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Research Article

Antibacterial and Anti-Quorum Sensing Activities of Ethanol Extract and its Fractions from Six Wild Fruits of Burkina Faso

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Abstract: The objective is to highlight the useful of tropical wild edible fruits with antibacterial properties. Six wild edible fruits from medicinal plant of Burkina Faso were extracted by ethanol and fractioned by using hexane, chloroform, ethyl acetate and methanol. The samples antibacterial effect was evaluated by using agar diffusion method and their anti-quorum sensing activity was measured spectrophotometrically. The bioactive compounds like flavonoid and polyphenolic compounds were quantified in different fractions from fruits. Interestingly, fruit extracts and fractions inhibited the growth of *Pseudomonas aeruginosa* POA1 tetracycline resistant and *Staphylococcus aureus* aztreonam resistant but the fractions did not improve the diameter of inhibition zone. According to this data, extracts and salicylic acid at 100 µg/mL compared to vehicle didn't affect bacterial growth but exhibited an anti-quorum sensing activity by inhibiting pyocyanin production and elastase activity. The pyocyanin production inhibition was increased from 11.68 to 54.54% and the elastase activity inhibition, from 21.05 to 40.35%. Interestingly, extract of *Adansonia digitata* was more active on the inhibition of pyocyanin production than salicylic acid while extract of *Vitellaria paradoxa* presented the same inhibition of elastase activity like salicylic acid ($p>0.05$). The phenolic compounds contribution to the diameter of inhibition zone of *Pseudomonas aeruginosa*, elastase activity and pyocyanin production inhibition was 0.56, 0.55 and 0.76 ($p<0.05$) respectively. These findings showed that tropical wild edible fruits are a reservoir of antibacterial bioactive compounds and could be exploited to fight microbial resistance.

Keywords: Bioactive compounds, edible fruit, elastase activity, microbial resistance, pyocyanin production, quorum sensing

INTRODUCTION

Antimicrobial resistance is a significant public health threat and a global crisis. According to WHO statistical investigations, significant morbidity and mortality have been associated to infections with antibiotic-resistant organisms (World Health Organization, 2014). In west Africa, the situation is startling due to the existence of a lot of barriers notably the gaps of data in antimicrobial resistance, surveillance networks and the existence of street medication (Percival *et al*., 2015). The antimicrobial resistance rate of *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella* spp. Non-Typhoidal *Salmonella* (NTS), *Salmonella enterica* serotype *Typhi*, *Streptococcus pneumoniae* and *Staphylococcus aureus* was around at 90% according to Bernabe *et al*. (2017) data.

In front of this invoking and complex situation, the researchers promoted the use of traditional medicinal plants as an alternative solution in the low income countries. In tropical regions, wild edible fruits are not only used in the human diet but are also employed in the folklore system of medicine for the treatment of microbial infections. The fruits pulp of *Adansonia digitata* L. (Bonbacaceae), *Detarium microcarpum* Guill. and Perr. (Caesalpiniaceae), *Saba senegalensis* (A. Dc) Pichon var. (Apocynaceae), *Vitellaria paradoxa* C.F. Gaertn. (Sapotaceae), *Ziziphus mauritiana* Lam. (Rhamnaceae) and *Parkia biglobosa* (Jacq.) R. Br. (Anacardiaceae) are used in tropical regions to treat dermatitis, dysentery, tuberculosis, meningitis, jaundice (Nadembega *et al*., 2011; Akah *et al*., 2012; Ajiboyea *et al*., 2014; Sundarambal *et al*., 2015). The previous antimicrobial demonstration of these medicinal plants was mainly focalized on the antibacterial and antifungal abilities of extracts from stem bark, bark, seed and leaves extracts (Akah *et al*., 2012; Igwo-Ezikpe *et al*., 2013). However, in some

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previous studies, the hydro ethanol and aqueous extracts of fruit pulp of *D. microcarpum* were able to inhibit bacteria development (Kini *et al*., 2010), ethanol extract from *P. biglobosa* pods inhibited *S. aureus* and *P. aeruginosa* growth (Igwo-Ezikpe *et al*., 2013), chloroform and methanol fraction from *Z. mauritiana* fruit pulp regulated *S. aureus* growth (Beg *et al*., 2016). The present investigation highlights the useful of tropical wild edible fruits with antimicrobial abilities. It was evaluated the Diameter of Inhibition Zone (DIZ) and the inhibition of *Pseudomonas aeruginosa* virulence factors production that were pyocyanin and elastase. The bioactive compounds like flavonoid and polyphenolic compounds were quantified in different organic fractions and ethanol extract.

MATERIALS AND METHODS

Chemicals and reagents: Folin-Ciocalteu reagent, Aluminum trichloride, Salicylic acid, Dimethyl Sulfoxide (DMSO), Luria Bertani (LB) agar, Elastin Congo Red (ECR) and Sodium carbonate were purchased from Sigma-Aldrich (Germany). Methanol, Ethanol, Chloroform, Ethyl acetate and Hexane were purchased by Prolabo (Paris, France). Silica gel® 60 F_{254}, 60 Å, Tetracycline 0 and Aztreonam were purchased by Merck, Germany. All solvents used were analytical grades.

Bacteria origin: *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus* ATCC 25923 were provided respectively from the Laboratoire de Biotechnologie Végétale (Université Libre de Bruxelles, Gosselies, Belgium) and the Centre National de Recherches Scientifiques et Technologies (Burkina Faso). *P. aeruginosa* PAO1 and *S. aureus* (37°C, agitation 175 rpm) were grown in LB broth.

Samples preparation: Fruits of wild plants *Detarium microcarpum*, *Adansonia digitata*, *Saba senegalensis*, *Vitellaria paradoxa*, *Parkia biglobosa* and *Ziziphus. mauritiana* were collected in Gampela region (25 Km, east of Ouagadougou, Burkina Faso). Fruits of *V. paradoxa*, *P. biglobosa* and *S. senegalensis* were collected during a winter period between June and August and fruits of *D. microcarpum*, *Z. mauritiana* and *A. digitata* were collected between January and March period. Fruits were also botanically identified in the laboratory of vegetal biology and ecology of the University Ouaga 1 Pr Joseph KI-Zerbo and some voucher herbal were deposited at department herbarium (*D. microcapum* CI: 15928, *A. digitata* CI: 15929, *Z. mauritiana* CI: 15930, *P. biglobosa* CI: 15931, *V. paradoxa* CI: 15932, *S. senegalensis* CI:15933). Within 4 h after harvesting, the fruits were washed with sterilized water and air dried after which pulps were scrapped and powdered for extraction.

Pulp powder of each fruit was soaked in ethanol (24 h, 25°C, continuous stirring). Extract was filtrated, concentrated to dryness in a vacuum evaporator. For the fractionation, 1g of each fruit ethanol extract was adsorbed in 10 g of silica gel (Silicagel® 60 F_{254}, 60 Å) and washed three times successively with hexane, chloroform, ethyl acetate and methanol. The fractions, obtained after filtration were evaporated to dryness and solubilized in adequate solvent for the phytochemical and anti-microbial investigations.

Antimicrobial assay:

Agar diffusion method: The sensitivity of different bacterial strains (*S. aureus* and *P. aeruginosa*, with optical density 0.02 nm) to ethanol crud extract and fractions of each fruit was measured by using disc diffusion method (Chingizova *et al*., 2017). Sterilized discs impregnated with each extract fraction (100 µg/disc) were incubated in Petri plates containing Luria Bertani agar spread with the inoculum (10^6 cellules/mL). Negative solvent controls (1% of DMSO) and positive controls antibiotic Tetracycline 0 and Aztreonam (30 µg/disc) were used. After 24 h of incubation at 37°C, the Diameters of Zones of Inhibition (DIZ) from sample action were measured.

Inhibition of pyocyanin production and elastase activity: The anti-quorum sensing activity of ethanol crud extract was assessed by measuring the pyocyanin production and the elastase activity in *P. aeruginosa* PAO1 culture. The pyocyanin quantification was assessed by using the method described by Krishnan *et al*. (2012)**.** Briefly, a single colony of *P. aeruginosa* PAO1 culture was inoculated in 1 mL nutrient broth containing each sample (100 µg/mL) and control tubes without sample were also maintained. After 24 h of incubation at 37°C, each tube was centrifuged at 3000 g for 10 min and pyocyanin was extracted with chloroform in the supernatant. The pyocyanin production was estimated at 520 nm and the inhibition percentages were calculated.

The inhibition of elastase activity of samples in culture supernatants was evaluated by using Elastin Congo Red method (ECR) as described by Adonizio *et al*. (2008). Briefly, culture supernatant mixed with ECR was incubated for 16 h and insoluble ECR was removed by centrifugation (3000 g, 5 min). The elastase activity was estimated at 415 nm and the inhibition percentages of elastase activity were calculated. Salicylic acid was used as reference compound. To assess the effect of samples on bacterial growth, the level of growth of *P. aeruginosa* PAO1 in the pellet was compared to the control (without sample) by measuring the optical density at 600 nm.

Polyphenolic content determination: The total phenolic and flavonoid contents were estimated by using the standard colorimetric method (Compaoré *et al*., 2016). The standard calibration curve (y = 201x - 21, 22; R^2>0.99; p<0.0001) was generated by using

Gallic acid (0-100 µg/mL) and total phenolic content was expressed as mg gallic acid equivalent to 10 g of fruit extract (mg GAE/10 g). Quercetin (0-100 mg/L) was used to plot a standard calibration curve ($y = 39.8x - 3.5$; $R^2 = 0.99$; $p<0.0001$) and total flavonoid content was expressed as mg of quercetin equivalent to 10 g of plant extract (mg QE/10 g). The data were obtained in triplicate test for each sample.

Statistical analysis: Experiments were performed in triplicate (n = 3) and data were presented as mean±S.D. Graph Pad Software (Graph Pad Software Inc., San Diego, CA, USA) was used for statistical analyses. The One-way ANOVA for repeated measures followed by Newman-Keuls post-test was used to verify the statistical difference on phenolic contents between extracts and the impact of extracts on bacterial growth, pyocyanin and elastase production. p value <0.05 was considered as being significant.

RESULTS

Diameters of Zone Inhibition (DIZ): The diameters of inhibition zones of samples were indicated in the Table 1. *P. aeruginosa* was more sensitive than *S. aureus* according to this data. *Z. mauritiana* and *S. senegalensis* extract and fractions would contain the best molecules inhibitor of *P. aeruginosa* and *S. aureus* growth. Probably, these bioactive molecules were extracted by ethanol, methanol, ethyl acetate and hexane. Chloroform fraction was the least active. Remarkably, the fractionation didn't increase the diameter of zone inhibition because all extract presented a better DIZ than fractions (p>0.05). All fractions and extracts of fruits exhibited a weak antimicrobial activity than references active antibiotics. Interestingly, fruit extracts and fractions could inhibit *P. aeruginosa* tetracycline resistant and *S. aureus* aztreonam resistant. These finding suggested that these extracts could be a potential source of new promoted antimicrobial compounds.

Anti-quorum sensitive activities: The anti-quorum effect of ethanol extracts was presented in the Table 2. According to this data, all extracts, salicylic acid and vehicles were showed the similar bacterial turbidity (p>0.05) suggesting that extracts and salicylic acid (100 µg/mL), didn't affect *P. aeruginosa* growth.

Table 1: Inhibition of bacteria growth ability and total polyphenolic content of fractions

		Diameter of inhibition zones (mm)		Total polyphenolic content	
Species	Extract/fractions	*P. aeruginosa* (Gram-)	*S. aureus* (Gram+)	TFC (mgQE/10 g)	TPC (mgGAE/10g)
A. digitata	Hexane fraction	10.00±0.00^{g}	8.50±0.71^{j}	3.85±0.07^{t}	200.38±11.67^{r}
	Chloroform fraction	Non-active	Non-active	34.54±1.86^{m}	2106.96±8.49^{h}
	Ethyl acetate fraction	9.50±0.71^{h}	9.50±0.71^{g}	14.68±0.14^{o}	1612.62±40.89^{j}
	Methanol fraction	10.00±1.41^{g}	Non-active	50.15±0.69^{j}	3683.15±21.11^{d}
	Ethanol extract	11.25±0.50^{d}	10.75±0.35^{e}	104.20±2.76^{f}	7604.10±20.54^{a}
D. microcarpum	Hexane fraction	10.50±0.71^{f}	8.50±0.71^{j}	9.88±3.68^{q}	71.69±5.04^{s}
	Chloroform fraction	Non-active	Non-active	42.63±3.27^{l}	52.71±4.07^{t}
	Ethyl acetate fraction	Non-active	8.50±0.71^{j}	195.22±3.03^{b}	1480.28±15.64^{k}
	Methanol fraction	9.50±0.71^{h}	8.00±0.00^{k}	10.14±0.43^{q}	394.01±12.59^{o}
	Ethanol extract	10.83±0.28^{e}	13.50±0.50^{b}	257.87±1.68^{a}	1998.68±9.25^{i}
P. biglobosa	Hexane fraction	10.00±0.00^{g}	9.50±0.71^{g}	51.40±0.90^{j}	278.32±23.93^{q}
	Chloroform fraction	9.50±0.71^{h}	Non-active	43.83±3.45^{l}	454.25±17.79^{n}
	Ethyl acetate fraction	Non-active	Non-active	36.27±2.13^{m}	2008.78±89.66^{i}
	Methanol fraction	Non-active	Non-active	5.51±0.24^{s}	373.28±13.93^{p}
	Ethanol extract	10.00±0.28^{g}	11.25±0.50^{d}	137.01±1.92^{d}	3114.63±35.75^{e}
S. senegalensis	Hexane fraction	9.50±0.71^{h}	Non-active	67.15±4.35^{h}	390.03±29.30^{o,p}
	Chloroform fraction	9.50±0.71^{h}	8.50±0.71^{j}	25.58±0.20^{n}	2329.90±13.26^{g}
	Ethyl acetate fraction	10.50±0.71^{f}	Non-active	5.82±0.13^{s}	679.21±39.08^{l}
	Methanol fraction	14.50±0.71^{b}	9.00±0.00^{i}	7.18±0.10^{r}	378.56±15.21^{p}
	Ethanol extract	8.16±0.28^{l}	11.66±0.28^{c}	105.73±1.20^{f}	3777.10±24.00^{c}
V. paradoxa	Hexane fraction	Non-active	Non-active	26.64±1.71^{n}	73.40±2.03^{s}
	Chloroform fraction	Non-active	Non-active	63.74±1.75^{i}	439.09±18.20^{n}
	Ethyl acetate fraction	9.00±0.00^{j}	Non-active	94.38±6.87^{g}	1959.19±84.42^{i}
	Methanol fraction	Non-active	9.50±0.71^{g}	7.31±0.24^{r}	413.94±16.68^{o}
	Ethanol extract	9.25±0.76^{i}	10.50±0.50^{f}	192.02±8.58^{c}	2885.62±30.25^{f}
Z. mauritiana	Hexane fraction	9.50±0.71^{h}	10.50±0.71^{f}	46.07±2.14^{k}	196.74±15.43^{r}
	Chloroform fraction	8.50±0.71^{k}	Non-active	10.92±1.06^{q}	625.58±18.75^{m}
	Ethyl acetate fraction	9.00±1.41^{j}	9.00±0.00^{i}	43.99±0.49^{l}	2816.77±77.62^{f}
	Methanol fraction	10.00±0.00^{g}	8.50±0.71^{j}	22.35±1.95^{n}	1648.55±39.08^{j}
	Ethanol extract	11.83±0.28^{c}	9.16±0.28^{h}	123.5±1.41^{e}	5287.64±37.71^{b}
Standards	Tetracycline	Non-active	26.00±1.41^{a}		
antibiotic	Aztreonam	20.50±0.71^{a}	Non-active		

Values are expressed as mean±S.D. (n = 3); Data in each column with different letters differed significantly for p<0.05; TFC (mgQE/10 g): Total flavonoid contents (milligram of quercetin equivalent/10 g); TPC (mgGAE/10 g): Total phenolic contents (milligram of Gallic Acid Equivalent/10 g)

Table 2: Anti-quorum sensing activity of fruits extracts

Sample (100 µg/mL)	Bacterial turbidity p>0.05	Inhibition of pyocyanin production (%)	Inhibition of elastase activity (%)
Vehicle (DMSO)	0.77±0.04	Non-active	Non-active
A. digitata	0.83±0.08	54.54±3.00^{e}	21.05±2.00
D. microcarpum	0.75±0.03	25.97±1.00^{c}	21.05±2.00
P. biglobosa	0.76±0.03	Non-active	Non-active
S. senegalensis	0.77±0.04	10.39±2.00^{d}	33.33±2.00^{c}
V. paradoxa	0.74±0.03	11.68±1.00^{d}	45.61±2.00^{a}
Z. mauritiana	0.77±0.06	24.67±1.00^{c}	40.35±1.00^{b}
Salicylic acid	0.80±0.05	35.04±2.00^{b}	38.60±3.00^{b}

Values are expressed as mean±S.D. (n = 3 independent experiments); Values within each column with different superscripted letters differ significantly (p<0.05) as determined by ANOVA

The pyocyanin production inhibition was increased from 11.68 to 54.54% and the elastase activity inhibition was from 21.05 to 40.35 %. *P. biglobosa* extract wasn't showed any anti-quorum activity. Interestingly, extract of *A. digitata* extracts inhibited more pyocyanin production than salicylic acid while extract from *V. paradoxa* presented the same inhibition of elastase activity than salicylic acid (p>0.05). All these findings suggested that the inhibition of pyocyanin production or elastase activity by the different fruits extracts would not due to the inhibition of bacterial growth but a plausible interference of extract with the quorum sensing system of bacteria thus inhibiting pyocyanin production and elastase activity.

Polyphenolic content evaluation: Fractions and extracts were screened for their total flavonoids and total phenolic contents (Table 1). The flavonoid content was increased form 3.85 mgQE/10 g (*A. digitata* hexane fraction) to 257.87 mgQE/10 g (*D. microcarpum* ethanol extract) and the phenolic content was increased from 52.71 mgGAE/10 g (*D. microcarpum* chloroform fraction) to 7604.10 mgGAE/10 g (Ethanol extract form *A. digitata*). According to the flavonoid content of ethanol extracts, it was found this order: *D. microcarpum*> Z. *mauritiana*>*V. paradoxa*>*A. digitata* (or *S. senegalensis*) >*P. biglobosa*. But according to the phenolic content of ethanol extract it was found the following order: *A. digiata*>*Z. mauritiana*>*S. senegalensis*>*P. biglobosa*>*V. paradoxa*>*D. microcarpum*. The fractionation demonstrated the notable variability of polarity of metabolites in the ethanol extracts.

DISCUSSION

This present investigation demonstrated the importance of the use of wild fruits in low income countries for health care. The fruits use could be a promote strategy to fight the bacteria resistance of human pathogenic microbes according to the inhibition of bacterial growth and the anti-quorum sensing effects of ethanol extracts and its fractions of 6 medicinal plant form Burkina Faso. It is the first time that anti-quorum sensing activities of extracts from *A. digiata*, *Z. mauritiana*, *S. senegalensis*, *P. biglobosa*, *V. paradoxa*, *D. microcarpum* were demonstrated.

The growth inhibition of *S. aureus* and *P. aeruginosa* found in the work confirmed the previous antimicrobial investigations. Indeed, aqueous extract from *D. microcarpum* fruit pulp inhibited *S. aureus* mhh 65.8T growth (Kini *et al*., 2010), ethanol extract of pods from *P. biglobosa* inhibited *S. aureus* and *P. aeruginosa* growth (Igwo-Ezikpe *et al*., 2013), chloroform and methanol fraction of *Z. mauritiana* fruit pulp presented a relative inhibition of *S. aureus* growth (Beg *et al*., 2016). The phytochemical investigation showed high metabolites contents notably flavonoids and phenolic in pulp fruits which was in agreement with previous studies where methanol extract of these 6 fruits pulp showed the highest flavonoids and phenolic contents (Lamien-Meda *et al*., 2008). In this investigation, flavonoids and phenolic contributed significantly to the anti-quorum sensing activity and to the inhibition of bacteria growth of ethanol extracts and its fractions from 6 fruits. So, the phenolic compound contribution to the DIZ of *P. aeruginosa*, elastase activity and pyocyanin production inhibition was 0.56; 0.55; and 0.76 (p<0.05) respectively. The flavonoid contributed significantly to the DIZ of *P. aeruginosa* only (0.63). The contribution of flavonoids and other phenolic compounds to the anti-quorum sensing activity and to the DIZ of bacteria was previously demonstrated. So, quercetin-3-Glycoside, rutin and quercetin found in tropical wild berries were responsible of the inhibition of *S. aureus* and *P. aeruginosa* growth (Radovanovic *et al*., 2013). Moreover, phenol acids such as gallic acid and cafeic acid have been reported to inhibit QS-regulated virulence factors expression in *P. aeruginosa* (Rodrigues *et al*., 2016).

Regarding to the central role of quorum sensing in bacterial virulence, inhibition of bacterial virulence factors production such as pyocyanin and elastase constitute an alternative and effective strategy to attenuate pathogenicity of bacteria resistant to available antibiotics. The effectiveness of fruits pulp extracts and their fractions in inhibition of bacterial growth and virulence factors production, could justify the traditional use of these fruits in West Africa in the treatment of several microbial diseases like dysentery, tuberculosis and meningitis (Sundarambal *et al*., 2015; Akah *et al*., 2012).

CONCLUSION

Wild edible fruits are rich in antibacterial compounds including total phenolic and total flavonoid. These phytochemicals constituents are both bactericidal potent and quorum sensing inhibitors. The pulp of wild edible fruits constitutes a reservoir of potential anti-microbial compounds for fighting microbial resistance. These funding show that wild edible fruits can be exploited for both their nutraceutical and their therapeutic potential.

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