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In vitro Evaluation of Antimicrobial and Antiproliferative Activities for Compounds Isolated from the *Ficus bubu* Warb. (Moraceae) Fruits: Chemotaxonomic Significance

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Abstract: *Background*: Plants are traditionally a major source of primary health care for local communities, but also for all people in all countries of the world. This study investigated the *in vitro* antimicrobial and antiproliferative activities of the methanol extract and of purified compounds from fruits of *Ficus bubu* Warb. *Methods*: The antimicrobial activities of the methanol extract and of purified compounds from fruits of *Ficus bubu*, were determined using microbroth dilution method against a set of bacteria and fungi pathogens including: *Enterococcus faecalis, Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermididis*, *Escherichia coli, Klebsiella pneumonia, Salmonella typhimurium*, *Candida albicans*, *Trichophyton rubrum*. The MIC values were evaluated after 24 h incubation at 37°C. Subsequently, MTT assay was used to estimate anti-proliferative activity of these methanol extracts and purified compounds on three human cancer cell lines (U373 glioblastoma, A549 NSCLC and SKMEL-28 melanoma). *Results*: Extensive chromatographic isolations provided two stilbenes, that were identified by NMR and mass spectrometry as *trans*-resveratrol **4a** and piceid **7a**, in addition to several other chemical compounds. It was found that *trans*-resveratrol **4a** exhibited the best antimicrobial activity (MIC value of 11 µg/mL), and presented a good anticancer activity (IC_{50} of 36 and 57 µM against A549 and SK-MEL-28 cancer cell lines, respectively). The peracetylation of isolated compounds was found to increase their antiproliferative activity. The peracetylated piceid **7b** was the most efficient with an IC_{50} of 16 µM against the cells of melanoma skin cancer SK-MEL-28 while the starting crude extract did not show any activity. In contrast, this crude extract exhibited good antimicrobial activity against all tested strains. *Conclusion*: The present study constitutes the first phytochemical report on the methanol extract from *F. bubu* fruits and establishes the preliminary basis for its medical use. Finally, it is worth mentioning that polyphenolic piceid **7a** is isolated for the first time from a plant of the genus *Ficus*.

Keywords: Bioactivity, cancer, chemotaxonomy, *Ficus bubu*, piceid hexaacetate, piceid, *trans*-resveratrol triacetate, *trans*-resveratrol.

INTRODUCTION

Many developing countries, including Cameroon, continue to have mortality patterns showing high levels of infectious diseases and strong risks of death during pregnancy and childbirth [1]. Plants are traditionally a major source of primary health care for local communities, and also all over the world. Furthermore, it is estimated that two-thirds of the world population still use traditional remedies, primarily due to low availability and to the high cost of pharmaceutical drugs [2].

It has been calculated that approximately 6% of the world's plants have been screened for their biological activities and only 15% have been phytochemically evaluated [3]. Based on ancient healing wisdom, research on plants with medicinal properties thus represents an interesting alternative source to obtain a large variety of molecules, and potentially to identify new chemical compounds with biological/ pharmacological properties. Hence, natural products from plants are well recognized as sources for drugs to treat major human diseases including cancers. Plant-derived pharmaceuticals, such as vincristine, irinotecan, etoposide and paclitaxel, are among the most effective medicines [4]. However, despite the availability of many antimicrobial and anticancer agents, the search for new drugs remains an active field of research, especially in order to increase the available chemi-

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cal diversity range and to find more effective and less toxic compounds.

A survey of recent taxonomic studies on Moraceae family shows a large plant family consisting of about 50 genera and more than 1400 species, with the important genera being *Ficus*, *Morus* and *Artocarpus* [5]. The genus *Ficus* gathers over 750 species, generally found in tropical regions. For instance, more than 600 species originated from Asia and Australia, while about 100 are from Africa [6], with 60 species present in Cameroon [7].

The use of *Ficus* species as food or as medicine comes from the Middle East, although *Ficus* is widely present in Traditional Chinese Medicine [8]. Indeed, the genus *Ficus* represents an important reservoir of various biologically active compounds, such as 3-keto-urs-12-ene, α- and β-amyrin, boswellonate, elasticoside, ficin, ficusamide, ficusoside, lupeol, lupenone, β-sitosterol, β-sitosterone and stigmasterol [9-12].

In Central and South America, the latex from some *Ficus* species was found to be used for its antihelmintic property in traditional medicine [13]; this parasiticidal activity may be explained by the presence of ficin [9]. More particularly, the latex of *Ficus bubu* Warb. is traditionally used for the treatment of hemorrhoids and asthma. However, to date, none of the chemical and biological studies of *Ficus bubu* Warb. have received significant attention in the scientific literature.

In the quest for new bioactive constituents, we analysed here the methanol (MeOH) extract of *F. bubu* fruits. This article describes the extraction and the characterization of compounds from this extract, and with the set-up of the chemotaxonomic significance. The antimicrobial and antiproliferative activities of the crude MeOH extract were evaluated, in addition to the biological activities of hemisynthetic derivatives of two isolated products.

MATERIALS AND METHODS

General

Extracts were purified using flash column chromatography (SiO_2, Merck 230-400 mesh and 70-230 mesh), and thin layer chromatography was performed using aluminium silica gel sheets (60 F_{254}). After spraying with an ethanolic solution (0.1% ethanol) combined with berberin HCl, spots were visualized under ultraviolet light (λ=254 and 365 nm) and dried at 100°C. A rapid (flash) column chromatography was performed using silica gel (size 60-200 μm) at moderate pressure. The chemical structures of isolated molecules were determined by comparing their spectra with those of the literature or by direct GC comparison with standards (after dilution in chloroform 0.5 mg in 2 mL of $CHCl_3$).

For NMR studies, solutions of 0.5 ml were used. Two-Dimensions NMR spectra were recorded as needs aroused: ^1H, ^1H COSY spectra (^1H, ^1H correlated spectroscopy), HMBC spectra (Heteronuclear Multi Bond Connectivity) and HMQC spectra (Heteronuclear Multiple Quantum Coherence). Melting points were determined using a stereomicroscope (an American Optical (Reichert) Forty). The ^1H and ^{13}C NMR spectra were recorded using two spectrometers (Bruker Avance 300 and Varian Inova 400), respectively, in $CDCl_3$ and CD_3OD using the residual isotopic solvent $CHCl_3$ and CH_3OH, as references for δ_H = 7.26 ppm, δ_C = 77.16 ppm, δ_H = 3.31 ppm, δ_C = 49.00 ppm. Chemical shifts were measured with tetramethylsilane as standard. Records of high-resolution mass spectra (positive mode) were obtained with direct infusion in a 6520 series Electrospray Ion Source (ESI)-Quadrupole Time-Of-Flight (Q-TOF) mass spectrometer (Agilent, Palo Alto, CA, USA). The difference between the observed mass was expressed in ppm. When this difference was below 3 ppm, the molecules were considered to have the predicted formula. The extracts were tested for their phytochemical composition using the methods described by Harborne [14].

Plant Material

The fruits of *Ficus bubu* were harvested during the last month of 2007 in Cameroon. The plants identification was established by a member of the National Herbarium of Cameroon (NHC), where voucher specimens (No. 29050 HNC) were kept. After Air-drying, the biological material (fruits of *Ficus bubu*) was crushed into a fine powder by using an electric grinder. Macerates of the dried aliquot was obtained using methanol twice for 48 h at room temperature (27 ± 2°C) [15]. After filtration (Whatman Number One), and evaporation at low pressure in a rotary evaporator (bath at 40°C), we obtained 530 g of extract. Concentrated extracts were obtained by drying at 40°C in a hot air oven. The dried extract was packaged in air proof containers and stored in a refrigerator at 4°C until further use.

Extraction and Isolation

The fractionation of the crude methanolic extract (430 g) was performed with column chromatography (CC) on silica gel (cyclohexane/EtOAc/MeOH gradient of increasing polarity) to produce four fractions N1–N4 on the basis of TLC analysis. The first Fraction N1 (78 g) was subjected to Column Chromatography with silica gel as solid phase and eluted with *n*-hexane/EtOAc 97:03 to yield several linear aliphatic alcohols (51 mg) with *n*-hexacosanol **1** as major compound ([16]; NMR spectroscopy and GC). Fraction N2 (29 g) was also submitted to CC with a gradient of *n*-hexane/EtOAc yielding a mixture of phytosterol (203 mg) with stigmasterol **2a** and β-sitosterol **2b** in proportion 50:50 at *n*-hexane/EtOAc 95:05 ([17] and GC), biochanin A **3** (38 mg) at *n*-hexane/EtOAc 88:12 [18] [19] and *trans*-resveratrol **4a** (370 mg) at *n*-hexane/EtOAc 80:20 [20]. Fraction N3 (20 g) was subjected to CC on silica gel eluted with *n*-hexane/EtOAc to afford ficusamide **5** (5 mg) at *n*-hexane/EtOAc 50:50 [12]. Fraction N4 (41 g) analysed on CC on silica gel eluted with $CHCl_3$/MeOH gradient to produce *trans*-resveratrol **4a** (843 mg) at $CHCl_3$/MeOH 97:03, sitosteryl -3-*O*-β-D-glucopyranoside **6** (1.87 g) at $CHCl_3$/ MeOH 95:05 [21] and piceid **7a** (345 mg) at $CHCl_3$/MeOH 90:10 [22]. Fraction N5 (98 g) obtained by elution with MeOH was a complex mixture. All isolated compounds are shown on (Fig. **1**).

Acetylation of *trans*-resveratrol (**4a**) and piceid (**7a**). *Trans*-resveratrol (**4a**) (3.7 mg) or piceid (**7a**) (8.9 mg) was dissolved in dry pyridine (1.0 mL) followed by the addition of Ac_2O (acetic anhydride, 1.0 mL). The mixture was stirred overnight at room temperature. After the usual workup [23], *i.e*. extraction, filtration on a short silica gel column provided the *trans*-resveratrol triacetate (**4b**) in pure form (5.6 mg):

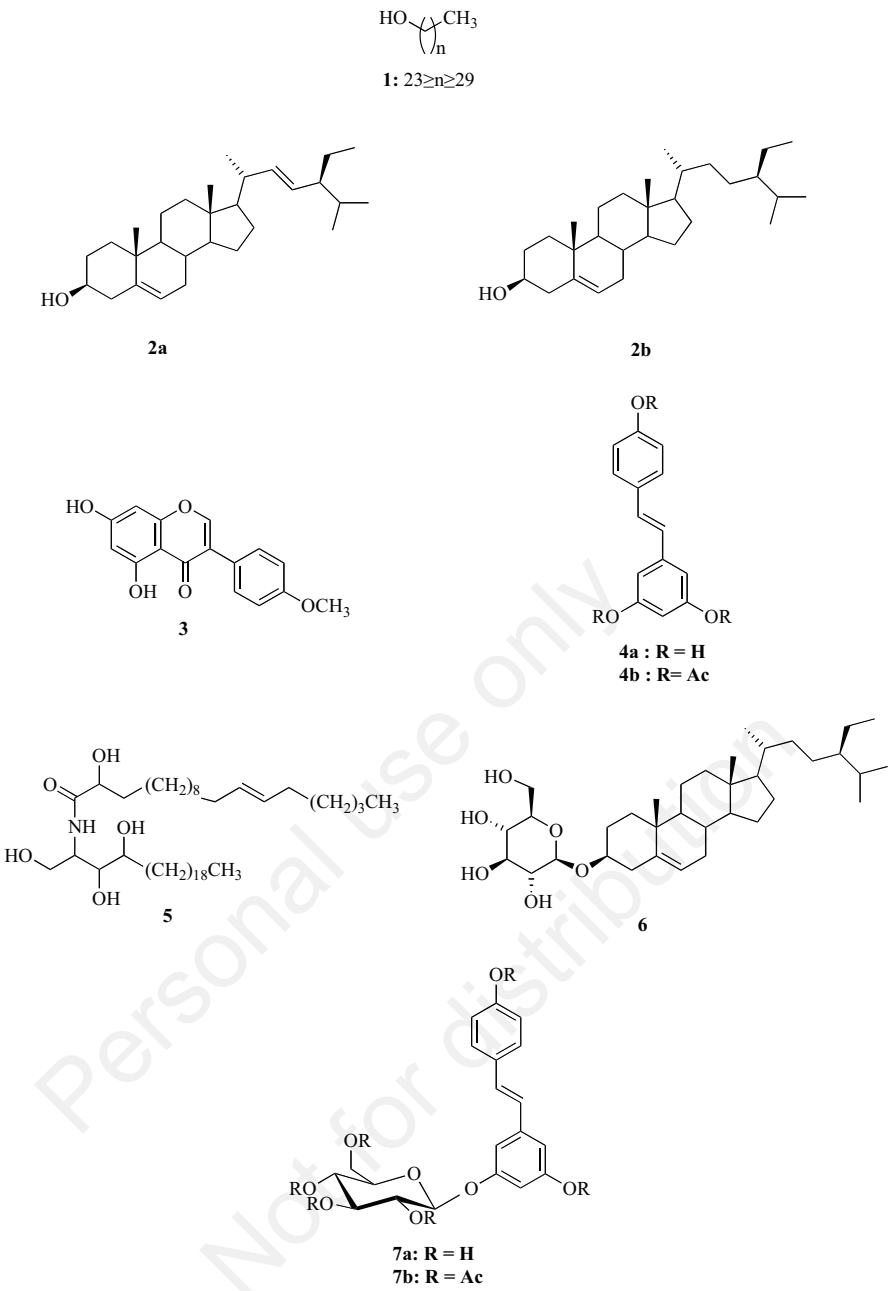

Fig. (1). Chemical structures of compounds **1**-**7**.

Rf 0.41 ($CHCl_3$-CH_3OH), white powder, m.p.113-114°C, HRESIMS, *m/z*: 372.1465 $[M+NH4]^+$ and piceid hexaacetate (**7b**) (12.3 mg): Rf 0.28 ($CHCl_3$-CH_3OH), white powder, m.p.98–99°C, HRESIMS, *m/z:* 665.1849 $[M+Na]^+$ respectively; ^1H and ^{13}C-NMR data are listed in Table **1**.

ANTIMICROBIAL ASSAY USING MICROBROTH DILUTION METHOD

Test Organisms and Preparations of Innocula

Nine microbial species were tested among which 4 Gram-positive, 3 Gram-negative bacteria, a yeast and a filamentous fungus. The Gram-positive bacteria were: *Enterococcus faecalis, Staphylococcus aureus, epidermididis* and *saprophyticus,* all isolated from urine and pus exudates of patients hospitalized in the Tiko CDC Hospital (Cameroon). The Gram-negative bacteria were: *Escherichia coli, Klebsiella pneumonia,* and *Salmonella typhimurium*, all obtained from urine and stool specimen from patients of the same hospital. The yeast, *Candida albicans*, was obtained from vaginal swab while the filamentous fungi, *Trichophyton rubrum*, was isolated from patients' legs. After growth on selective media, isolated microorganisms were identified using selective biochemical tests. The microorganisms were kept on agar slants from which single colonies were collected and suspended in 5 ml nutrient broth (NB, Oxoid), and incubated for 24 h (at 37ºC) in order to obtain fresh cultures of the different isolates. They were then used for antimicrobial susceptibility tests.

Table 1. *^{1}H-NMR* **(at 300 MHz)** *and ^{13}C-NMR* **(at 75MHz) Data for compounds 4b and 7b.** δ **in ppm,** J **in Hz.**

	4b		7b	
Position	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
1		140.4		139.7
2	6.82 (1H, *br s,* H-2)	113.9	6.98 (1H, *br s,* H-2)	112.6
3		156.3		157.5
4	5.25 (lH, *br s,* H-4)	115.9	6.66 (lH, *br s,* H-4)	109.6
5		151.4		151.3
6	6.79 (1H, *br s,* H-6)	116.9	6.97 (1H, *br s,* H-6)	114.4
7	6.91 (1H, *d*, $J_{7,8}$ = 16.0, H-7)	128.3	6.95 (1H, *d*, $J_{7,8}$ = 16.3, H-7)	127.4
8	7.01 (1H, *d*, $J_{7,8}$ = 16.0, H-8)	129.3	7.05 (1H, *d*, $J_{7,8}$ = 16.3, H-8)	129.5
1’		134.3		134.4
2’, 6’	7.47 (2H, *d*, $J_{2',3'}$ = 8.5, $J_{5',6'}$ = 8.5, H-2', H-6')	130.6	7.49 (2H, *d*, $J_{2',3'}$ = 8.4, $J_{5',6'}$ = 8.4, H-2', H-6')	127.5
3’, 5’	7.09 (2H, *d*, $J_{2',3'}$ = 8.5, $J_{5',6'}$ = 8.5, H-3', H-5')	124.6	7.10 (2H, *d*, $J_{2',3'}$ = 8.4, $J_{5',6'}$ = 8.5, H-3', H-5')	121.8
4’		150.5		150.4
Glucose				
1”			5.14 (1H, *d*, $J_{1'',2''}$ = 7.0, GIc H-1")	98.9
2”			5.28 (1H, *m*, Glc H-2")	71.1
3”			5.30 (lH, *m*, Glc H-3")	72.7
4”			5.17 (IH, *t*, $J_{4'',5''} = J_{3'',4''}$ - = 8.5, Glc H-4")	68.3
5”			3.92 (I1H, *ddd,* Glc H-5")	72.1
6”			4.28 (1H, *dd,* $J_{5'',6a''}$ = 1.5, $J_{6a'',6b''}$ = 12, Glc H-6a") 4.18 (1H, *dd,* $J_{5'',6b''}$ = 5.6, Glc H-6b")	62.0
3CH_3CO	2.33 (s, 9H)	20.4-20.6		
3CH_3CO		169.5-169.7		
6CH_3CO			2.02-2.30 (18H)	20.5-21.0
6CH_3CO				169.0-170.4

Reference Drugs Used for Antimicrobial Assay

Nystatin (Sigma, USA), clotrimazole (Sigma, USA) and gentamycin (Sigma, USA) were used as reference antimicrobials against yeasts, filamentous fungi and bacteria species, respectively.

Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC) Determinations

The minimum inhibitory concentration (MIC) was determined by the microbroth dilution method [24, 25] in Mueller Hinton or Sabouraud broth supplemented with 10% glucose and 0.5% phenol red. For susceptibility testing, we started by distributing the supplemented Mueller Hinton or Sabouraud broth (50 µL) from the first to the twelfth on a 96 wells microplate. First, dry extract was dissolved in DMSO (20%) (100 µL) and later on in Mueller Hinton broth, to obtain a final concentration of 10^4 µg/mL for FBFr and 6400 µg/mL for compounds **4b**, **7a** and **7b** and 5632 µg/mL for compound **4a** (the extract nomenclature found on footnotes of Tables **2-3**). Solutions (50 µL) were added to the first well and successive dilutions were then carried out by transferring the solution (50 µL) from the first to the eleventh well. The same volume (50 µL) was discarded from the eleventh well. The last well served as growth control since no sample (extract or the reference antibiotic) was added. A microbial suspension (50 µL, 10^5 colony forming units/ml), obtained from an overnight growth at 37°C was added to each well. Tests were incubated aerobically at 37°C before being read, during 24 and 48 h, for bacteria and fungi cultures, respectively. The MIC was considered as the lowest concentration of the sample that prevented the change in colour from red to yellow (formation of acidic metabolites) indicating the microbial growth.

Table 2. Antimicrobial activities (Gram (+) bacteria) of total extract and pure compounds isolated from the MeOH crude extract of *Ficus bubu* fruits.

		Gram positive bacteria											
		E. faecalis			*S. aureus*			*S. saprophyticus*			*S. epidermididis*		
		MIC	MMC	MMC/ MIC	MIC[c]	MMC[c]	MMC/ MIC	MIC	MMC	MMC/ MIC	MIC	MMC	MMC/ MIC
		Concentration (µg/mL)											
FBFr[a]	5	39	78	2	624	1248	2	39	78	2	624	1248	2
4a[a]	1	22	44	2	88	176	2	11	22	2	88	88	1
4b[a]	2	25	50	2	50	100	2	25	50	2	50	100	2
7a[a]	3	100	200	2	400	800	2	200	400	2	200	1600	8
7b[a]	4	12.5	25	2	50	100	2	100	400	4	50	100	2
Gentamycin[b]	6	39	78	2	39	78	2	39	78	2	39	78	2

[a] (FBFr) MeOH crude extract of *Ficus bubu* fruits; (**4a**) *trans*-resveratrol; (**4b**) *trans*-resveratrol triacetate; (**7a**) piceid; (**7b**) piceid hexaacetate.

[b] Reference antibiotics (gentamycin for bacteria).

[c] MIC is considered as the lowest concentration of the sample, that inhibits the visible growth of the strain and MMC is considered as the lowest concentration of the sample capable of causing the death of at least 99.99% of a tested inoculum.

Table 3. Antimicrobial activities (filamentous fungi, yeast and Gram (-) bacteria) of total extract and pure compounds isolated from the MeOH crude extract of *Ficus bubu* fruits.

		Filamentous fungi			Yeast			Gram (-) bacteria								
		T. rubum			*C. albicans*			*E. coli*			*K. pneumoniae*			*S. typhimurium*		
		MIC[c]	MMC[c]	MMC/ MIC	MIC	MMC	MMC/ MIC	MIC	MMC	MMC/ MIC	MIC	MMC	MMC/ MIC	MIC	MMC	MMC/ MIC
		Concentration (µg/mL)														
FBFr[a]	5	156	312	2	156	312	2	312	624	2	624	1248	2	624	1248	2
4a[a]	1	44	44	1	88	176	1	44	88	2	22	44	2	88	176	2
4b[a]	2	50	50	1	50	100	2	100	200	2	25	50	2	50	100	2
7a[a]	3	90	90	1	50	100	2	50	100	2	50	100	2	400	1600	4
7b[a]	4	*Nd	*Nd	/	25	50	2	50	50	1	25	50	2	50	100	2
Nystatin[b]	7	/	/	/	39	78	2	/	/	/	/	/	/	/	/	/
Gentamycin[b]	6	/	/	/	/	/	/	78	156	2	78	156	2	78	156	2
Clotrimazole[b]	8	39	78	2	/	/	/	/	/	/	/	/	/	/	/	/

[a] (FBFr) MeOH crude extract of *Ficus bubu* fruits; (**4a**) *trans*-resveratrol; (**4b**) *trans*-resveratrol triacetate; (**7a**) Piceid; (**7b**) Piceid hexaacetate.

[b] Reference antibiotics (nystatin for yeast, gentamycin for bacteria and clotrimazole for filamentous fungi).

[c] MIC is considered as the lowest concentration of the sample, that inhibits the visible growth of a microbe and MMC is considered as the lowest concentration of the sample capable of causing the death of at least 99.99% of a tested inoculum.

*Nd Not determined.

The minimum microbicidal concentration (MMC), indicating the lowest concentration of chemical compound able to cause the deaths of at least 99.99% of a growth, was also determined. After the MIC determination, a volume (10 µL) from each microwell showing no visible growth was inoculated on fresh drug-free Mueller Hinton agar (for bacteria cultures) and Sabouraud agar (for fungi) plates and incubated at 37°C for 24 h. Plates showing no growth indicated bactericidal effect of the fraction (sensitive) [25]. Each experiment was performed in triplicate until consistency is achieved, *i.e.* three times the same value.

Determination of *in vitro* Anticancer Activity

Human Cancer Cell Lines

Three human cancer cell lines were used in this study, namely the U373 (ECACC code 08061901) of human glioblastoma brain cancer, the A549 (DSMZ code ACC107)

NSCLC of human lung cancer and the SKMEL-28 (ATCC code HTB-72) of human melanoma cancer. These cancer cell lines had various degrees of drug resistance to distinct proapoptotic stimuli. The cells were cultured in supplemented media RPMI (Invitrogen, Merelbeke, Belgium) with 10% heat inactivated fetal calf serum (Invitrogen). Supplements of 4 mM glutamine, 100 µg/mL gentamicin, and penicillin-streptomycin (200 U/mL and 200 µg/mL, respectively) (Invitrogen) were added to all cultures.

Determination of the IC_{50} *in vitro* Growth Inhibitory Concentration

The inhibitory concentrations IC_{50} of all extracts were determined with a MTT assay (3-[4,5]-dimethylthiazol-2yl-diphenyl tetrazolium bromide, Sigma, Belgium), as detailed previously [26, 27], in a panel of three human cancer cell lines after 72 h of incubation of cancer cells in presence of the extract. Briefly, MTT viability assay is based on the capacity of living cells to reduce MTT into purple formazan crystals within their mitochondria. After dissolution of the crytals formed in dimethylsulfoxide, optical densities are measured by a plate reader at 570 nm (Biorad 680XR, Nazareth, Belgium) with a reference wavelength lecture at 630 nm. The assay was performed once in sextuplicate (the average value was recorded), on each cell line over a 72 h period of incubation with the compound/extract and nine concentrations (from 0.01 to 100 µg/mL, with semilog increases) were available for each cancer cell line. The 50% growth inhibitory concentration (50%) is the concentration that reduced by 50% the global growth of the cell line under study after 72 h of exposure to the sample test in comparison to the control condition.

RESULTS

The crude methanol (MeOH) extract from *F. bubu* fruits exhibited potent growth inhibitory antimicrobial activity (Tables **1** and **2**) against the Gram-positive bacteria, *E. faecalis* and *S. saprophyticus*, both with MIC values of 39 µg/mL, but also against *T. rubrum* and *C. albicans* (MIC=156 µg/mL), *E. coli* (MIC=312 µg/mL), *S. aureus*, *K. pneumonia*, *S. epidermididis* and *S. typhimurium* (MIC=624 µg/mL). However, the same MeOH extract did not display significant *in vitro* tumor cell growth inhibitory activities (Table **4**).

Subsequently, the MeOH extract was subjected to chromatographic separation and seven compounds were isolated and further chemically characterized by NMR and by mass spectrometry. The chemical structures of these compounds are represented on Fig. **1**. Peracetylation of **4a** and **7a**, yielded compounds **4b** and **7b,** respectively, that were then analyzed by HRESIMS mass spectrometry. The HRESIMS gave rise to a pseudo-molecular ion peak *m/z* 372.1465 $[M+NH4]^+$ and *m/z* 665.1849 $[M+Na]^+$ suggesting the resulting molecular formula $C_{20}H_{18}O_6$ and $C_{32}H_{34}O_{14,}$ respectively. Comparison of NMR spectra of **4a** and **4b** indicated the presence of three free hydroxyl groups in **4a** while those of **7a** and **7b** indicated the presence of six free hydroxyl groups in **7a**.

Results of the antimicrobial activities for the molecules **4a**, **4b**, **7a** and **7b** are shown in Tables **2** and **3**. The four compounds display differences in their antimicrobial activities against the tested pathogens. Considering the observed MMC/MIC ratios that were less or equal than 2, all the 4 compounds showed bacteriostatic/fungistatic or bactericidal/fungicidal properties against all investigated microorganisms and demonstrated a considerable antimicrobial activity with MIC values in the range of 11-400 µg/mL. It is interesting to note that all compounds showed a considerable antibacterial activity higher than that of the reference drug, gentamycin : **4a** with *S. saprophyticus* (MIC value of 11 µg/mL), *E. faecalis*, *K. pneumoniae* and *E. coli* (MIC values of 22 µg/mL); **4b** with *E. faecalis*, *K. pneumonia*, *S. saprophyticus* (MIC values of 25 µg/mL) and with *S. typhimurium* (MIC value of 50 µg/mL); **7a** with *E. coli* and *K. pneumonia* (MIC values of 50 µg/mL); **7b** with *E. faecalis* (MIC value of 12.5 µg/mL), *K. pneumonia* (MIC value of 25 µg/mL), *E. coli* and *S. typhimurium* (MIC values of 50 µg/mL). Concerning the antifungal activity, all samples displayed a significant antifungal activity against *C. albicans* with MIC values in the

Table 4. ***In vitro*** **growth inhibitory antiproliferative activity of** ***trans*****-resveratrol (4a) and piceid (7a) isolated from the MeOH crude extract of** ***Ficus bubu*** **fruits and of their peracetate derivatives using a panel of 3 cancer cell lines.**

Human cancer cell lines (IC_{50} *in vitro* growth inhibitory concentrations (µM)[a])				
#	U373 (glioma)	A549 (lung)	SK-MEL-28 (melanoma)	Mean IC_{50}
Trans-resveratrol (**4a**)	*Nd	36	57	46
Trans-resveratrol triacetate (**4b**)	42	28	28	33
Piceid (**7a**)	> 100	> 100	> 100	> 100
Piceid hexaacetate (**7b**)	31	22	16	25
IC_{50} *in vitro* growth inhibitory concentrations (µg/ml)[a]				
MeOH crude extract of fruit of *Ficus bubu*	> 100	> 100	> 100	> 100

[a] The IC_{50} *in vitro* growth inhibitory concentrations were determined using the MTT colorimetric assay. The human cell lines include the U373 (ECACC code 08061901) glioma, the A549 (DSMZ code ACC107) NSCLC and the SKMEL-28 (ATCC code HTB-72) melanoma models. The IC_{50} concentration represents the concentration of extract or compound needed to decrease by 50% the cell population growth after having cultured the cells for 72 h in the absence (control) or in the presence of the extract or the compound of interest.

*Nd Not determined.

range of 25-88 μg/mL (nystatin was the reference drug with MIC value of 39 μg/mL) and against *T. rubum* with MIC values between 44-90 μg/mL (clotrimazole was the reference drug with MIC value of 39 μg/mL). These results reveal the powerful and very interesting antifungal potency of these compounds.

More specifically, *trans*-resveratrol **4a** possesses antimicrobial activity higher than that of piceid **7a** against all microbial strains except *C. albicans*. The acetylation of piceid **7a**, giving the compound **7b**, increases the growth-inhibitory action against all microbial strains (by a factor of ~8 against *E. faecalis*, *S. aureus*, *S. typhimurium*, by a factor of ~4 against *S. epidermididis* and by a factor of ~2 against *S. saprophyticus*, *K. pneumonia* and *C. albicans*). In contrast, the acetylation of *trans*-resveratrol **4a**, *i.e*. compound **4b**, showed a mixed effect. The activity increased by a factor of 1.8 to 8 on *S. aureus*, *S. epidermididis*, *S. typhimurium* and *C. albicans* but decreased by a factor of 0.4 to 0.9 on *E. faecalis*, *S. saprophyticus*, *T. rubum*, *E. coli* and *K. pneumonia.*

Table **4** shows the cellular antiproliferation activity IC_{50} of the crude MeOH extract and of the 4 compounds tested against three cancer cell lines. The samples exhibited a large variation of activity. Indeed, Piceid **7a** and the MeOH extract of *F. bubu* fruit FBFr have very low activity against all tested human cell lines with a mean IC_{50} value higher than 100 μg/mL. In contrast, compounds **4a**, **4b** and **7b** showed good anticancer activity against the three human cells lines with mean IC_{50} values between 16-57 μM.

DISCUSSION

Compounds **1**, **2a**, **2b**, **3**, **5**, **6** (Fig. **1**) have already been reported for their antimicrobial activities against Gram-positive and Gram-negative bacteria specimens or against yeasts: *i.e*. *n*-hexacosanol **1** [28, 29], mixture of phytosterol with stigmasterol **2a** and β-sitosterol **2b** [30], biochanin A **3** [31, 32], ficusamide **5** [12] and sitosteryl 3-*O*-β-D-glucopyranoside **6** [33]. Phytosterols (stigmasterol **2a** and β-sitosterol **2b**) were previously mentioned as protective agents against lung, stomach, ovarian and breast cancer developments [34]. The anticancer properties of biochanin A **3** [35-37] and ficusamide **5** were also described previously [12]. Sitosteryl 3-*O*-β-D-glucopyranoside **6** was reported to show significant anti-cancer activity against Epstein–Barr virus-early antigen EBV-EA cancer cells lines [38].

Trans-resveratrol **4a** is a polyphenolic phytochemical compound isolated from grapes and many other plants [39], which is usually synthesized upon stress conditions or in response to pathogenic infection [40]. It is reported to have antitumor [41-43] and antimicrobial activities [44, 45]. The resveratrol molecule is currently tested to prevent colon cancer in phase-II of clinical trials [46]. It could be underlined that some acylated derivatives were reported to have better antiproliferative activities than resveratrol on human DU-145 prostate cancer cells [47]. Piceid **7a** is also known to possess antimicrobial activity [48] and was also tested in several tumor cell lines [49].

We carried out the acetylation of *trans*-resveratrol **4a** and piceid **7a** isolated from MeOH extract of *F. bubu* fruits, obtaining the peracetylated derivatives **4b** and **7b,** respectively. A structure-activity relationship investigation was performed through determination of their antimicrobial and antitumor activities which were not studies before on the mentioned microorganisms and cell lines. All the 4 tested compounds were active. Due to the fact that the MMC/MIC ratio of each sample against several tested organisms (*i.e*. **4a** against *S. epidermididis*, *T. rubum*, *C. Albicans*, **4b** against *T. rubum* and **7a** against *T. rubum* as well as **7b** against *E. coli*) was less than 2, they can be considered to be bactericidal/fungicidal [50]. In agreement with other published bioactivity results [51], the *trans*-resveratrol **4a** has antiproliferative effect, showing an IC_{50} of 36 and 57 μM against cell lines A549 and SK-MEL28, respectively. In contrast, no antiproliferative activity was observed for the piceid **7a**.

It has also been noted that the health benefits observed for moderate wine consumers may be partially attributed to the resveratrol molecule [52]. This study reveals that, resveratrol analogue such as *trans*-resveratrol triacetate **4b** is more active than trans-resveratrol **4a**. Acetylation of *trans*-resveratrol enhances its antiproliferative activity, from 36-57μM to 28 μM (Table **4**). Although no data exists regarding the activity of *trans*-resveratrol **4a** against cancer cells U373, an IC_{50} of 42 μM was determined for compound **4b**. Similarly, the conversion of inactive piceid **7a** into acetylated derivative **7b** afforded to obtain antiproliferative activity against cancer cells U373, A549 and SK-MEL-28, with IC_{50} of 31, 22, and 16 μM, respectively (Table **4**). The acetylated piceid **7b** arises as the most active compound with an IC_{50} of 16 μM against the cells of melanoma skin cancer. Clearly, the peracetylation modification significantly enhances the antiproliferative activity, but also the antimicrobial effect in the most cases. It can be suggested that the antimicrobial and antiproliferative activities of these compounds could be improved by the presence of acetyl groups on their structures.

CONCLUSION

We reported the first ethnopharmacological investigation of *Ficus bubu,* a plant traditionally used in Cameroon against microbial infections. Previous studies have already stated the presence of various compounds belonging to different classes in plants of the genus *Ficus* (Table **5**). Here, we elucidated several chemical structures of molecules isolated from *F. bubu* fruit (structures 1 - 7) (Fig. **1**), thereby increasing our knowledge on phytochemical of *Ficus bubu*. Indeed, the extraction and the identification of six different structures from *F. bubu* represent the first phytochemical work on this plant and may be used as foundation for further chemotaxonomic studies. Note that we isolated for the first time polyphenolic piceid 7a from a plant of the genus *Ficus*.

Furthermore, it was found that the *trans*-resveratrol **4a** presented a good anticancer activity (IC_{50} of 36 and 57 μM against A549 and SK-MEL-28, respectively), while the starting crude extract did not show any activity, may be due to the presence of antagonist molecules. In contrast, this extract exhibited good antimicrobial activity against all strains (MICs ranging between 39 and 624 μg/mL). *Trans*-resveratrol **4a** exhibits the best antimicrobial activity (MIC 11 μg/mL) against *S. saprophyticus* and the highest antiproliferative activity, with IC_{50} of 16, 22 and 31 μM against SK-MEL-28, A549 and U373, respectively. The important

Table 5. Class compounds previously reported from the Ficus genus.

Class	Compound	Source/reference
Phenylpropanoïds	Ficuscarpanoside	*F. microcarpa* [53]
	Guaiacylglycerol	
Apocarotenoïd	Ficusone	*F. microcarpa* [54]
γ-lactones derivatives	Ficuspirolide	
	Ficosoline	
Triterpenes	Ptiloepoxide	*F. microcarpa* [55]
	Moretenolactone	*F. insipida* [56]
	Rhoiptelenol	*F. thunbergii* [57]
	3β-acetylursa-14-en-16-one	*F. fistulosa* [58]
Sterols	Lanosta-8,24-dien-11-on-3β-ol acetate	
	3β-Acetoxycycloart-25-en-24-ol	*F. pumila* [59]
	Campesterol	
	β-Sitosterol	*F. carica,* *F. sycomorus* [60] *F. salicifolia*
Glucosides	Bergenine	*F. racemosa* [61]
	Racemosic acid	
Chromenes	Ficuformodiol A	*F. formosana* [62]
	Ficuformodiol B	
Flavonoïds	Ficuisoflavone	*F. microcarpa* [63]
	5,7,3',4',5'-Pentamethoxyflavone	*F. maxima* [64]
	5,7-Dihydroxy-4'-methoxy-3'-(2,3-dihydroxy-3-methylbutyl) isoflavone	*F. nymphaefolia* [65]
	Chrysine	*F. pantoniana* [66]
Alcaloïds	Antofine	*F. septica* [67]
	O-Methyltycophorinidine	*F. hispida* [68]
	Ficine	*F. pantoniana* [66]
Lignanes	Ficusal	*F. microcarpa* [69]
α-tocopheroïds	α-tocopherol	*F. microcarpa* [70]
Phenolic compound	Threo-2,3-bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol	*F. beecheyana* [71]
Sesquiterpenes	Pumilaside B	*F. pumila* [72]
Saponin	Elasticoside	*F. elastica* [12]
Sphingolipids	Ficusamide	
	Ficusoside	
Coumarins	S)-(-) oxypeucedanin hydrate	*F. exasperate* [73]

antimicrobial activity obtained in this study sustains the traditional use of this plant against infectious diseases. Finally, the obtained activities compared to those of the reference substances appear particularly promising for further investigations.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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