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Short communication

Antimicrobial activity of bark extracts of *Syzygium jambos* (L.) Alston (Myrtaceae)

Corine Djadjo Djipa [a], Michel Delmée [b], Joëlle Quetin-Leclercq [a,*]

[a] *Laboratory of Pharmacognosy, CHAM Unit, Université Catholique de Louvain, UCL 72.30. Av. E. Mounier 72, 1200 Brussels, Belgium*

[b] *Microbiology Unit, Université Catholique de Louvain, UCL 54.90. Av. Hippocrate 54, 1200 Brussels, Belgium*

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Abstract

Syzygium jambos (L.) Alston (Myrtaceae) is a widespread medicinal plant traditionally used in sub-Saharan Africa to treat infectious diseases. Acetone and aqueous extracts from the bark of *S. jambos* were tested for antimicrobial activity in vitro by the agar dilution method in petri dishes. Both extracts showed some activity against the tested micro-organisms. They proved to be particularly effective on *Staphylococcus aureus*, *Yersinia enterocolitica* and coagulase negative staphylococci among which *Staphylococcus hominis*, *Staphylococcus cohnii* and *Staphylococcus warneri*. These properties seem to be related to the high tannin content of *S. jambos* extracts (77 and 83% for the aqueous and acetone extracts, respectively, determined according to the European Pharmacopoeia method) which were generally more active than *Hamamelis virginiana*, *Krameria triandra*, *Alchemilla vulgaris* and *Rubus fruticosus* extracts containing 48, 44, 46 and 28% tannins, respectively. Furthermore, elimination of tannins totally suppressed these antimicrobial activities.

Keywords: *Syzygium jambos*; Antibacterial activity; Tannins; African medicinal plants

1. Introduction

Syzygium jambos (L.) Alston (= *Eugenia jambos*) is widespread in sub-Saharan Africa (Benin, Democratic Republic of Congo and Cameroon) where its bark is traditionally used, as the bark of *Syzygium guineense* with which it is often confused, to treat pernicious attack, amenorrhea, abdominal pain and diarrhoea (Adjanohoun, 1988, 1989). It is also distributed in Reunion Island, Central America (i.e. Guatemala) and Asia (i.e. Malaysia, Nepal) where fruits are eaten (Maskey and Shah, 1982). Beside studies on the fruit volatiles and sugars (Maskey and Shah, 1982; Vernin et al., 1991; Wong and Lai, 1996), the only part of the plant chemico-pharmacologically studied was the leaves. An infusion of *S. jambos* leaves has been tested for antidiabetic activity by a glu-

* Corresponding author. Tel.: +32-2-764-7232.

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cose tolerance test in a randomised, parallel, double-blind clinical trial in nondiabetic subjects, but no significant effect was detected (Teixeira et al., 1990). Aqueous, methanol and ethyl acetate extracts of *S. jambos* leaves from Guatemala have been shown to possess anti-inflammatory activity in an adjuvant-carrageenan-induced inflammation model in rats (Slowing et al., 1994a). From these active extracts, several flavonoids were isolated among which myricetin and quercetin 3-*O*-β-D-xylopyranosyl(1-2)α-L-rhamnopyranosides (Slowing et al., 1994b, 1996). These two flavonoids proved to be more effective than phenylbutazone and indomethacine used as controls (Slowing et al., 1994b, 1996). The methanol extract of leaves was also recently shown to contain ellagic acid derivatives: 3,3′,4′-tri-*O*-methylellagic acid-4-*O*-β-D-glucopyranoside and 3,3′,4′-tri-*O*-methylellagic acid (Chakravarty et al., 1998). Furthermore, several ellagitannins (pedunculagin, casuarinin, tellimagrandin I, strictinin, casuarictin, 2,3-HHDP-glucose and traces of tellimagrandin II) were detected, as in several other Myrtaceae, in a plant extract of *S. jambos* from Japan (Okuda et al., 1982). Other investigations showed that ethanol extracts of *S. jambos* leaves possessed antiviral activity on herpes simplex type I and inhibited the replication of vesicular stomatitis virus but had no effect on poliovirus replication (Abad et al., 1997).

In spite of the use of the bark of this plant to treat infectious diseases, no investigation was made on the content of the bark and its antibacterial properties. Thus, we decided to investigate the antimicrobial properties of extracts of bark of *S. jambos*.

2. Methodology

2.1. Plant material

Bark of *S. jambos* (L.) Alston (Myrtaceae) was collected in Mbam region (Cameroon) and authenticated by M. Bamps of 'Jardin Botanique de Belgique' where a voucher specimen was deposited. The plant material was dried and pulverised.

Dried *Hamamelis virginiana* L. (Hamamelidaceae) leaves, *Rubus fruticosus* L. s.l. (Rosaceae) and *Alchemilla vulgaris* auct. non L. (Rosaceae) herbs were obtained from S.A. Tilman (Baillonville, Belgium) while dried *Krameria triandra* RUIZ et PAV. (Krameriaceae) bark was purchased from S.A. Denolin (Brussels, Belgium). Samples were pulverised before extraction.

2.2. Extracts preparation

2.2.1. Aqueous extracts

Aqueous decoctions (10%) were prepared. After filtration, solutions were lyophilised.

2.2.2. Acetone extract

Powdered bark material was extracted after 24 h maceration by percolation with acetone 70% v/v at room temperature. Acetone was then removed under reduced pressure and the remaining aqueous solution was lyophilised.

2.2.3. Elimination of tannins

Dried aqueous extract (300 mg) was dissolved in a minimum volume of methanol and applied to a 15 g polyamide column. Elution was performed with methanol until the eluate was clear. The eluate was then evaporated under reduced pressure and gave 153 mg of residue (51%).

The final powders were stored at −20°C.

Before testing, the extracts were solubilised in water to give 10 mg/ml stock solutions. Each solution was sterilised on a 0.22 μm membrane filter.

2.3. Micro-organisms

The following reference strains from international collections were used:

2.3.1. Gram positive bacteria

Enterococcus faecalis strains ATCC 29212 and NCTC 775^T, *Enterococcus faecium* strain NCTC 7171^T, *Enterococcus gallinarum* strain LMG 13129 and *Staphylococcus aureus* strain ATCC 25923.

2.3.2. Gram negative bacteria

Pseudomonas aeruginosa strain ATCC 27583.

In addition, we also used strains from our laboratory collection obtained by clinical isolation.

2.3.3. Gram positive bacteria

Enterococcus durans strain D78 (vancomycin resistant), *E. faecalis* strain V583, *E. faecium* strain C1/17, *E. gallinarum* strain BM 4174, *S. aureus* strains 1, 2, 3, 5, 6, Vero 104, Vero 105, Vero 353, TP (oxacillin resistant), TP (oxacillin sensitive), Vero 544–553, 104 MC and 105 MC, coagulase negative *Staphylococcus* strain 1, *Staphylococcus cohni* strain 212, *Staphylococcus hominis* strains 214, 217, and 218, *Staphylococcus saprophyticus* strain 219 (oxacillin resistant), *Staphylococcus warneri* strains id 2607, 213, 215, and 216.

2.3.4. Gram negative bacteria

Enterobacter aerogenes strains TP, MC and 41, *Enterobacter cloacae* strains Vero and MC, *Escherichia coli* strains MC, TO, 54, 52, 43, 34, 27, 21, 14, 10 and 6, *Klebsiella oxytoca* Vero and 118, *Klebsiella pneumoniae*, *Morganella morganii* strains TP TP1, TP2, 13, 26, 93 and 180, *P. aeruginosa* strain TP, *Salmonella Enteritidis* strains TP and MC, *Citrobacter freundii* strains TP and MC, *Shigella sonnei* strains Vero and MC, *Yersinia enterocolitica* strains E001/97, E004/97, E003/98, E204/97, E203/97, E202/97, E201/97 E170/98 and E 169/98.

Strains were stored in glycerol and maintained at −20°C prior to use for antimicrobial tests.

2.4. Reference antibiotics

Ampicillin (Bristol-Myers, Belgium) and erythromycin (Calbiochem Behring Corp., Hoechst, France) were used.

2.5. Culture media

The medium was the Muller-Hinton 2 agar (bioMerieux., France).

2.6. Antimicrobial susceptibility tests

The agar dilution method (National Committee for Clinical Laboratory Standards, 1990) was used to determine the minimal inhibitory concentration (MIC: the minimal concentration completely inhibiting the growth of the micro-organism) of *S. jambos*, *A. vulgaris*, *R. fruticosus*, *H. virginiana* and *K. triandra* extracts, erythromycin and ampicillin. We also determined the minimal active concentration (MAC: the minimal concentration reducing the growth of the micro-organism as compared to controls) for *S. jambos* extracts.

The micro-organisms were grown overnight on Tryptone soya broth (Oxoid Ltd., England). Inocula of 10^3–10^4 CFU were spotted with a multipoint inoculator A400 (Denley Instruments Ltd., England) on Muller-Hinton agar supplemented with the extracts or antibiotics at concentrations ranging from 1000 to 0.977 μg/ml for the extracts and from 256 or 16 to 0.25 μg/ml for the antibiotics. Blanks were included. The plates were incubated for 18–24 h at 37°C. Tests were performed at least in duplicate.

3. Results and discussion

In traditional medicine, people usually use aqueous decoctions to treat patients. That is why we first prepared an extract from an aqueous decoction. Phytochemical analysis of this extract by TLC and colorimetric reactions showed that tannins were the major compounds (both hydrolysable and condensed tannins were identified) but we also detected a small amount of saponins. Because of the presence of tannins, another extract was prepared with acetone 70%, a better solvent for tannins (Bruneton, 1993). Quantitative determination of tannins in these extracts by the hide powder-phosphotungstic acid method described in the European Pharmacopoeia (Pharmacopée européenne, 1997) gave 77 and 83% of tannins for the aqueous and acetone extracts, respectively. These extracts were tested on a panel of positive and negative gram bacteria and then on different strains of the sensitive bacteria (Table

Table 1
In vitro antibacterial activities of *S. jambos* extracts aqueous (SJD) and acetone (SJA) extracts

Strains	SJD MIC[a]	MAC[b]	SJA MIC	MAC	Antibiotics Erythromycin	Ampicillin
					MIC	MIC
C. freundii TP	>1000	125	>1000	125	>16	>32
E. aerogenes TP	>1000	125	>1000	125	>16	>32
E. aerogenes 41	>1000	125	>1000	125	>16	>32
E. cloacae Vero	>1000	125	>1000	125	>16	>32
E. coli TP	>1000	125	>1000	125	>16	4
E. coli 6	>1000	125	>1000	62	64	2
E. coli 10	>1000	125	>1000	62	64	2
E. coli 14	>1000	125	>1000	62	128	>256
E. coli 21	>1000	125	>1000	62	128	8
E. coli 27	>1000	125	>1000	62	64	4
E. coli 34	>1000	125	>1000	62	64	4
E. coli 43	>1000	125	>1000	62	64	>256
E. coli 52	1000	31	1000	31	>16	>32
E. coli 54	>1000	125	>1000	62	32	2
E. durans D78 vancomycin resistant	>1000	250	>1000	250	>16	0.25
E. faecalis ATCC 29212	>1000	125	>1000	125	>16	0.5
E. faecalis V583	>1000	250	>1000	250	2	1
E. faecalis NCTC 775[T]	>1000	250	>1000	62	0.5	0.5
E. faecium NCTC 7171[T]	>1000	250	>1000	62	2	1
E. faecium C1/17	>1000	250	>1000	62	>16	16
E. gallinarum LMG 13129	>1000	62	>1000	62	0.5	2
E. gallinarum BM 4174	>1000	62	>1000	250	0.25	1
K. oxytoca Vero	>1000	125	>1000	125	>16	>32
K. pneumoniae	>1000	125	>1000	125	>16	8
M. morganii TP1	1000	125	>1000	62	>16	>32
M. morganii TP2	1000	31	>1000	31	>16	>32
M. morganii 13	750	31	>1000	31	>256	>256
M. morganii 26	1000	31	>1000	31	>256	256
M. morganii 93	1000	31	>1000	31	>256	256
P. aeruginosa TP	>1000	125	>1000	125	>16	>32
P. aeruginosa ATCC 27853	>1000	125	>1000	125	>16	>32
S. aureus ATCC 25923	500	62	500	31	0.25	0.25
S. aureus TP oxacillin sensitive	750	62	750	62	0.5	0.25
S. aureus TP oxacillin resistant	500	62	750	62	0.25	4
S. aureus 1	500	62	750	62	>16	>32
S. aureus 2	750	250	750	62	0.25	0.25
S. aureus 3	1000	62	750	62	>16	4
S. aureus 5	750	62	750	62	>16	16
S. aureus 6	750	62	500	62	>16	16
S. aureus Vero 104	750	31	500	62	2	>32
S. aureus Vero 105	750	125	750	62	0.5	1
S. aureus Vero 353	500	250	500	62	>16	>32
S. aureus Vero 544	1000	62	750	31	>16	>32
S. aureus Vero 545	1000	62	750	62	>16	>32
S. aureus Vero 546	750	62	750	62	>16	16
S. aureus Vero 547	750	62	750	62	>16	>32
S. aureus Vero 548	750	62	750	62	>16	>32
S. aureus Vero 549	750	31	750	31	>16	>32

Table 1 (*Continued*)

Strains	SJD MIC[a]	MAC[b]	SJA MIC	MAC	Antibiotics Erythromycin	Ampicillin
					MIC	MIC
S. aureus Vero 550	750	31	750	31	>16	32
S. aureus Vero 551	750	31	750	31	>16	>32
S. aureus Vero 552	750	62	750	31	0.25	32
S. aureus Vero 553	1000	62	750	62	1	32
S. cohnii 212	250	31	250	31	0.25	0.25
S. enteridis TP	>1000	125	>1000	125	>16	1
S. hominis 214	250	31	250	31	0.25	0.25
S. hominis 217	15.5	8	15.5	8	0.25	0.25
S. hominis 218	31	8	15.5	8	<0.25	<0.25
S. saprophyticus 219 oxacillin resistant	750	62	750	62	>16	0.25
S. sonnei Vero	>1000	125	>1000	125	>16	4
S. warneri id 2607	1000	125	750	62	1	2
S. warneri 213	250	31	250	31	>16	0.25
S. warneri 215	15.5	8	15.5	8	>256	0.25
S. warneri 216	1000	62	750	31	0.25	2
Y. enterocolitica E001/97	1000	31	1000	31	>16	>32
Y. enterocolitica E004/97	1000	31	1000	31	>16	>32
Y. enterocolitica E003/98	500	31	750	31	64	32
Y. enterocolitica E201/97	250	125	500	8	<0.25	<0.25
Y. enterocolitica E202/97	750	125	500	31	64	128
Y. enterocolitica E203/97	750	31	750	31	64	64
Y. enterocolitica E204/97	750	31	750	31	64	64

[a] MIC: minimum inhibitory concentration (in μg/ml).
[b] MAC: minimum concentration which lowered the growth of the micro-organism (in μg/ml).

1). We observed a decrease in the growth of all bacteria and an inhibition of growth for some of them: *S. aureus*, *S. saprophyticus*, *Y. enterocolitica*, *M. morganii*, one strain of *E. coli* and coagulase negative staphylococci among which *S. hominis*, *S. cohnii* and *S. warneri*. A particularly interesting activity is observed on two *S. hominis* strains and one erythromycin resistant *S. warneri* strain.

Comparison of the MIC and MAC values of aqueous and acetone extracts shows that both extracts have similar activities. Small differences are only observed with some strains where the acetone extract is often more active than the aqueous one.

To determine the type of compounds responsible for the activity of these extracts, we eliminated tannins on a polyamide column according to the method described by Houghton and Raman (Houghton and Raman, 1998). The resulting extract was inactive.

As tannins are well known to possess general antimicrobial properties (Scalbert, 1991), we also wondered if the observed properties of the extracts of *S. jambos* were specific or if they were a general feature of tannins. That is why we tested on some selected strains, including very sensitive strains, aqueous extracts of tannin containing plants available in our laboratory such as *H. virginiana*, *K. triandra*, *A. vulgaris* and *R. fruticosus*. These extracts contained 48, 44, 46 and 28% tannins, respectively, according to the method of the European Pharmacopoeia (Pharmacopée européenne, 1997). The results (Table 2) show that *S. jambos* extract is generally more active on very sensitive strains (MIC $\leq$ 1000 μg/ml), but exceptions are observed as the lower MIC value of *H. virginiana* on *M. morganii* 180 and the equal MIC values of *H. virginiana* on *S. aureus* 105 MC and *S. Warnerii* 215 and of *A. vulgaris* on *M. morganii* 180. Furthermore, some of these extracts are active on strains

Table 2
In vitro antibacterial activities of ampicillin (A) and aqueous extracts of *S. jambos* (SJD), *H. virginiana* (H), *K. triandra* (K), *A. vulgaris* (Al) and *R. Fructicosus* (Ru)[a]

No.	Strains	A	SJD	H	Ru	Al	K
1	*C. freundi* MC	>64	>1000	>1000	>1000	>1000	>1000
2	*E. aerogenes* MC	64	>1000	>1000	>1000	>1000	>1000
3	*E. cloacae* MC	64	>1000	>1000	>1000	>1000	>1000
4	*E. coli* MC	8	>1000	>1000	>1000	>1000	>1000
5	*E. faecolis* ATCC 29212	0.5	>1000	1000	>1000	>1000	250
6	*Klebsiella oxytoca* 118	64	>1000	>1000	>1000	>1000	>1000
7	*M. morganii* 180	>64	1000	750	>1000	1000	>1000
8	*M. morganii* TP	>64	>1000	750	>1000	1000	>1000
9	*Salmonella enteritidis* MC	>64	>1000	500	>1000	>1000	>1000
10	*Shigella sonnei* MC	2	1000	1000	>1000	>1000	>1000
11	*S. aureus* ATCC25923	0.5	250	750	>1000	>1000	250
12	*S. aureus* 104 MC	>64	125	750	>1000	>1000	250
13	*S. aureus* 105 MC	2	125	125	>1000	>1000	250
14	*S. hominis* 214	0.5	62	125	1000	125	250
15	*S. hominis* 217	0.5	15	125	>1000	>1000	250
16	*S. hominis* 218	0.5	31	62	1000	750	250
17	*S. warneri* 215	0.5	31	31	1000	125	250
18	*Y. enterocolitica* E 170/98	64	62	125	>1000	1000	>1000
19	*Y. enterocolitica* E 169/98	64	62	125	>1000	1000	1000

[a] MIC, minimum inhibitory concentration (in μg/ml).

less sensitive to *S. jambos* extract (MIC > 1000 μg/ml): *H. virginiana* on the strains of *S. enteridis*, *E. faecalis* and *M. morganii* TP, *K. triandra* on the strain of *E. faecalis* and of *A. vulgaris* on *M. morganii* TP. This first indicates that even though all extracts do not possess the same antimicrobial spectrum, the observed activities seem to be generally related to the total tannin content. This also shows that strains do not seem to have the same sensitivity to the different type of tannins present in the extracts and that the antimicrobial properties of *S. jambos* extracts can probably be explained by the presence of high concentrations of antimicrobial tannins.

4. Conclusions

Aqueous and acetone extracts of bark of *S. jambos* reduced and in some cases inhibited the growth of all tested micro-organisms including bacteria implicated in cutaneous and diarrhoeal infections. These extracts were also shown to be generally more active than other tannin containing extracts like *H. virginiana*, *K. triandra*, *A. vulgaris* and *R. fruticosus* extracts. These properties seem to be due to the presence of high concentrations of antimicrobial tannins.

These facts support the claims of the traditional medicines using *S. jambos* bark extracts to treat infectious diseases.

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