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Carotenoids content and antibacterial activity from galls of *guiera senegalensis* j.f. Gmel (combretaceae).

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Abstract

Guiera senegalensis is a well known medicinal plant which is used as a drug in Burkina Faso. The purpose of the present study was to investigate the carotenoids content and antibacterial activity from galls of Guiera senegalensis. The hydroacetonic extract (HAE) exhibited the highest of β-carotene (4.67±0.25 mg/g) and lycopene (0.99±0.0735 mg/g) content. The extracts and fractions of galls exhibited antimicrobial activity against some gram positive and gram negative bacteria. We observed an inhibitory activity of the extracts against Bacillus cereus ATCC 13061 and Staphylococus aureus ATCC 6538 which showed a resistance to the penicillin and ampicillin. The ratio of the MBC and the MIC showed that the hydroacetonic extract is bactericidal against all the bacterial strains used except for Proteus mirabilis ATCC 35659. The aqueous decoction extract is bactericidal against all the bacterial species tested. The present study thus suggested that galls from G senegalensis may be used as a new potential source of natural nutraceutical components.

Keywords: *Guiera senegalensis*, Galls, Antibacterial activity, carotenoid content

Introduction

Plants materials are a rich source of phytochemicals such as carotenoids, phenolic compounds, vitamins, anthocyanins which scavenge free radicals. Carotenoids are fat soluble natural pigments, which are synthesized by plants and are responsible for the bright colors of various fruits and vegetables [1]. Carotenoids are reported to be of additional importance either as antioxidants, to reduce the infectious diseases by enhancing immune response [2]. Beta-carotene is the major dietary precursor of vitamin A. Vitamin A deficiency (VAD) is a major public health problem in the developing world. Beta-carotene supplementation of the diet in areas at risk of VAD decreases morbidity and mortality related to several pathological conditions [3]. Lycopene does not have provitamin A activity. Epidemiological studies revealed that the consumption of lycopene-rich foods is inversely associated with the risk of atherosclerosis, cardiovascular disease, prostate cancer, cognitive impairment [4]. The health benefits of lycopene are attributed to its ability to protect cells against oxidative damage [5]. In recent years, multiples resistances in human pathogenic microorganisms have developed. The screening of plant products for antimicrobial activity is necessary to research the new anti-infective agents.

The *Guiera senegalensis* (Combretaceae) have been used in Burkina Faso as antioxidant and antimicrobial agent since ancient times. Clinically, GS is used to treat fatigue, depression, anemia, nervous system disorders and bacterial diseases [6]. The aim of this work was to assess the carotenoid content and antibacterial activities from galls of *Guiera senegalensis* J.F.Gmel (Combretaceae). The antibacterial activity was tested against some Gram-positive and Gram-negative bacteria.

Material and Methods

Chemical and reagents

Mueller Hinton agar and solvent used were supplied by Fluka Chemie, Buchs (Switzerland). All other reagents were of analytical and HPLC grades.

Microorganisms

Five serotype strains of bacteria used in this study are: *Escherichia coli* ATCC 25922; *Salmonella typhimurium* ATCC 13311; *Staphylococus aureus* ATCC 6538, *Bacillus cereus* 13061, *Proteus mirabilis* ATCC 35659.

Sample preparation

The plant material is constituted of galls of *Guiera senegalensis.* The galls were dried and ground to powder. The obtained powder was extracted with acetone 80% via maceration (hydroacetonic extract) and distilled water via decoction (aqueous decoction extract). The hydroacetonic and the aqueous decoction extracts obtained were respectively dissolved in distilled water and successively extracted with the ethyl acetate, butanol. Each extract was dried to give: ethyl acetate fraction (EAF), butanol fraction (BF) and final water fraction (WF). The obtained extracts were stored in a refrigerator at +4°C until use.

β-Carotene and lycopene content determination

β-Carotene and lycopene were determined according to the method of [7]. The dried extract or fraction (100 mg) was vigorously shaken with 10 mL of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm. Content of β-carotene and lycopene were calculated according to the following equations:

lycopene (mg/ 100 ml) = -0.0458 A663 + 0.372 A505 - 0.0806 A453

β-carotene (mg/100 ml) = 0.216 A663 - 0.304 A505 + 0.452 A453.

The assays were carried out in triplicate; the results were mean values ± standard deviations and expressed as mg of β-Carotene /g and mg of lycopene/g of extract or fraction.

Antibacterial study

Before testing, pure cultures were realized with all the strains in Mueller Hinton Agar and Tryptic Soy Broth. The inocula were prepared by adjusting the turbidity of the suspension to match the 0.5 M_C Farland standards.

Antibacterial tests

The agar diffusion method was used to evaluate the antibacterial activity of extracts using 2ml of the inocula [8]. Mueller Hinton Agar sterilized in flask with autoclave (Prestige Medical 2100 classic, England) and cooled to 45-46°C were distributed to sterile Petri dishes (preciser in diameter). The filter paper discs were individually impregnated with 10µL of the extract and then placed onto the agar plates which had previously inoculated with the strain bacterial. The plates were incubated at 37°C for 24h (Memmert, Schwabach). The diameter of the inhibition zones were measured in millimeters. Negative controls were prepared using discs impregnated with 10% DMSO and virgin discs (blank control).

Minimum Inhibitory Concentrations (MIC) of the extracts from galls of *Guiera senegalensis* was determined using the agar-well diffusion method [9]. All the extract were diluted in Dimethyl Sulfoxide (DMSO) 25% to obtain series of concentrations of 150, 75, 37.5, 18.75, 9.38, 4.69 and 2.34 mg. mL^{-1}

The MIC was taken as lowest concentration of extract at which no visible growth was observed after 24 h incubation at 37°C.

Sterile Petri dishes (d=10cm, Bibby Sterilin, UK) were prepared with a base layer of Müller-Hinton agar (Difco). Bacteria at density of 106-108 cfu were inoculated on solid agar. Holes (6mm) were made in the agar with a sterile cork borer and filled with 50 µL of different dilutions of the extracts. Petri dishes were incubated at 37°C for 24h. The diameters of the circular inhibition obtained were measured. Commercial antibiotic discs of Gentamicin and Amoxicillin were used as positive controls. DMSO 25% was used as negative control.

The MBC determination was carried out by transferring to the fresh MH broth aliquots of bacterial suspensions from the test tubes containing extract concentrations equal or higher (up to 1000µg.mL^{-1}) than the MIC. Each extract was tested in triplicate; the experiment was performed four times. The antibacterial activities of the extracts can be estimate by considering the MBC/MIC ratio.According to [10] the effect of extract is known as bactericidal when the ratio included between 1 to 2 and bacteriostatic when the ratio included between 4 to 16.

Statistical analysis

All measurement were performed in triplicates, results are given as the mean and standard deviations of the obtained values. Data manipulation was performed by means of Microsoft Excel. Data were analyzed using ANOVA test (Fisher). $P < 0.05$ was considered significant

Results and Discussions

β-carotene and lycopene content

Epidemiological evidence indicates that diets rich in β-carotene, the principal pro-vitamin A active member of the carotenoids, afford protection against the development of coronary heart disease and various epithelial cell cancers [11]. Lycopene is a natural antioxidant due to its ability to act as a free radical scavenger [12].

Table 1

Table 1: β-Carotene and Lycopene content from galls of Guiera senegalensis

Carotenoids	β-Carotene (mg/g)	Lycopene (mg/g)
HAE	4.67±0.25[a]	0.99±0.0735[a]
EAF / HAE	01.18±0.10[b]	0.69±0.0041[b]
BF / HAE	2.53±0.039[b]	0.60±0.0388[b]
WF / HAE	0.13±0.0015[b,c]	0.31±0.0038[c]
ADE	0.1±0.0029[c]	0.25±0.0019[c,d]
EAF / ADE	Nd[c]	0.13±0.0014[d]
BF / ADE	0.02±0.0023[c]	0.21±0.0093[d]
WF / ADE	Nd[c]	0.25±0.0249[c,d]

Data are mean ± SEM (n = 3).
HAE: Hydroacetonic extract, EAF/HAE: Ethyl acetate fraction from HAE, BF/HAE: Butanol fraction from HAE, ALF/HAE: Aqueous layer fraction from HAE, ADE: aqueous decoction extract, EAF/ADE: Ethyl acetate fraction from ADE, BF/ADE: Butanol fraction from ADE, WF/ADE: Water fraction from ADE
Nd: No detected
Values showing the same letter are not significantly different ($P > 0.05$) from one other in the same columns.

shows the β-carotene and lycopene content of the extracts and fractions from galls of Guiera senegalensis. The hydroacetonic extract (HAE) contain the highest β-carotene (4mg /g) and lycopene (0.99 mg/g). The β-carotene and lycopene content of HAE is significantly higher when compared to the other extracts content. The extracts from galls of G. Senegalensis are rich in β-carotene and lycopene compared to mushrooms [13, 1 4]. A good and significant correlation coefficient between β-carotene and lycopene content was found (R^2= 0.8071, p=0.0001). The daily consumption of the extracts of *Guiera senegalensis* could thus help to reduce the risk of cardiovascular diseases and prostate cancer.

Antibacterial Activity

The antibacterial effect from galls of *Guiera senegalensis* was tested against two species of Gram-positive bacteria and three species of Gram-negative bacteria.
The mean zones of inhibition against bacteria were ranging from 00±00 to 17.667 ± 0.58mm. Table 2 summarizes the bacterial growth inhibition by extracts and fractions from galls of *G. senegalensis.*
The bacterium growth inhibition produced by *G. senegalensis* extracts varied in relation to the type of extract and to the bacterium strain used.
Hydroacetonic extract (HAE) is particularly significant active against *Staphylococcus aureus* ATCC 6538 (13.50 ± 0.5 mm) than against the others bacterial strain.
The aqueous decoction extract exhibited significant antibacterial effects against *Bacillus cereus* ATCC 13061 (17.667 ± 0.58 mm) than the other bacterial strain and when compared to the hydroacetonic extract and the various fractions.
The ethyl acetate fraction from hydroacetonic extract (EAF/HAE) significantly showed the strongest inhibition activity of the growth of *Salmonella typhimurium* ATCC 13311 (14.67 ± 1.53) except when compared to EAF/ADE, WF/ADE, and BF/HAE. It was also more active against *Escherichia coli* ATCC 25922 (13.667 ±0.58) than the other extracts and fractions.
The water fraction from aqueous extract (WF/ADE) showed good activity against *Staphylococcus aureus ATCC 6538* (14.67 ± 0.58 mm) but no significant difference was observed when compared to those obtained by HAE and BF/ADE.
Proteus mirabilis ATCC 35659 showed a big sensitivity to the combination of the ethyl acetate fraction and butanol fraction from aqueous decoction extract (FA2/FB2) follow of the ethyl acetate (EAF/ADE) and the butanol (BF/ADE) fractions from aqueous decoction extract. The combination of the ethyl acetate fraction and butanol fraction from the hydroacetonic extract did not involve an improvement of their antibacterial activity against the various bacterial species tested.
We also observed an inhibitory activity of the extracts against *Bacillus cereus* 13061 and *Staphylococus aureus* ATCC 6538 which showed a resistance to the penicillin and ampicillin.
The production of β-lactamases is one of potential virulence factors that make the producing strains resistant even to the 3rd generation of cephalosporins [15].
The different extracts and fractions from galls of *G senegalensis* which is active against *Bacillus cereus* and *Salmonella aureus* probably contain some inhibitors of β-lactamases reducing the virulence of this pathogen strain.
The MIC obtained (Table 3) varied from 3.125 to 6.25 and 1.5652 to 6.25 mg/ml, respectively for HAE and ADE against pathogens. The MBC values (Table 3) also ranged from 6.25 to 25 mg/ml for HAE and 1.5625 to 25 mg/ml with the ADE.
The chloroform extract of *Ibicella lutea* showed activity against *E. coli* and *S. aureus* with a MIC of 5 mg/ml [16]. This MIC is highest compared to those of aqueous decoction extract against the same strain suggesting that the galls of *Guira senegalensis* possess a strong bacterial activity against these bacterial species than *Ibicella lutea.*
The aqueous decoction extract (ADE) possess the weak MIC and MBC value (1.5625 mg/ml) against *Bacillus cereus*.
The ratio of the MBC and the MIC of the extracts and fractions varies from 1 to 8. The hydroacetonic extract is bactericidal against all the bacterial strains used except for *Proteus mirabilis* ATCC 35659. The aqueous decoction extract is bactericidal against all the bacterial species tested. This ratio shows that the two types of extracts are more active against the positive bacterial than against negative bacterial except against *Proteus mirabilis* ATCC 35659.

Table 2: Diameter Inhibition Zone of bacteria from galls of Guiera senegalensis.

Extracts/Bacteria species	BC	ST	EC	SA	PM
HAE	10.333^{e} ±0.58	10,000c ± 2	10,333c ± 0.58	13,50a,b ±0.5	9,00b,c ±0.0
EAF / HAE	12,000d ±0.0	14,667 a ±1.53	13,667a ±0.58	12,667b ±1.53	8,00d,e ±0.0
BF / HAE	15,667b ±0.58	12,667a,b±0.58	11,00b,c±1.0	14,667a±0.58	8,00d,e ±0.0
EAF1/ BF1	10,667e ±0.58	8,667c ±0.58	9,00d ± 1.0	9,000d ± 1.0	8,00d,e ± 0.0
WF / HAE	10,667e± 0.58	13,000a,b ±3.0	0,000e ±0.0	12,667b ±0.58	7,667e,f ± 0.58
ADE	17,667a ±0.58	11,000b,c ±1.0	9.33 C ±1.53	12,00b,c ±0.0	7,00f ±0.0
EAF/ ADE	8,000g ± 0.0	13,000a,b ± 0.0	10,00c,d ± 0.0	)0d ±0.0	10,000a± 1.0
FB/ ADE	11,000e ±1.0	11,00b,c ± 0.0	0,000e ± 0.0	13,00a,b ± 0.0	9,667a,b ± 0.58
EAF2/ BF2	9,000f ± 0.0	9,000c ± 1.0	9,00d ± 0.0	10,667c,d ± 2.52	10,000a± 1.0
WF/ADE	14,667c ± 0.58	11,00b,c ± 1.0	12,00b ± 0.0	14,667a ± 0.58	8,667c,d ± 0.58
Ampicillin	R	>53	>53	R	>53
Penicillin	R	>53	>53	R	>53

HAE: Hydroacetonic extract, EAF/HAE: Ethyl acetate fraction from HAE, BF/HAE: Butanol fraction from HAE, WF/HAE: Water fraction from HAE, ADE: aqueous decoction extract, EAF/ADE: Ethyl acetate fraction from ADE, BF/ADE: Butanol fraction from ADE, WF/ADE: Water fraction from ADE EC: Escherichia coli ATCC 25922, ST: Salmonella typhimurium ATCC13311, SA: Staphylococus aureus ATCC 6538, BC: Bacillus cereus ATCC 13061, PM: Proteus mirabilis ATCC 35659 R= Resistant . The values represent the diameters of the zones of inhibition in mm. HAE: Hydroacetonic extract, ADE: Aqueous decoction extract

Table 3:Bactericidal and bacteriostatic activity from galls of *Guiera senegalensis*.

Bacterial strains	HAE					ADE			
	MIC	MBC	MBC/MIC	Effect		MIC	MBC	MBC/MIC	Effect
ST	3.125	6.25	2	Bactericidal		6.25	25	4	Bactericidal
BC	6.25	12.5	2	Bactericidal		1.5625	1.5625	1	Bactericidal
SA	6.25	6.25	1	Bactericidal		3.125	3.125	1	Bactericidal
PM	3.125	25	8	Bacteriostatic		6.25	12.5	2	Bactericidal
EC	3.125	6.25	2	Bactericidal		6.25	12.5	2	Bactericidal

EC: Escherichia coli ATCC 25922, ST: Salmonella typhimurium ATCC13311, SA: Staphylococus aureus ATCC 6538, BC: Bacillus cereus ATCC 13061, PM: Proteus mirabilis ATCC 35659
MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration

The antibacterial activity of extracts could be explained by the presence of rutin (quercetin-3-rutinoside) in the extracts from galls of *G senegalensis* [17] which possess antimicrobial activity against some Gram-positive and Gram-negative bacteria [18]. The quercetin also contained in the galls of *Guiera senegalensis* possesses antibacterial activity against E. coli by inhibition of DNA gyrase [19].

It inhibited bacterial DNA polymerases [20]. The antibacterial activity from galls of *Guiera senegalensis* may be attributed to their high total phenolic contents and strong antioxidant activity [21].

The difference in sensitivity between Gram-positive and Gram-negative bacteria might be attributed to the differences in morphological constitutions between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane containing lipopolysaccharide components, on the other hand, the Gram-positive bacteria only have an outer peptidoglycan layer which is not as an effective permeability barrier as the former [22, 23, 24].

These results are of interest since they have been obtained with crude extracts and are not a pure product and it could be considered to have a good potency level.

Conclusion

In conclusion, the results of this study represent the first evidence that extracts from galls of *G senegalensis* possess effective antibacterial activities. It is therefore suggested that galls from G

senegalensis may be used as an inexpensive and easily accessible source of effective natural β-carotene and lycopene. The results obtained in this work scientifically support the traditionally used of this plant to treat infection-related diseases in Burkina Faso. The galls of *Guiera senegalensis* could be a good source of bioactives components with antimicrobial potency.

Contribution Of Co-Authors

Hilou Adama: Technical assistance and correction of the article draft
Coulibaly Ahmed Yacouba: Technical assistance.
Konaté Kiessoum: Technical assistance. He provide the bacteria strains for the antibacterial activity from galls of Guiera senegalensis*.*
Compaoré Moussa Marie-Yacinthe: Technical assistance
Nacoulma Odile Germaine: Head of LABIOCA and supervisor of this work.

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