins, sterol and/or triterpenes and reducing sugars in this extract may explain its antimicrobial activities.This is the first report about antimicrobial activity of ethyl acetate extract of the stem bark of *Cylicodiscus gabunensis*. The results support the traditional use of this plant to treat infection diseases.

At present, our group is concerned with the fractionation and the isolation of pure compounds and the elucidation of their structures in order to better evaluate their pharmacological activity in vitro and in vivo.

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