Free Radical Scavenging Capacity and Anti-Biofilm Potentiality of Six Wild Edible Fruits from Burkina Faso

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A B S T R A C T

The development of antibiotic resistance raises an urgent need for new treatment strategies. The purpose of this current investigation was to evaluate the free radicals scavenging ability and the anti-biofilm potent of fractions of ethanol extract from six tropical edible fruits. The capacity of extract to quench free radicals was measured spectrophotometrically by using four antioxidant models. Furthermore, the ability of fruit extract to inhibit *Pseudomonas aeruginosa* biofilm formation was examined by using the crystal violet assay. Ethyl acetate and methanol fractions of *Parkia biglobosa*, *Zizipus mauritiana, Saba senegalensis* and *Adansonia digitata* showed strong antioxidant activities through their DPPH, ATBS and nitric oxide radicals scavenge abilities as well as their inhibitory potent of deoxyribose degradation. Moreover, fractions of fruit extracts inhibited significantly the *P. aeruginosa* biofilm formation (up to 72% inhibition) without affect the bacterial growth. Chloroform fraction of *Z. mauritiana* showed the highest anti-biofilm activity (72.11 ± 0.20% inhibition). In general, chloroform and methanol fractions of fruit extract exhibited more anti-biofilm activity than hexane and ethyl acetate fractions. Tropical edible fruits are potent sources of effective antimicrobial agents. Extensive phytochemical investigation is necessary to isolate the effective antimicrobial drugs for fighting multi-resistant pathogens.

Introduction

The development of resistance to antibiotics has become a significant public health problem worldwide as it increases mortality and morbidity due to the reduction of the effectiveness of treatments (Romero *et al.,* 2016). This antibiotic resistance is a consequence of the adaption of infectious pathogens to the types of antimicrobial drugs.

Bacteria use different mechanisms to challenge antimicrobial agents. The formation of biofilms is one of many bacteria defense mechanisms that are responsible for the development of chronic diseases (Nikoli *et al.,* 2014). Biofilm formation shows extreme resistance to the antimicrobial drugs and to the human's defense system (Nogueira *et al.,* 2017). A biofilm is a sessile form of bacterial existence, in which bacteria are covered by a

matrix containing proteins, nucleic acids and polysaccharides (Packiavathy *et al.,* 2012). Biofilm formation confers to bacteria an extreme resistance resulting in the decrease of the antimicrobials penetration into the deep layers of biofilms and the increase of the bacterial enzymes concentration that become more effective for antibiotics inactivation (Slobodníková *et al.,* 2016).

Bacteria covered by biofilm matrix develop up to 1,000 times more antimicrobial resistance than free-floating (Sánchez *et al.,* 2016). This defense mechanism generates serious consequences for antibiotic therapy and severely complicates treatment options (Ahmad *et al.,* 2014). Regarding to the increase in complexity of most microbial resistance to conventional therapy, it is necessary to develop new strategies to combat microbial resistance. The use of preventive strategy to inhibit bacterial common behavior without killing them is an opportunity promoting to attenuate the pathogenicity of bacteria resistant to available antibiotics. Regarding to the central role of biofilm formation in bacteria resistance development, inhibition of biofilm formation by anti-biofilm agents is an innovative approach.

Research on edible plants as potential sources of new and effective antimicrobials with novel modes of action is interesting to researchers owing their traditionally uses in low income countries to treat numerous infectious diseases. Fruits pulp of *Detarium microcarpum*, *Adansonia digitata*, *Saba senegalensis*, *Vittelaria paradoxa*, *Parkia biglobosa* and *Ziziphus mauritiana* are used in West Africa to treat numerous infectious diseases including dermatitis, dysentery, leucoderma, malaria, tuberculosis and meningitis (Nadembega *et al.,* 2011; Rouamba *et al.,* 2018). This current investigation aimed to evaluate the anti-biofilm potent of fractions from six edible fruit pulp extracts. Furthermore, the free radical scavenging

capacity of fractions of fruit extracts was evaluated.

Materials and Methods

Plants material

The fresh fruits of wild plants *D. microcarpum*, *A. digitata*, *S. senegalensis*, *V. paradoxa*, *P. biglobosa* and *Z. mauritiana* were harvest in Gampela zone situated at 25 Km, east of Ouagadougou (Burkina Faso). Fruits of *V. paradoxa*, *P. biglobosa* and *S. senegalensis* were collected during June and August lapse period and fruits of *D. microcarpum*, *Z. mauritiana* and *A. digitata* during January and March lapse period. The botanical identification of fruits was performed by Pr Jeanne Millogo of the laboratory of vegetal biology and ecology from the University Ouaga 1 Pr Joseph KI-Zerbo (Ouagadougou, Burkina Faso). Avoucher herbal were deposited at department herbarium and the following identifications codes were saved: *D. microcapum* CI: 15928; *A. digitata* CI: 15929; *Z. mauritiana* CI: 15930; *P. biglobosa* CI: 15931; *V. paradoxa* CI: 15932; *S. senegalensis* CI: 15933.

Bacterial growth

Pseudomonas aeruginosa PAO1, procured from the Laboratoire de Biotechnologie Végétale (Université Libre de Bruxelles, Gosselies, Belgium) was used to determine the anti-biofilm action of samples. *P. aeruginosa* PAO1 was cultured routinely in Luria-Bertani (LB) broth (37 °C, agitation 175 rpm).

Chemicals

Chemicals were from analytical grade. Silica gel® 60 F254, 60 Å was purchased by Merck, Germany. Trolox, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, quercetin, gallic acid, dimethyl sulfoxide (DMSO), deoxyribose, iron sulfate, sodium

nitroprusside, Griess reagent for nitrite and Luria Bertani (LB) agar were purchased by Sigma–Aldrich (St. Louis, USA). Crystal violet, sodium phosphate dibasic, sodium phosphate monobasic, thiobarbituric acid, Trichloroacetic acid, potassium persulfate, 2,20-azinobis (3-ethylbenzoline-6-sulphonate) (ABTS), acetic acid, salicylic acid, ethanol, methanol, hexane, chloroform and ethyl acetate were purchased by Prolabo (Paris, France).

Extraction and fractionation

The pulp powder (25 g) of each fruit was macerated in 250 mL of ethanol (24 h, 25°C, continuous stirring). Extract was filtrated with a micro pore filter paper and concentrated to dryness in a vacuum evaporator. The ethanol extract of each fruit (1g) was adsorbed in 10 g of silica gel (Silicagel® 60 F254, 60 Å) and fractioned with hexane, chloroform, ethyl acetate and methanol. Each fraction was evaporated to dryness and solubilized in adequate solvent for the biochemical investigations.

Free radicals scavenging assay

DPPH radical scavenging assay

The capacity of each fruit fraction to quench DPPH radical was assessed spectro photometrically at 517 nm as described by Compaoré *et al.,* (2016). Quercetin was used to plot a standard curve ($y = -27.94x + 8.15$; $r2 > 0.99$; $P < 0.000\ 1$) and data were expressed in mg equivalent of quercetin per 10 gram (mg QE/10g) of extract.

Nitric oxide radical scavenging assay

The ability of sample to scavenge nitric oxide radical was evaluated at 546 nm as described previously (Parul *et al.,* 2012). Ascorbic acid was used to generate a calibration curve ($y = -$ $0.022\text{ln}x\ 0.224$; $r2 > 0.99$; $P < 0.000\ 1$) and data were expressed in mg equivalent of ascorbic acid per 10 gram (mg AAE/10g) of extract

Deoxyribose degradation inhibition

The sample capacity to inhibit the deoxyribose degradation was measured at 532 nm as described by Perjesi and Rozmer (Perjési*et* Rozmer 2011). Gallic acid was used to generate a standard curve ($y = 0.21x + 37.57$; $r2 > 0.99$; $P < 0.000\ 1$) and data were expressed in mg equivalent of gallic acid per gram (mg AGE/g) of extract.

ABTS radical quenching assay

The potentiality of sample to quench the ABTS radical cation was assessed spectro photometrically at 734 nm following the standard method described by Compaoré *et al.,* (2016). Trolox was used to plot a calibration curve ($y = -72.38x + 54.57$, $r2 = 0.99$; $P < 0.000\ 1$) and data were expressed as mmol trolox equivalent per gram (mmol TE/g) of extract.

Anti-biofilm assay

The ability of sample to inhibit the *P. aeruginosa* biofilm formation was performed by using the crystal violet assay as described previously (Mutalib *et al.,* 2015). Briefly, 20 µL of each fruit fraction were incubated with 10 µL of fresh bacterial suspension in sterile 96 flat wells microtiter plates containing 170 µL of LB broth per well. Following incubation at 37 °C for 24 h, the bacterial turbidity was measured at 600 nm to verify any bactericidal action of fruits extracts. For the biofilm assay, content of each well was gently removed and well was washed with 200 µL of sterile distilled water to remove non-adherent bacteria. Biofilms formed by adherent cells in plate were fixed with methanol for 15 min and

stained with 0.1% crystal violet for 30 minutes at room temperature. Excess stain was removed and 300 µL of acetic acid were added. Optical densities of stained adherent bacteria were measured at 630 nm using an ELISA microplate reader (BioTek Instruments, USA). Salicylic acid was used as standard and anti-biofilm activity of sample was expressed in percentage of biofilm inhibition.

Data analysis

All experiments were performed in triplicate (n = 3) and data expressed as mean values ± standard deviation.

Microsoft Excel 2013 was used to generate standard curves and to calculate means and standard deviations. One-way ANOVA followed by Newman Keuls post-test of GraphPad software (GraphPad Software Inc, San Diego, CA, USA) was used to performed statistical analysis. *P value < 0.05* was considered as being significant.

Results and Discussion

Free radicals scavenging activities

The potentiality of the plant extracts to quench the free radicals was evaluated by using four antioxidant methods and the data were shown in the table 1. DPPH radical scavenging activities were increasing from 43.68 ± 1.02 to 67.51 ± 0.39 mgQE/10g. The ethyl acetate fraction of *P. biglobosa* and *Z. mauritiana*, and the methanol fraction of *Z. mauritiana* showed the best DPPH radical scavenging activity. However, methanol fraction of *S. senegalensis* showed the best nitric oxide radical scavenging activity (4084.16 ± 72.86 mgAAE/10g) followed by methanol fraction of *Z. mauritiana* (3105.00 ± 92.58 mgAAE/10g). Remarkably, in each plant extract, methanol fractions exhibited more

nitric oxide radical quenching activity than the other fractions. Regarding to the inhibition of deoxyribose degradation model, the hexane fraction of *P. biglobosa*, the methanol fraction of *S. senegalensis* and the ethyl acetate fraction of *Z. mauritiana* showed the best antioxidant activity while the best ABTS radical quenching activity was observed with the methanol fraction of *A. digitata* (52.31 ± 1.71 mmolTE/g) followed by the methanol fraction of *Z. mauritiana* (43.22 ± 0.85 mmol TE/g).

In general, the methanol and ethyl acetate fractions exhibited the best antioxidant activity. These finding suggested that the antioxidant phytomolecules of the plant extracts were more extractible by polar solvents (methanol and ethyl acetate) than non-polar solvents (chloroform and hexane).

Anti-biofilm activity

The anti-biofilm effect of fractions of fruit pulp extracts was showed in the table 2. According to this data, extracts, salicylic acid and vehicle (DMSO 1 %) showed the similar bacterial turbidity (*P > 0.05*) suggesting that plant extracts and salicylic acid didn't inhibit the bacterial growth. Furthermore, fractions of fruit extracts and salicylic acid reduced significantly the bacterial biofilm formation. The percentage of inhibition of biofilm formation increased from 16.23 ± 0.60 % to 72.11 ± 0.20 %. The chloroform fraction of *Z. mauritiana* showed the highest anti-biofilm activity. Remarkably, chloroform and methanol fractions exhibited more anti-biofilm activity than ethyl acetate and hexane fractions. All these finding suggested that the ability of fruits extract to inhibit the bacterial biofilm formation would not due to the inhibition of *P. aeruginosa* growth but a plausible disruption and/or an inactivation of biofilms formation.

Table.1 Free radicals scavenging activity of fruits pulp fractions

Plants	Fractions	DPPH (mgQE/10g)	NO$^\bullet$-I (mgAAE/10g)	DRD-I (mgGAE/g)	ABTS mmolTE/g
A. digitata	Hex	52.01 ± 0.81^n	1.97 ± 0.14^p	17.45 ± 2.11^r	32.58 ± 1.85^f
	CHCl$_3$	60.91 ± 0.68^h	37.91 ± 3.72^i	82.78 ± 1.24^n	18.38 ± 1.65^h
	EtAc	63.74 ± 0.35^d	19.70 ± 3.91^j	196.34 ± 0.94^b	$35.35 \pm 0.83^{d.c.e}$
	MeOH	63.18 ± 1.28^e	113.95 ± 2.46^g	152.00 ± 1.10^h	52.31 ± 1.71^a
D. microcarpum	Hex	43.69 ± 3.88^p	Non active	134.41 ± 1.09^j	$42.92 \pm 0.05^{b.c}$
	CHCl$_3$	43.68 ± 1.02^p	$13.81 \pm 0.54^{k.l}$	158.83 ± 9.04^e	17.72 ± 2.79^h
	EtAc	62.77 ± 1.33^f	Non active	35.83 ± 0.98^p	Non active
	MeOH	66.62 ± 1.18^b	110.01 ± 3.39^h	170.00 ± 2.42^d	$36.07 \pm 0.97^{d.c}$
P. biglobosa	Hex	54.79 ± 0.56^k	2.69 ± 0.12^o	212.02 ± 8.70^a	13.80 ± 2.53^j
	CHCl$_3$	50.23 ± 0.54^o	280.02 ± 7.11^e	5.62 ± 0.98^s	$16.48 \pm 1.07^{h.i}$
	EtAc	67.51 ± 0.39^a	Non active	74.40 ± 1.53^o	6.33 ± 0.99^l
	MeOH	63.72 ± 1.31^d	510.27 ± 7.57^d	146.65 ± 0.92^h	37.60 ± 1.36^d
S. senegalensis	Hex	55.38 ± 0.68^j	0.96 ± 0.04^r	137.85 ± 0.74^i	$41.62 \pm 2.63^{b.c}$
	CHCl$_3$	52.37 ± 0.23^m	1.15 ± 0.06^q	Non active	34.23 ± 0.81^e
	EtAc	65.92 ± 1.71^c	118.69 ± 4.04^f	153.16 ± 0.89^g	29.14 ± 1.68^g
	MeOH	59.42 ± 6.59^i	4084.16 ± 72.86^a	210.12 ± 0.76^a	29.43 ± 1.63^g
V. paradoxa	Hex	62.22 ± 1.04^g	Non active	88.51 ± 2.60^m	9.80 ± 1.13^k
	CHCl$_3$	52.49 ± 1.34^m	Non active	116.44 ± 0.98^l	$36.06 \pm 2.61^{d.c}$
	EtAc	66.43 ± 0.53^b	14.34 ± 0.53^k	169.59 ± 0.40^d	39.66 ± 2.43^c
	MeOH	65.72 ± 2.75^c	873.93 ± 10.29^c	74.31 ± 7.85^o	$36.10 \pm 2.11^{d.c}$
Z. mauritiana	Hex	53.19 ± 5.40^l	$7.27 \pm 0.24^{m.n}$	155.45 ± 2.11^f	13.61 ± 0.15^j
	CHCl$_3$	59.25 ± 0.30^i	20.54 ± 1.27^j	131.73 ± 1.81^k	29.41 ± 1.48^g
	EtAc	67.36 ± 0.53^a	7.78 ± 0.38^m	209.73 ± 0.60^a	37.76 ± 1.27^d
	MeOH	67.44 ± 0.39^a	3105.00 ± 92.58^b	192.91 ± 0.47^c	43.22 ± 0.85^b

Values are expressed as mean values ± SD (n = 6 independent experiments). Values in each column with different superscripted letters were statistically different (P < 0.05) as determined by using One-Way ANOVA followed by the Newman-Keuls post-test. Hex: Hexane; CHCl$_3$: Chloroform; EtAc: Ethyl acetate; MeOH: Methanol; NO$^\bullet$-I: Nitric oxide radical inhibition; DRD-I: Deoxyribose degradation inhibition; mgQE/g: milligram quercetin equivalent/gram; mgAAE/g: Milligram ascorbic acid equivalent/gram; mgGAE/g: milligram gallic acid equivalent/gram, mmolTE/g: millimoltrolox equivalent/gram.

Table.2 Anti-biofilm activity of fruits fractions

Samples	Fractions	Turbidity (DO 600 nm) *P > 0.05*	Anti-biofilm activity (%)
Vehicle (DMSO 1 %)		0.84 ± 0.02	
A. digitata	Hexane	0.83 ± 0.01	16.23 ± 0.61[o]
	Chloroform	0.88 ± 0.07	31.67 ± 2.00[l]
	Ethyl acetate	0.76 ± 0.02	53.33 ± 0.58[e,f]
	Methanol	0.84 ± 0.01	31.20 ± 1.63[l]
D. microcarpum	Hexane	0.90 ± 0.11	31.63 ± 0.17[l]
	Chloroform	0.89 ± 0.02	61.50± 1.38[b]
	Ethyl acetate	0.88 ± 0.03	30.91 ± 1.0[m,n]
	Methanol	0.80 ± 0.07	59.95 ± 0.54[c]
P. biglobosa	Hexane	0.83 ± 0.07	49.92 ± 1.39[h]
	Chloroform	0.94 ± 0.27	54.98 ± 0.46[d]
	Ethyl acetate	0.89 ± 0.02	37.95 ± 0.52[k]
	Methanol	0.90 ± 0.06	55.29± 0.29[d]
S. senegalensis	Hexane	0.85± 0.01	62.73 ± 1.89[b]
	Chloroform	0.91 ± 0.05	52.20 ± 1.41[f]
	Ethyl acetate	0.85 ± 0.02	42.97 ± 0.23[j]
	Methanol	0.85 ± 0.04	52.53 ± 0.43[f]
V. paradoxa	Hexane	0.86 ± 0.02	51.79 ± 1.13[f,g]
	Chloroform	0.84 ± 0.06	54.26 ± 1.60[e]
	Ethyl acetate	0.87 ± 0.02	48.64 ± 1.10[h]
	Methanol	0.86 ± 0.03	47.04 ± 1.37[i]
Z. mauritiana	Hexane	0.83 ± 0.07	56.25 ± 1.52[d]
	Chloroform	0.80 ± 0.04	72.11 ± 0.20[a]
	Ethyl acetate	0.92 ± 0.02	51.83 ± 2.11[f]
	Methanol	0.78 ± 0.02	60.82 ± 0.54[b,c]
Standard salicylic acid		0.83 ± 0.05	41.07 ± 0.26

Values are expressed as mean values ± SD (n = 6 independent experiments). [a-o]Values with different superscripted letters were statistically different (P < 0.05) as determined by using One-Way ANOVA followed by Newman-Keuls post-test.

Currently, edible fruits have emerged as potential sources of bioactive compounds including antioxidant, antimicrobial, anti-inflammatory and anti-cancer compounds (Nile, 2014). More previous investigations on antibacterial plants and their active constituents have been documented but they have focused entirely on planktonic bacteria (Al-sohaibani Murugan, 2012). Studies on properties of plants extract to inhibit bacterial biofilm formation without kill bacteria are

less documented. The current investigation highlighted the use of tropical edible fruits as anti-biofilm potent. Data demonstrated that tropical wild edible fruits are potent sources of antimicrobial agents for combatting the microbial resistance. Chloroform and methanol fractions showed in this study more anti-biofilm activity than hexane and ethyl acetate fractions suggesting that fruits extracts contain both polar and non-polar anti-biofilm compounds. Fractions of fruit extracts by

inhibiting the bacterial biofilm formation could promote new strategies to eradicate infectious diseases caused by antibiotic multi-resistant bacteria. Fruit extracts from these medicinal plants may influence biofilm formation mainly by degrading the extracellular matrix, inhibiting peptidoglycan synthesis, efflux pumps and extracellular polymeric substances synthesis, and/or modulating quorum sensing (Nikoli *et al.,* 2014).Fruit extracts could contain backbone compounds that can modify the surface charge of biofilm matrix polymers and thereby inhibiting bacterial adherence (Abdel-aziz*et* Aeron, 2014). Lectin present in the bacterial membrane is essential for biofilm formation. Previous studies demonstrated that polysaccharides from antimicrobial plants can inhibit lectin-dependent adhesion of pathogens and biofilm formation (Abdel-aziz*et* Aeron, 2014). Fruits pulp of tropical edible fruits was recognized to possess high polysaccharide contents (Cui *et al.,* 2015). Their polysaccharide contents could justify their anti-biofilm activity observed in this study. It has been reported that metal cations such as calcium, magnesium and iron have been implicated in maintaining and in reinforcing biofilm matrix integrity (Abdel-aziz*et* Aeron, 2014).

Fruit extracts exhibited in this study free radical scavenging potent that may due to their mono-electronic transfer ability or their ion metal chelating property. Fruit extracts may chelate or reduce metal cations implicated in maintaining matrix integrity and thereby affect the integrity of biofilm matrix. Moreover, the N-acetyl-D-glucosamine-1-phosphate acetyl transferase is known as an essential peptidoglycan and lipopolysaccharide precursor *in P. aeruginosa* pathogens responsible of the biofilm formation and maturation (Riddhi and Liang, 2011). Fruit extracts may inhibit this enzyme leading to biofilm matrix disruption.

This study highlighted the free radical quenching and the anti-biofilm properties of fractions from tropical edible fruits extracts. According to the interesting anti-biofilm activities of fruits used in this study, they constitute potential sources of effective anti-microbial agents for combating microbial resistance. Further phytochemical investigations will involve the isolation and identification of these anti-biofilm compounds.

Competing interest

The authors declare no competing interests

Authors' contributions

AR, VO and EC conceptualized the study including sample analysis, compiled and entered the data. MC and MK conceived and designed the experiments. AR drafted the manuscript. All authors read and approved the final manuscript.

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