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Antimicrobial Activity of Dichloromethane-Methanol (1:1 v/v) Extract from the Stem Bark of *Coula edulis* Bail. (Olacaceae)

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Abstract: In order to confirm the traditional uses of *Coula edulis*, the CH_2Cl_2-MeOH (1:1 v/v) extract of stem bark of this plant and its column fractions were screened for antimicrobial activity. The plant was dried and extracted by maceration in CH_2-Cl_2-MeOH (1:1 v/v). The dry extract was fractionated by silica gel column chromatography. Phytochemical screening was performed using common chemical standard methods. Antimicrobial activity was assayed by disc diffusion method and broth macro dilution method. From the results, it appeared that the crude extract of *Coula edulis* stem bark displayed antibacterial activities against four clinical isolates of bacteria and antifungal activities against six strains of *Candida* species. The Minimum Inhibitory Concentration (MIC) values ranged from 12.5 to 25 mg mL^{-1} for bacteria and 1.56 to 6.25 mg mL^{-1} for yeasts. The fractionation of crude extract gave eight fractions. Fractions F3 and F4 showed higher antibacterial activities while fractions F5 and F6 displayed higher antifungal activity compared to the crude extract. Their MICs ranged from 0.19 to 12.5 mg mL^{-1}. Phytochemical screening indicated that the crude extract contains tannins, flavonoids, anthraquinones, anthocyanins, sterols and phenols. *Coula edulis* crude extract has the ability to inhibit bacterial and yeast growth. Fractionation enhanced the antimicrobial activity in some fractions. These results justify the traditional use of this plant for the treatment of infectious diseases.

Key words: *Coula edulis*, phytochemical screening, antibacterial, antifungal, bacteria and yeasts

INTRODUCTION

The search for plants with antimicrobial activity has gained increasing importance in recent years due to the development of antimicrobial drug resistance and often the occurrence of undesirable side effects of some antibiotics (Soberon *et al*., 2007). For example, over the last three decades, methicillin resistant *Staphylococcus aureus* has caused major problems in hospitals throughout the world (Waldyogel, 1995). With the advent of ever-increasing resistant bacterial and yeast strains, there has been a corresponding rise in the universal demand for natural antimicrobial therapeutics (Kishore *et al*., 1996; Soberon *et al*., 2007). Indeed, even though pharmacological industries and researchers have produced a good number of antibiotics in the last three decades, resistance to these drugs by micro organisms is increasingly high. Herbal medicine has been widely used and formed an integral part of

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primary health care in many countries (Akinyemi *et al*., 2005) and may constitute a reservoir of new antimicrobial substances to be discovered. According to WHO, medicinal plants would be the best source of a variety of drugs (Nascimento *et al*., 2000). On the other hand, about 80% of developing countries, citizens used traditional medicine based on plant products. This explains why numerous studies have been conducted on various medicinal plant extracts, screening their antimicrobial activities to better understand their properties and efficacy and also with the hope to discover new antimicrobial compounds.

Coula edulis Bail. (Family, Olacaceae), commonly known as African walnut, is a medicinal plant that originated from Tropical Western Africa. It is an evergreen tree growing to a height of 25-38 m and native to Tropical Western Africa. It can be found in the top canopy of forest as well as the lower story and has no special soil requirements. Ethnobotanical studies indicate that the stem or fruits of *C. edulis* are commonly used in West Africa for the treatment of stomach ache, skin diseases and tonic effect (Iwu, 1993). The bark is used to produce rinses or enemas for loin pains or kidney problems (Davidson, 1999). There is no report regarding the antimicrobial activities of this plant. Therefore, the aim of the present work is to evaluate the antimicrobial activities of the crude dichloromethane-methanol (1:1 v/v) extract of the stem bark of *C. edulis* and its column fractions on several bacteria and yeasts that can cause stomach ache, skin diseases and urinary problems in man.

MATERIALS AND METHODS

Plant Material

The stem bark of *Coula edulis* was collected in Buea (South West Province of Cameroon) in January 2005. Plant material was identified at the Cameroon National Herbarium in Yaoundé where a voucher specimen was kept under the accession number 19357 HNC.

Micro Organisms

The micro-organisms used in this study consisted of two gram positive (*Enterococcus faecalis*, *Staphylococcus aureus*) and six gram negative (*Pseudomonas aeruginosa*, *Proteus mirabilis*, *Shigella flexneri*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*) bacteria (clinical isolates) collected from Centre Pasteur (Yaoundé-Cameroon). Also, two strains of *Candida albicans* (ATCC 9002 and ATCC 1663.86) and six clinical isolates (*Candida parapsilosis*, *C. lusitaniae*, *C. tropicalis*, *C. glabbrata*, *C. krusei*, *C. albicans*) of yeasts originally obtained from Centre Pasteur (Paris-France) were tested. The bacterial and yeast isolates were grown at 35°C and maintained on nutrient agar (NA, Conda, Madrid, Spain) and Sabouraud Dextrose Agar (SDA, Conda) slants, respectively.

Extraction and Fractionation

The stem bark of *C. edulis* Bail was dried at room temperature (25±2°C) for 3 weeks and crushed. Five kilograms of obtained powder was macerated into 12 L dichloromethane-methanol (Merck) (1:1 v/v) mixture for two days and this process was repeated twice. After filtration, the filtrate was successively evaporated to dryness at 40°C (for the dichloromethane) and at 65°C (for the methanol) under reduced pressure using rotary vacuum evaporator. The dried crude extract was stored at +4°C. The crude extract (104 g) was then subjected to column chromatography (30×8 cm column) using 500 g of silica gel 40 (particle size 0.2-0.5 mm). The column was successively eluted with hexane (1000 mL), Hexane-ethyl acetate [19:1 v/v (2500 mL), 9:1 v/v (3750 mL), 4:1 v/v (1500 mL), 7:3 v/v (1750 mL), 3:2 v/v (1750 mL), 1:1 v/v (1750 mL), 2:3 v/v (2750 mL) and 1:4 v/v (5000 mL)] mixtures, ethyl acetate (5000 mL), ethyl acetate-methanol [9:1 v/v (2750 mL), 4 : 1(2250 mL), 13:7 (2500 mL), 1:1 v/v (2500 mL), 3:7 v/v (5250 mL)] mixtures and methanol (750 mL). One hundred and seventy one fractions of 250 mL each were collected and combined on the basis of their Thin Layer

Chromatography (TLC) profiles to afford eight main fractions. Fractions from 1-59, 60-122, 123-124, 125-126, 127-138, 139-149, 150-156 and 157-171 were referred as F1, F2, F3, F4, F5, F6, F7 and F8, respectively. TLC analyses were carried out on silica Gel 60 GF_{254} precoated plates (20×20 cm) and visualised under a UV light (254 and 366 nm), UV lamp Model 52-58 mineralight and sprayed with 50% v/v H_2SO_4 followed by heating at 100°C.

Phytochemical Screening of Extracts

The Phytochemical screening of the crude extract and its column fractions were carried out using standard methods (Silva *et al.*, 1998; Bruneton, 1999). The plant material was screened for the presence of different classes of compounds including alkaloids, flavonoids, sterols, triterpenes, coumarins, anthraquinones, tannins, anthocyanins, saponins and phenols.

Antimicrobial Assay

The susceptibility tests were performed by disc diffusion method as recommended by National Committee for Clinical Laboratory Standards (1993) with slight modifications. Stock solutions of the extracts (crude extract and fractions) were prepared in 5% v/v aqueous dimethyl sulphoxide (DMSO, Fisher chemicals) at concentration of 125 mg mL^{-1}. The inocula of micro-organisms were prepared from 24 h old broth cultures. The absorbance was read at 530 nm and adjusted with sterile distilled water to match that of a 0.5 McFarland standard solution. From the prepared microbial solutions, other dilutions with sterile distilled water were prepared to give a final concentration of 10^6 Colony-Forming Units (CFU) per millilitre for bacteria and 2×10^5 spores per millilitre for yeasts. Bottles containing 19.8 mL of sterile Sabouraud Dextrose Agar (Conda, Madrid, Spain) or Mueller Hinton Agar (MHA) (Conda, Madrid, Spain) were maintained in a steam bath set at 40°C to prevent solidification of the medium and then inoculated aseptically with 0.2 mL of bacteria or yeast suspension followed by thorough mixing. Sterile Petri dishes (Diameter, 90 mm) were filled to 20 mL final volume of each bottle to give a solid plate. Discs of 6 mm in diameter previously impregnated with 10 μL of stock solution of extracts were placed aseptically on the solid plates and allowed for 2 h at +4°C for the extract to diffuse. The Petri dishes were then incubated at 35°C for 24 h (for bacteria) and for 48 h (for yeasts). The final disc charges were 1.25 mg of extract per disc. The susceptibility was recorded by measuring the clear zone of growth inhibition on agar surface around the discs.

All the experiments were carried out in triplicates. Gentamicin (Sigma-Aldrich, Steinheim, Germany) and Nystatin (Merck, Darmstadt, Germany) at 10 μg per disc (for bacteria and yeasts respectively) were used as positive controls and 5% v/v aqueous DMSO as a negative control.

Determination of the Minimum Inhibitory Concentration (MIC)

MIC was determined by broth macro dilution method with slight modifications from the one described by Gulluce *et al.* (2003). The two-fold serial dilutions in concentration of the extracts (25-0.19 mg mL^{-1}) were prepared in Mueller Hinton Broth (MHB) (Conda, Madrid, Spain) for bacteria and Sabouraud Dextrose Broth (SDB) (Conda, Madrid, Spain) for yeasts. For every experiment, a sterility check (5% v/v aqueous DMSO and medium), negative control (5% v/v aqueous DMSO, medium and inoculum) and positive control (5% v/v aqueous DMSO, medium, inoculum and water-soluble antibiotics) were included. In general, the 24 macro well plates (Nunclon, Roskilde, Danmark) were prepared by dispensing into each well 880 μL of an appropriate medium, 100 μL of test extracts (crude extract or fractions) and 20 μL of the inoculum (10^6 cfu per mL for bacteria and 5×10^5 spores per mL for yeasts). The content of each well was mixed thoroughly with a multi-channel pipette and the macro well plates were covered with the sterile sealer and incubated at 35°C for 24 h (for bacteria) and 48 h (for yeasts) under shaking by using a plate shaker (Flow Laboratory, Germany) at 300 rpm. Microbial growth in each well was determined by observing and comparing the test wells

with the positive and negative controls. The absence of microbial growth was interpreted as the antibacterial or antifungal activities. The MIC was the lowest concentration of the test substances that prevented visible growth of micro-organisms. Minimum Bactericidal Concentrations (MBCs) or Minimum Fungicidal Concentrations (MFCs) were determined by plating 10 μL from each negative well and from the positive growth control on Mueller Hinton Agar (for bacteria) and Sabouraud Dextrose Agar (for yeasts). MBCs or MFCs were defined as the lowest concentration yielding negative subcultures or only one colony. All the experiments were performed in triplicate. Gentamicin and Nystatin at the concentration ranging between 400 and 0.79 μg mL^{-1} served as positive controls for antibacterial and antifungal activities respectively.

Statistical Analysis

The inhibition diameters of crude extract and its column fractions were expressed as the Mean±Standard Deviation and compared using Student-Waller Duncan test at $p \leq 0.05$.

RESULTS

The crude extract of *Coula edulis* and eight fractions obtained presented variable physical aspects (Table 1). The phytochemical screening of crude extract showed the presence of phenols, flavonoids, sterols, tannins, anthocyanins and anthraquinones but alkaloids, saponins, coumarins and triterpenes were absent. These main classes of compounds varied within the fractions.

Table 2 reports the inhibition zones of crude extract and its column fractions determined for eight clinical isolates of bacteria and eight *Candida* species. The results showed that *Escherichia coli* and *Klebsiella pneumoniae* were resistant since no inhibition zone was observed. Other micro-organisms

Table 1: Physical characteristics and phytochemical analysis of crude extract of *Coula edulis* stem bark and its column fractions

Extracts	Aspect and colour	Yield[a] (%, w/w)	Number[b] of spots and bands in TLC	Positive tests for	Negative tests for
Crude extract	Brown and paste	6.16	9	Phenols, sterols, flavonoids, anthocyanins, anthraquinones, tannins	Alkaloids, saponins, coumarins, triterpenes
F1	Orange and oily	4.70	4	Sterols	Alkaloids, saponins, coumarins, triterpenes, flavonoids, anthocyanins, anthraquinones, tannins, phenols
F2	Blackish and paste	19.32	3	Sterols, flavonoids, phenols	Alkaloids, coumarins, triterpenes anthocyanins, anthraquinones, saponins, tannins
F3	Darkish-brown and paste	4.23	5	Phenols, flavonoids, anthraquinones	Alkaloids, saponins, coumarins, triterpenes, anthocyanins, sterols, tannins
F4	Darkish-brown and paste	4.61	6	Phenols, tannins, flavonoids, anthraquinones	Alkaloids, saponins, coumarins, triterpenes, anthocyanins, sterols,
F5	Brownish-orange and paste	21.44	4	Phenols, tannins, flavonoids, anthocyanins,	Alkaloids, saponins, coumarins, triterpenes, sterols, anthraquinones
F6	Brownish-orange and paste	17.30	3	Phenols, tannins, flavonoids, anthocyanins	Alkaloids, saponins, coumarins, triterpenes, sterols, anthraquinones
F7	Greyish-brown and paste	16.54	2	Phenols, tannins, flavonoids	Alkaloids, saponins, coumarins, triterpenes, anthocyanins, sterols, anthraquinones
F8	Greyish-brown and paste	4.88	7	Phenols, tannins, flavonoids	Alkaloids, saponins, coumarins, triterpenes, anthocyanins, sterols, anthraquinones

[a]: The crude extract and fractions are reported as a percentage with respect to the plant material and dichloromethane-methanol extract respectively. [b]: Thin-layer chromatography plates were performed with ethyl acetate-methanol solvent system as mobile phase at different polarity. Spots and bands were visualized by UV irradiation (254 and 366 nm) and by spraying with 50% (v/v) sulphuric acid reagent followed by heating at 100°C

Table 2: Diameter of the inhibition zones* (mm) of crude extract of *Coula edulis* stem bark and its column fractions

	Test substances									
Name of the organism	Crude extract	F1	F2	F3	F4	F5	F6	F7	F8	Reference**
Bacteria										
Pseudomonas aeruginosa	12.33±0.57^{c}	0^{e}	11.33±1.52^{dc}	13.00±0.00^{b}	12.67±1.15^{bc}	11.33±1.52^{dc}	11.67±1.52^{dc}	0^{e}	0^{e}	24.00±1.00^{a}
Proteus mirabilis	12.33±0.57^{d}	0^{f}	12.33±0.57^{d}	18.33±0.57^{a}	16.00±1.00^{b}	14.33±0.57^{c}	12.67±2.08^{dce}	10.67±0.57^{e}	18.67±0.57^{a}	18.67±0.57^{a}
Klebsiella pneumoniae	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	20.00±1.00^{b}
Shigella flexneri	11.33±1.52^{f}	0^{h}	17.00±1.00^{c}	19.67±1.52^{b}	21.00±1.73^{ab}	13.00±0.00^{e}	14.67±1.52^{d}	11.33±1.52^{f}	7.67±1.52^{g}	23.33±1.52^{a}
Salmonella typhi	10.67±0.57^{cd}	0^{e}	10.00±2.00^{cd}	10.67±1.52^{cd}	14.33±1.15^{b}	9.33±1.52^{d}	11.67±1.52^{c}	0^{e}	0^{e}	23.66±0.57^{a}
Escherichia coli	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	21.33±1.52^{a}
Staphylococcus aureus	12.33±1.52^{d}	0^{f}	16.67±0.57^{bc}	17.67±1.52^{b}	17.00±2.00^{bc}	16.67±3.05^{bc}	14.33±3.05^{cd}	10.67±1.15^{e}	0^{f}	25.33±1.52^{a}
Enterococcus faecalis	9.67±1.52^{d}	0^{e}	17.33±1.15^{b}	17.33±1.15^{b}	17.33±1.52^{b}	14.00±1.00^{c}	14.33±0.57^{c}	0^{e}	0^{e}	20.66±1.15^{a}
Yeasts										
Candida albicans ATCC 9002	10.00±0.00^{c}	0^{e}	0^{e}	0^{e}	7.67±1.52^{d}	13.67±0.57^{b}	14.67±1.52^{b}	10.33±0.57^{c}	0^{e}	23.33±1.52^{a}
Candida albicans 1663.86	20.33±0.57^{b}	0^{h}	8.67±0.57^{g}	10.33±0.57^{f}	9.00±0.00^{g}	16.33±0.57^{d}	19.00±1.00^{c}	14.00±1.00^{e}	14.00±1.00^{e}	23.00±0.00^{a}
Candida albicans	10.67±0.57^{d}	0^{g}	0^{g}	0^{g}	10.00±0.00^{e}	15.33±0.57^{c}	16.67±0.57^{b}	8.33±0.57^{f}	9.67±0.57^{e}	25.66±0.57^{a}
Candida parapsilosis	14.67±0.57^{d}	0^{f}	0^{f}	13.67±0.57^{e}	14.33±1.15^{cde}	15.33±0.57^{c}	19.33±0.57^{b}	13.67±0.57^{e}	0^{f}	25.00±1.00^{a}
Candida tropicalis	9.67±0.57^{d}	0^{e}	0^{e}	0^{e}	0^{e}	12.00±0.00^{c}	14.67±0.57^{b}	0^{e}	0^{e}	21.33±1.52^{a}
Candida krusei	8.67±.57^{c}	0^{d}	0^{d}	0^{d}	0^{d}	8.33±0.57^{c}	11.33±0.57^{b}	0^{d}	0^{d}	19.00±1.00^{a}
Candida glabbrata	7.00±0.00^{c}	0^{d}	0^{d}	0^{d}	0^{d}	10.67±1.15^{b}	11.67±1.15^{b}	0^{d}	0^{d}	23.66±1.15^{a}
Candida lusitaniae	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	15.67±0.57^{c}	19.00±1.73^{b}	0^{d}	0^{d}	32.66±1.52^{a}

The results are the mean values of triplicate tests measured in two directions after 24-48 h incubation at 35°C, *: Zone diameter±SD at 1.25 mg per disc, **: Gentamicin and Nystatin were used as reference drugs for bacteria and yeasts respectively. For the same line, values affected by the same superscripts letter (a-h) are not significantly different (test of student-Waller-Duncan at $p>0.05$). 1.25 mg of extract per disc is the minimum charge of disc after testing 5 and 0.625 mg of extract per disc (results have not shown)

Table 3: Minimal Inhibitory Concentration (MIC)/Minimum Bactericidal or Fungicidal concentration (MBC or MFC) of extracts (mg mL^{-1}) of *Coula edulis* stem bark

	Test substances									
Name of the organism	Crude extract	F1	F2	F3	F4	F5	F6	F7	F8	Reference[a]
Bacteria										
Pseudomonas aeruginosa	25/>25	-	1.56/3.25	0.78/1.56	0.78/1.56	1.56/6.25	1.56/6.25	-	-	0.025/0.025
Proteus mirabilis	12.5/25	-	0.78/3.25	0.19/0.39	0.19/0.39	0.19/0.78	0.78/1.56	0.78/3.25	12.5/12.5	0.10/0.10
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	-	0.025/0.025
Shigella flexneri	12.5/25	-	0.39/1.56	0.19/0.78	0.19/0.39	0.78/1.56	0.78/0.78	0.78/3.12	0.78/6.25	0.050/0.050
Salmonella typhi	25/>25	-	1.56/6.25	1.56/3.25	1.56/3.25	0.78/0.78	3.12/12.50	-	-	0.050/0.050
Escherichia coli	-	-	-	-	-	-	-	-	-	0.025/0.025
Staphylococcus aureus	12.5/>25	-	0.39/0.78	0.39/0.39	0.39/1.56	0.39/0.78	0.78/3.25	0.79/1.56	-	0.050/0.050
Enterococcus faecalis	25/>25	-	0.78/0.78	0.39/0.39	0.39/0.78	0.39/1.56	0.78/1..56	-	-	0.0125/0.0125
Yeasts										
Candida albicans ATCC9002	1.56/3.12	-	-	-	6.25/6.25	1.56/3.12	1.56/3.12	3.12/12.50	-	0.00156/0.00156
Candida albicans ATCC 1663.86	1.56/3.12	-	12.5/12.5	0.78/1.56	0.39/0.78	0.39/0.39	0.39/0.78	0.39/0.78	3.12/6.25	0.00156/0.00156
Candida albicans	1.56/1.56	-	-	-	-	3.12/3.12	3.12/12.5	3.12/12.5	12.50/12.5	0.00156/0.00156
Candida parapsilosis	3.12/3.12	-	-	6.25/12.5	3.12/6.25	1.56/3.12	0.78/3.12	6.25/6.25	-	0.0125/0.0125
Candida tropicalis	3.12/3.12	-	-	-	-	3.12/6.25	6.25/12.50	-	-	0.00625/0.00625
Candida krusei	3.12/6.25	-	-	-	-	3.12/3.12	6.25/12.50	-	-	0.00312/0.00312
Candida glabbrata	1.56/1.56	-	-	-	-	0.78/0.78	1.56/1.56	-	-	0.0125/0.0125
Candida lusitaniae	1.56/1.56	-	-	-	-	0.78/1.56	0.39/1.56	-	-	0.00156/0.00156

- : Absence of inhibition at concentrations up to 25 mg mL^{-1} for crude extract and 12.5 mg mL^{-1} for column fractions, [a]: Gentamicin and Nystatin were used as reference drugs for bacteria and yeasts respectively

tested showed sensitivity for at least two extracts with inhibition zones ranging from 7 to 21 mm. *Staphylococcus aureus*, *Proteus mirabilis* and *Shigella flexneri* were in general, found to be more sensitive among the bacteria tested while *Candida albicans* ATCC 9002 and *Candida parapsilosis* showed the best susceptibility among the yeasts tested. The fractionation increased the antibacterial and antifungal activities of the crude extract in fractions F2, F3, F4, F5 and F6. However, these activities were low in fractions F7 and F8. No activity was noticed in fraction F1 for all the micro organisms tested.

The bacterial and fungal growth inhibitions by the crude stem bark extract and column fractions indicated by the MIC values are summarized in Table 3. In general, the results obtained confirm the considerations retained in Table 2. *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Shigella flexneri*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida* species were all inhibited by crude extract and fractions F5 and F6 with the MIC/MBC or MFC values ranging from 0.19 to 25/0.39 to >25 mg mL^{-1}. *Klebsiella pneumoniae* and *Escherichia coli* were not inhibited at concentrations up to 25 mg mL^{-1} for crude extract and 12.5 mg mL^{-1} for column fractions.

DISCUSSION

Differences were noticed between the crude extract and its fractions as far as the antibacterial and antifungal activities are concerned. This can be linked to the differences in chemical composition of these substances. The crude extract was more active on yeast while the fractions were more active on bacteria, showing that fractionation increased the antibacterial activity and in some cases decreased the activity against yeast. These results suggest possible synergetic effects between some of the extract constituents for antifungal activity. The antifungal activity is more concentrated in fractions F5 and F6, indicating that the antifungal active principle may belong to anthocyanins group. On the other hand, fractionation may have increased the concentration and the activity of antibacterial principles in the fractions. This activity is found in almost all the fractions but particularly in fractions F2 to F6. These fractions are characterised by the presence of phenols, flavonoids and in some cases tannins. These groups of compounds are known to possess antibacterial activities (Rojas *et al*., 1992; Scalbert, 1991) and they may act by complexing with extracellular and soluble proteins as well as cell wall of micro-organisms (Cowan, 1999). In addition, tannins also complex with polysaccharides (Ya *et al*., 1988). Comparable results were obtained by Ogunleye *et al*. (2003) while working on *Ximenia americana*. It is also important to mention the presence of anthraquinones and anthocyanins in some of the fractions since Ali *et al*. (2000), Mohamed (2003) and Lenta *et al*. (2007) reported the antibacterial and antifungal activities of some individual anthraquinones and anthocyanins.

The results also indicated that crude extract and its column fractions are less active compared to reference drugs. This may be due to the low concentration of active compound(s) in these extracts suggesting that purification of bioactive compound(s) from the more active fractions are needed.

The fact that the fractions F5 and F6 showed relatively good antifungal activity against the eight clinical isolates of *Candida* species is interesting because there are currently only a few antifungal agents e.g., Amphotericin B, fluconazole, itraconazole that are effective on candidoses particularly those caused by *C. albicans*. The treatment of candidoses often requires a combination of these agents. Resistance and significant adverse effects including gastrointestinal disturbances, nephrotoxicity and arachnoiditis have been observed following treatment with the above antifungal drugs, while the success rate has been rather limited (Rahalison *et al*., 1991; Carrillo-Munoõz *et al*., 2006).

From this study, we can conclude that the crude stem bark extract of *C. edulis* possesses antibacterial and antifungal properties. It is interesting to notice that the fractionation enhanced the antibacterial and antifungal activities and then can be used at the local level to produce phytomedecine

that can be used at affordable prise by the populations to cure stomachache, skin diseases and urinary tract problems in man. Purification of bioactive compound(s) from the more active fractions is underway and further investigations may improve our understanding of possible antimicrobial and antifungal activities.

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REFERENCES

Akinyemi, K.O., O. Oladapo, C.E. Okwara, C. Ibe and K.A. Fasure, 2005. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant-*Staphylococcus aureus* activity. Complement Alt. Med., 5: 5-8.

Ali, A.M., N.H. Ismail, M.M. Mackeen, L.S. Yazan, S.M. Mohamed, A.S.H. HO and N.H. Lajis, 2000. Antiviral, cytotoxic and antimicrobial activities of anthraquinones isolated from the roots of *Morinda elliptica*. Int. J. Pharm., 4 (38): 298-301.

Bruneton, J., 1999. Pharmacognosie, Phytochimie, Plantes Medicinales. 3rd Edn. Tec et Doc. Lavoisier Paris, pp: 1120.

Carrillo-Munoõz, A.J., G. Giusianõo, P.A. Ezkuõrra and G. Quinõdos, 2006. Antifungal agents: Mode of action in Yeast cells. Rev. Esp. Quimioterap, 2 (16): 130-139.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12 (4): 564-582.

Davidson, A., 1999. Oxford Campanion to Food. Gabon Nut, pp: 328.

Gulluce, M., M. Sokmen, D. Dafera, R.G. Aga and F. Ozkan, 2003. *In vitro* antibacterial, antifungal and antioxidant activities of herbal parts and Callus cultures of *Satureja hortensis* L. J. Agric. Food Chem., 51: 3958-3965.

Iwu, M.M., 1993. Handbook of African Medicinal Plants. CRC Press. Inc., Florida, London, Tokyo, pp: 435.

Kishore, N., J.P.N. Chansouria and N.K. Dubey, 1996. Antidermatophytic action of the essential oil of *Chenopodium ambrosioides* and an ointment prepared from it. Phytother. Res., 10: 453-455.

Lenta, B.N., B. Weniger, C. Antheaume, D.T. Noungoue, S. Ngouela, C.N.J. Assob, V.C. Senecheau, A.P. Fokou, P.K. Devkota, E. Tsamo and N. Sewald, 2007. Anthraquinones from the stem bark of *Stereospermum zenkeri* with antimicrobial activity. Phytochemistry, 11 (68): 1595-1599.

Mohamed, S.M., 2003. Chemical and biological evaluation of sweet pea *Lathyrus odoratus* flowers. Bull. Nat. Res. Centre, Cairo, Egypt, 5: 577-590.

Nascimento, G.G.F., J. Locatelli, P.C. Freitas and G.L. Silva, 2000. Antibacterial activity of plants extracts and phytochemical on antibiotic-resistant bacteria. Braz. J. Microbiol., 31: 247-256.

National Committee for Clinical Laboratory Standards (NCCLS), 1993. Methods for Dilution. Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 3rd Edn. Approved Standard M7-A3, Villonova, PA.

Ogunleye, D.S., O. Onabanjo and S.F. Ibitoye, 2003. Studies of antimicrobial and chemical constituents of *Ximenia americana*. Trop. J. Pharm. Res., 2 (2): 239-241.

Rahalison, L., M. Hamburgern and K. Hostettmann, 1991. A Bioautographic agar overlay method for the detection of antifungal compounds from Higher plants. Phytochem. Anal., 2: 199-203.

Rojas, A., L. Hernandez, R. Pereda-Miranda and R. Mata, 1992. Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. J. Ethnopharmacol., 35: 275-283.

Scalbert, A., 1991. Antimicrobial properties of tannins. Phytochemistry, 30 (12): 3875-3883.

Silva, G.L., Lee IK-Soo and D.A. Kinghorn, 1998. Special Problems with the Extraction of Plants. From: Methods in Biotechnology, Natural Products Isolation. Humana Press Inc. Totowa N. J., 4: 343-363.

Soberon, J.R., M.A. Sgariglia, D.A. Sampietro, E.N. Quiroga and M.A. Vattuone, 2007. Antibacterial activity of plant extracts from Northwestern Argentina. J. Applied Microbiol., 102: 1450-1461.

Waldyogel, F.A., 1995. *Staphylococcus aureus* (Including Toxic Shock Syndrome). In: Douglas and Benett's Principles and Practice of Infectious Disease, Mandell, G.L., J.E. Benett, R. Dolin and D.S. Mandell (Eds.). Curchill Livingstone, New York, pp: 1754-1777.

Ya, C., S.H. Gaffney, T.H. Lilley and E. Haslam, 1988. Carbohydrate Polyphenol Complexation. In: Chemistry and Significance of Condensed Tannins, Hemingway, R.W. and J.J. Karchesy (Eds.). Plenum Press, New York, pp: 553.