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Antimicrobial and cytotoxic extractives from *Ficus sycomorus* L. (Moraceae)

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ABSTRACT: Our search for antimicrobial and cytotoxic leads from *Ficus sycomorus* led to the isolation of twenty-four known compounds (**1**– **24**) from its methanolic stem bark and root extracts. The structures of all the isolated compounds were established using their 1D- and 2D-NMR spectroscopic data while the crude extracts, some fractions and compounds were tested for their antibacterial, antifungal and cytotoxicity activities. In the antimicrobial assay, compound **18** displayed good to moderate activity with MIC values of 31.25 μg/ml against *Escherichia coli*, 125 μg/ml against *Salmonella typhimurium* cpc and *Pseudomonas aeruginosa*, while compounds **10**, **13** and **14** demonstrated moderate activities on almost all the tested strains, with the highest potency observed for compound **13** on *E. coli* (MIC = 125 μg/ml). However, although compounds **18** and **19** gave a significant inhibition of the VERO cell line ATCC CRT-1586 with CC_{50} values of 52.60 μg/ml and 69.10 μg/ml, respectively, all the extract samples and other tested compounds did not show distinguishable cytotoxicity. Finally, the chemophenetic significance of the isolated compounds is discussed.

1. INTRODUCTION

Over 100 trillion microbes are estimated as spending most of their lifetime on and within human beings including some of them involved in either human health or in diseases affecting humans through numerous mechanisms (Gill et al., 2006; Ley et al., 2006; Wang et al., 2017). Infectious diseases commonly spread among humans are caused by the production of toxins by microbes in the human host also called dysbiosis of the human microbiota which will have a significant incidence on the immune system, leading to antibiotic resistance or causing the new emerging infectious or microbial diseases like HIV, hepatitis and many others (Brenchley & Douek, 2012; Cohen, 2016; Hand et al., 2012; Hu et al., 2016). Moreover, the resistance of infectious pathogens to existing drugs has been the major cause of the increasing rate of microbial diseases and the emergence of new ones around the world (Kemayou et al., 2021; Tabekoueng et al., 2020). The medicinal plants including those of the genus *Ficus* have been long used as the first line of treatment in folk medicine to manage several microbial illnesses and might represent a good source of new antimicrobial lead compounds to address the need for new potent drugs to face that resistance (Happi et al., 2021; Mbobda et al., 2021; Mbougnia et al., 2021). *Ficus sycomorus* (Moraceae) is one of the 840 species of *Ficus* genus which grows as a semi-deciduous tree with green or yellow to orange bark and is widely distributed in tropical regions of Africa (Hossain, 2019). The tree can reach up to 20 m in height and 6 m wide. Its leaves are deep green and heart-shaped; the fruit is large (2 to 3 cm in diameter), maturing from buff-green to yellow or red; the flowers are spherical, greenish and unisexual (Hossam et al., 2019). Different parts of *F. sycomorus* are introduced in the preparations of traditional medicines for the cure of microbial infections like diarrhoea, dysentery, urinary tract infections, cough, skin rashes, ulcers and tuberculosis (Abubakar et al., 2015; Fowler, 2007). Previous studies undertaken on the aerial parts of *Ficus* species revealed the presence of different classes of compounds including triterpenoids, coumarins and flavonoids (Chiang et al., 2005; Chiang & Kuo, 2002; Popwo et al., 2019). Those reported compounds exhibited a large range of activities including antimicrobial, antiviral, antioxidant, and anti-proliferative activities (Dzubak et al., 2006; Yan et al., 2014). As a way forward in our search for antimicrobial and cytotoxic leads from Cameroonian medicinal plants (Tegasne

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et al., 2020; Wouamba et al., 2020), we have investigated the stem bark and roots of *F. sycomorus* for its chemical constituents and their antibacterial, antifungal and cytotoxicity activity against a panel of microbial strains and VERO cell line. The methodology for isolation and analyses of the compounds, the discussion on results obtained and the chemophenetic significance of this study are all herein presented.

2. MATERIALS AND METHODS

2.1. General instrumentation

Instruments used during this study for isolation and analyses of the isolated compounds can be found in the supplementary information (Appendix A).

2.2. Plant material

The stem bark and the roots of *F. sycomorus* were collected at Malentouen (GPS coordinates: 5°30′20″N, 10°00′00″E, Elevation 1100 m), a locality of Foumban, West region, Cameroon, in January 2017. Mr Victor Nana, a botanist of the National Herbarium of Cameroon, Yaounde, made the identification of the plant and a specimen has been kept under the voucher number 13750/HNC.

2.3. Extraction and isolation

The air-dried and powdered stem bark (2.10 kg) and roots (2.00 kg) of *F. sycomorus* were separately macerated twice in methanol for 48 h and 24 h, respectively. The crude extracts of stem bark (200.55 g) and roots (34.55 g) were obtained after removing the solvent. The purification processes of the two extracts were separately conducted and led to the isolation of 24 distinct compounds as summarized in Figure 1 and Figure 2.

Briefly, part of the stem bark extract (195.00 g) was submitted to flash chromatography using EtOAc, EtOAc/MeOH (70:30, v/v) and MeOH as solvents, to afford EtOAc fraction (25.15g); EtOAc/MeOH fraction (80.05 g) and MeOH fraction (85.10 g). A part of the EtOAc fraction (22.15 g) was chromatographed on silica gel column chromatography with an increasing amount of EtOAc in *n*-Hexane (Hex) from 5% to 100% (v/v). A total of 232 fractions (100 ml each) have been collected and combined on the basis of TLC analyses into seven sub-fractions (F1–F7). Sub-fraction F1 [225.55 mg, 5% of EtOAc in Hex (v/v)] was purified on silica gel column chromatography, with a gradient solvent system starting by 2.5 % of EtOAc in Hex (v/v) and gave compounds **14** (8.5 mg), the mixture of **2** and **4** (20.1 mg) and **8** (12.8 mg), then, with the solvent system 5% of EtOAc in Hex (95:5, v/v) to give compound **9** (13.5 mg). The mixture of **12** and **15** (20.0 mg) precipitated from F2 [135.10 mg, Hex–EtOAc (90:10, v/v)] purified following elution with an isocratic solvent system of Hex–EtOAc (92.5:7.5, v/v). Following the same process, the sub-fraction F3 [134.50 mg, Hex–EtOAc (85:15, v/v)] gave compounds **1** (8.2 mg) and **10** (10.5 mg). The mixture of compounds **3** and **5** precipitated from sub-fraction F4 [76.55 mg, Hex–EtOAc (80:20, v/v)] while compounds **11** (9.1 mg), **21** (11.5 mg) and the mixture of **23** and **24** (21.5 mg). Were obtained from the sub-fraction F5 [126.50 mg, Hex–EtOAc (75:25, v/v)]. Furthermore, compound **22** (5.8 mg) was obtained from sub-fraction F6 [90.50 mg, Hex–EtOAc (70:30, v/v)] and compound **16** (80.7 mg) precipitated from F7 [95.55 mg, Hex– EtOAc (25:75, v/v)].

Likewise, part of the methanol crude extract of roots (30.00 g) was fractionated following the same procedure as performed with the stem bark extract. Therefore, the EtOAc fraction from the root gave six fractions (F1'–F6'). From purification using silica gel column chromatography as described earlier, Fraction F1' [105.50 mg, Hex–EtOAc (90:10, v/v)] afforded compound **12** (7.2 mg), **13** (7.6 mg), **9** (10.1 mg) and **7** (13.8 mg). Fraction F2' [200.50 mg, Hex–EtOAc (85:15, v/v)] led to the obtention of compounds **17** (14.6 mg), **18** (8.56 mg), **19** (13.4 mg) and **20** (25.20 mg), while fraction F3' [155.50 mg, Hex–EtOAc (80:20, v/v)] led to compounds **3** (9.2 mg) and **5** (12.3 mg). Compounds **23** (15.5 mg) and **24** (25.3 mg) were obtained after elution of the fraction F4' [99.50 mg, Hex–EtOAc (75:25, v/v)] and compounds 6 and 16 were obtained from Fraction F5' [90.50 mg, Hex–EtOAc (70:30, v/v)] and the recrystallization of F6' [75.50 mg, Hex–EtOAc (40:60, v/v)], respectively.

2.4. Spectral data of isolated compounds

The ^{1}H and ^{13}C NMR spectra (Figures 1S–45S, Appendix A) as well as the full assignations (Tables 1S–24S, Appendix A) of all the carbon and hydrogen atoms of the isolated compounds **1**– **24** are provided in the supplementary information attached to this paper (Appendix A).

2.5. Antimicrobial and cytotoxicity evaluations

Some compounds obtained in sufficient amounts for biological tests have been evaluated for their antibacterial, antifungal and cytotoxicity potencies. The full protocol for each test can be consulted in the supplementary material (Appendix A). For instance, eight bacterial strains namely *Salmonella typhimurium* cpc, *S. enteritidis* cpc, *S. typhi* cpc, *Staphylococcus aureus* MR, *S. aureus* (ATCC25922), *Klebssiella pneumonae* (ATCC13883), *Escherishia coli* (ATCC35218), *Pseudomonas aeruginosa* ; seven fungal strains including *Candida albicans*, *C. krusei*, *C. parasilosis*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes*, *Microsporium audouinii*, *Epidermophyton flocosum* as well as the VERO cell line ATCC CRT-1586 have been used during the three biological evaluations.

The antibacterial assay has been done using the broth microdilution method was used for susceptibility testing of bacteria species in 96 well-microtiter sterile plates as described by (Newton, 2002). The lowest concentration at which no visible colour change was observed was considered the Minimum Inhibitory Concentration (MIC) while the smallest concentration at which no colour change was observed was considered the Minimum Bactericidal Concentration (MBC). The tests were performed in duplicates. The ratio MBC/MIC was calculated to determine the bactericidal (MBC/MIC $\leq$ 4)

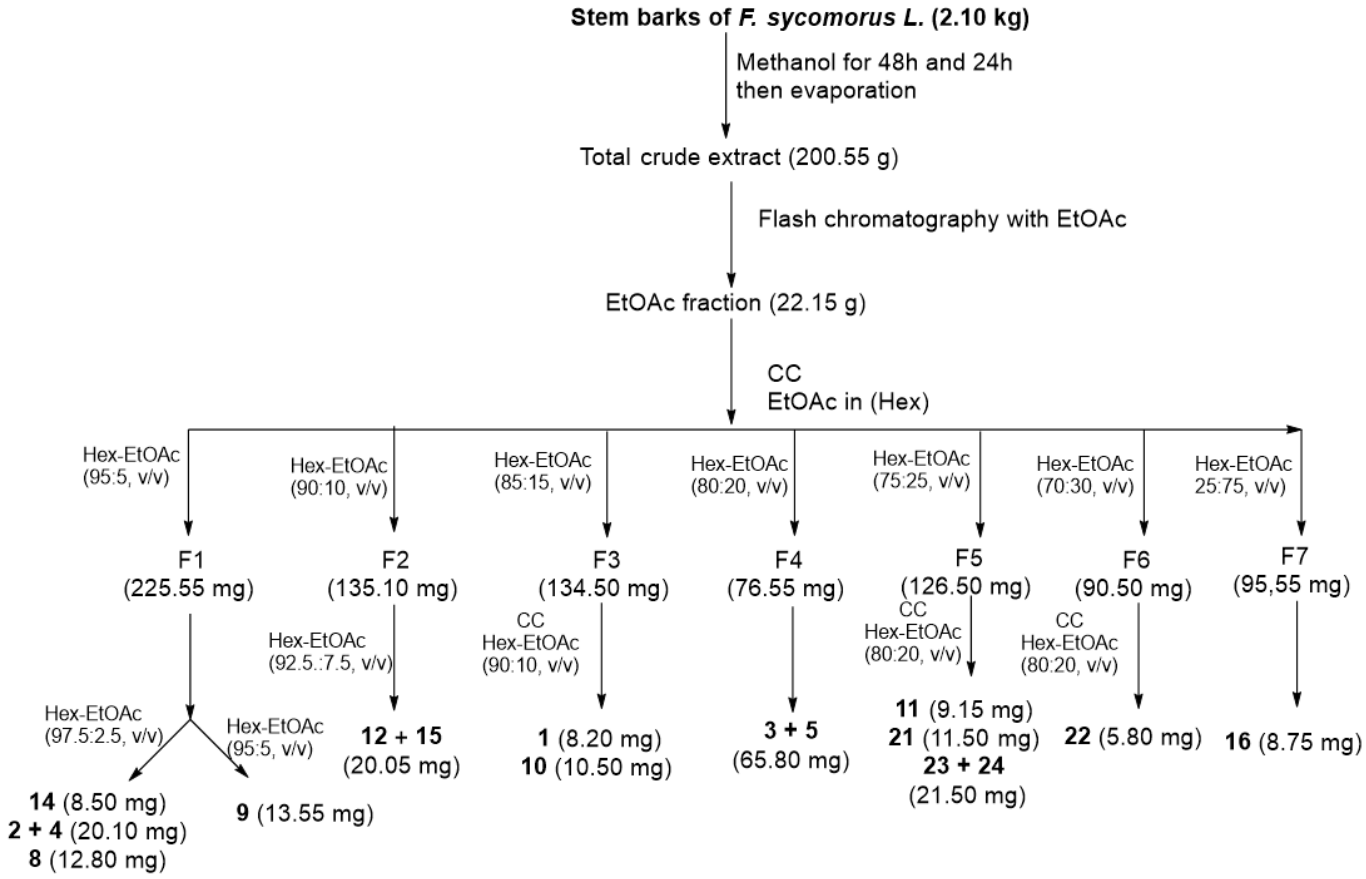

Figure 1. Summarized protocol for isolation of compounds from *F. sycomorus* bark

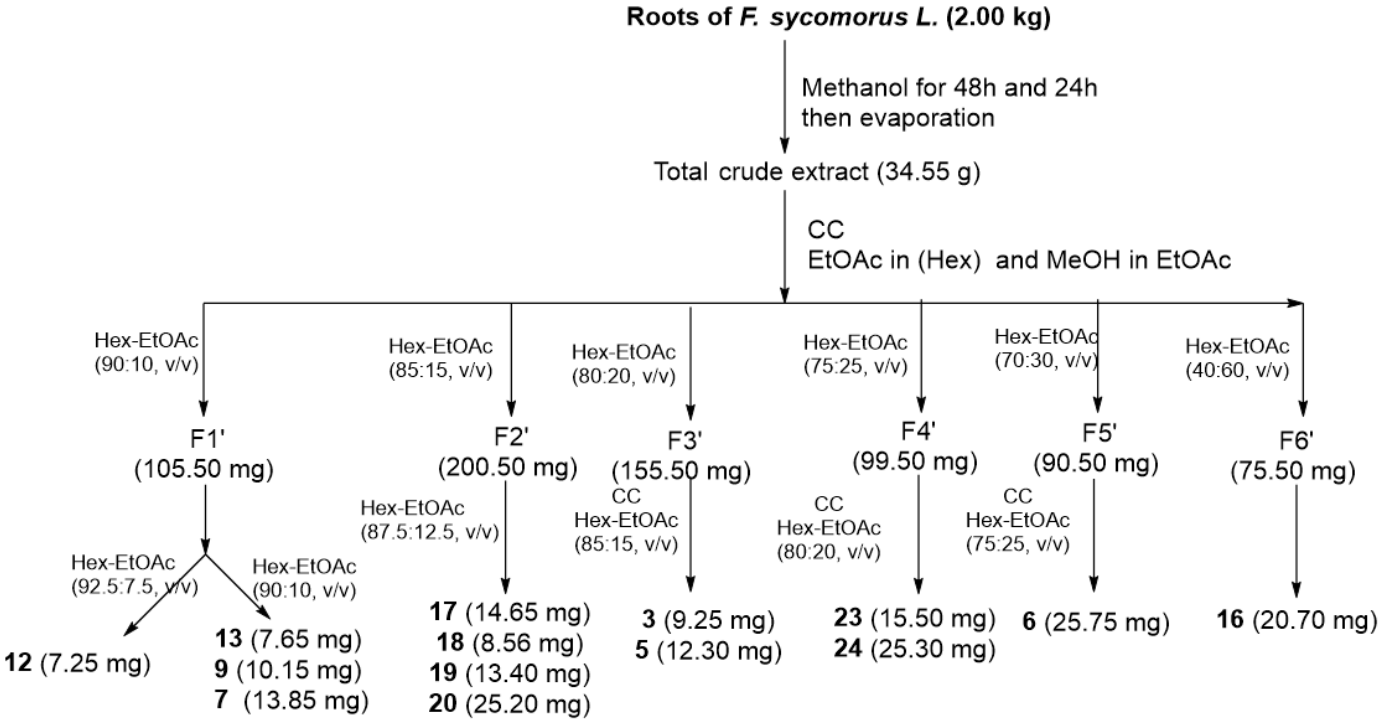

Figure 2. Summarized protocol for isolation of compounds from *F. sycomorus* root

and bacteriostatic (MBC/MIC > 4) effects (Mativandlela et al., 2006).

In antifungal activity evaluation, the MIC of each sample was determined by using broth microdilution techniques according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical and Laboratory Standards, NCCLS) for yeasts (M27-A2). MIC values were assessed visually after the corresponding incubation period and were taken as the lowest product concentration at which there was no growth or virtually no growth, while the lowest concentration that yielded no growth after the subculturing was taken as the Minimum Fungicidal Concentration (MFC). The assay was repeated three times.

Finally, cytotoxicity activity was investigated on VERO cell line ATCC CRT-1586 using a rezasurin-based assay as previously described by Mosmann (1983). The results were expressed as a percentage of viability of the control cells and CC_{50} values were calculated as a sigmoidal dose-response curve using GraphPad Prism 4.03 software using the nonlinear regression log (inhibitor) vs. response algorithm.

3. RESULTS AND DISCUSSION

3.1. Phytochemical study

The chemical investigations of the stem bark and roots of the medicinal plant *Ficus sycomorus* led to the isolation and identification of twenty-four distinct compounds (Figure 3) including ten triterpenoids belonging to five different classes including one taraxastane-type triterpenoid named epi-ψ-taraxastanonol or 3-oxo-20*R*-hydroxytaraxastane (**1**) (Anjaneyulu et al., 1999), two ursane-type triterpenoids α-amyrin acetate (**2**) and ursolic acid (**3**) (Tabekoueng et al., 2020; Wouamba et al., 2020), three oleanane-type triterpenoids β-amyrin acetate (**4**), oleanolic acid (**5**) and 2-*O*-trans-*p*-coumaroyl maslinic acid (**6**) (Yan et al., 2014), one friedelane-type triterpenoid named canophyllol (**7**) (Ngouamegne et al., 2008), as well as three lupane-type triterpenoids lupeol (**8**), betulinic acid (**9**) and lupeol acetate (**10**) (Javed et al., 2021; Mbougnia et al., 2021). Additionally to the ten triterpenoids, we isolated one diterpenoid called ent-kauran-2β,3α,16α-triol (**11**) (Dongmo et al., 2019); five steroids including stigmasterol (**12**), stigmast-22-ene-3,6-dione (**13**) (Lima et al., 2013), stigmast-7-en-3-one (**14**) (Wu et al., 1990), β-sitosterol (**15**) and β-sitosterol-3-O-β-D-glucopyranoside (**16**) (Mbougnia et al., 2021); four flavonoids among which three isoflavones namely alpinumisoflavone (**17**) (Kuete et al., 2008), derrone (**18**) (Chibber & Sharma, 1980) and 3'-(3-methylbut-2enyl)-biochanin A (**19**) (Abiy et al., 1998), as well as one flavone named atalantoflavone (**20**) (Nsangou et al., 2021). Further compounds have been characterized as one benzoquinone identified as 2,6-dimethoxybenzoquinone (**21**) (Dongmo et al., 2019); one phenylethanol derivative named 2-(4-hydroxyphenyl)-ethyldocyoctadecanoate (**22**) (Acevedo et al., 2000) and two peptide derivatives viz. asperphenamate (**23**) and asperglaucide (**24**) (Popwo et al., 2019).

The results showed that the plant produces chemical constituents with a high structural diversity from terpenoid derivatives (triterpenoids and steroids) identified as the major classes of compounds to flavonoids and other phenolic compounds. The significant change observed in the core structures from one compound to another is mainly oxidation of some functional groups and it might play an important role in their potencies and the understanding of their action mechanisms (Dzouemo et al., 2022; Happi et al., 2020).

3.2. Antimicrobial and cytotoxicity activities of the isolated compounds

Eleven compounds (**1**–**4**, **10**, **13**, **14**, **17**– **19** and **22**), the crude methanolic extracts of stem bark and roots as well as the EtOAc fraction from stem bark have been tested for their potency against eight bacterial strains and seven fungal strains as well as for their cytotoxicity against the VERO cell line ATCC CRT-1586.

The antibacterial assays have been carried out against bacterial strains listed in section 2.4 and ciprofloxacin was the standard drug. The results (Table 1) indicated that compound **18** showed good activity against *Escherichia coli* (MIC = 31.25 μg/ml) and moderate potency against *Salmonella typhimurium* cpc and *Pseudomonas aeruginosa* (MIC = 125 μg/ml, each). Compounds **10**, **13** and **14** showed moderate activities on almost all the tested strains and compound **13** was the most potent against *E. coli* (MIC = 125 μg/ml). All the two crude extracts demonstrated moderate activities against almost all the tested strains (MIC = 250 μg/ml, MBC/MIC = 2) on *S. enteritidis* and *E. coli*. These results showed that the other compounds in the extracts might play an antagonistic effect on compound **18** (MIC = 31.25 μg/ml) which is the most active we found so far in this study.

Furthermore, the results of the antifungal activity (Table 2) showed that the methanol extract of the stem bark exhibited good activity against *Candida albicans* with a MIC value of 31.5 μg/ml and a fungicidal effect with an MFC/MIC ratio of 4. Almost all the isolated compounds showed weak activity against some yeast and dermatophytes with MIC values from 125 μg/ml to 500 μg/ml and fungicidal with an MFC/MIC ratio of either 2 or 4. The good activity of the methanol extract from the stem bark may justify its use in folk medicine for the treatment of infectious illnesses.

Finally, the samples were evaluated for their cytotoxic activity on VERO cell line ATCC CRT-1586. Their potencies are reported in Table 3. According to the results, all the tested extracts showed cell viability greater than 100 μg/ml, indicating that they have no risk for living cells. Compounds **18** and **19** showed the higher inhibition of cell lines with CC_{50} values of 52.60 μg/ml and 69.10 μg/ml, respectively, indicating that they have a risk for living cells.

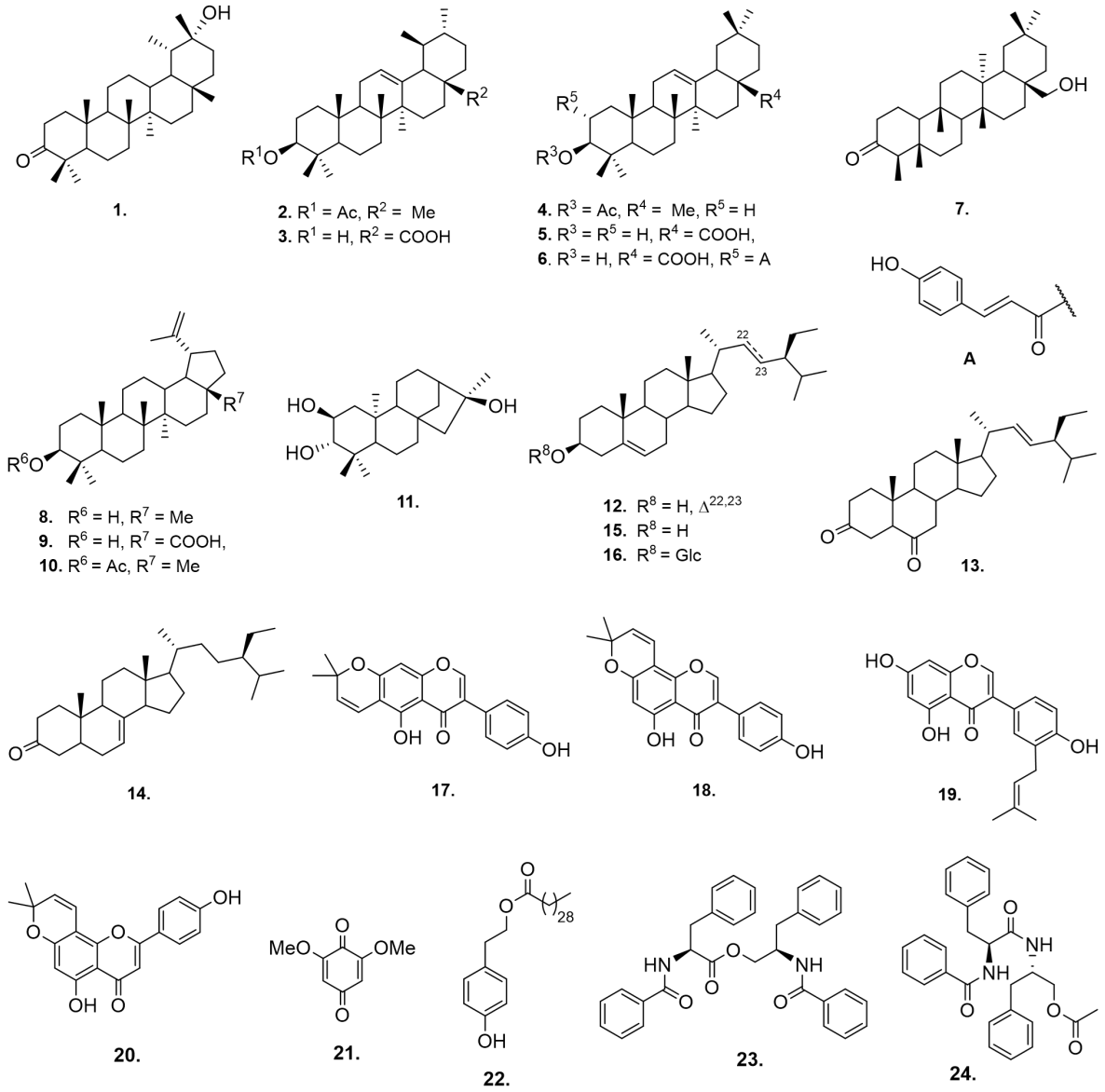

Figure 3. Structures of compounds **1**– **24** from stem bark and roots of *F. sycomorus*.

Table 1
Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of root extract, stem bark extract and isolated compounds

Samples	Parameters	STM	SE_{cpc}	ST_{cpc}	SAMR	SA	KP	EC	PA
Methanolic Root extract	MIC (μg/mL)	500	250	500	500	500	500	250	500
	MBC (μg/mL)	>500	500	>500	>500	>500	>500	500	>500
	MBC/MIC	ND	2	ND	ND	ND	ND	2	ND
Methanolic Stem bark extract	MIC (μg/mL)	>250	>250	>250	>250	>250	>250	>250	>250
	MBC (μg/mL)	ND	ND	ND	ND	ND	ND	ND	ND
	MBC/MIC	ND	ND	ND	ND	ND	ND	ND	ND
EtOAc fraction of the stem bark	MIC (μg/mL)	>250	>250	>250	>250	>250	>250	>250	>250
	MBC (μg/mL)	ND	ND	ND	ND	ND	ND	ND	ND
	MBC/MIC	ND	ND	ND	ND	ND	ND	ND	ND
1	MIC (μg/mL)	>125	>125	>125	>125	>125	>125	>125	>125
	MBC (μg/mL)	ND	ND	ND	ND	ND	ND	ND	ND
	MBC/MIC	ND	ND	ND	ND	ND	ND	ND	ND
2 + 4	MIC (μg/mL)	500	500	500	250	500	500	500	250
	MBC (μg/mL)	>500	>500	>500	500	>500	>500	>500	500
	MBC/MIC	ND	ND	ND	2	ND	ND	ND	2
3	MIC (μg/mL)	>125	>125	125	>125	>125	>125	>125	>125
	MBC (μg/mL)	ND	ND	250	ND	ND	ND	ND	ND
	MBC/MIC	ND	ND	2	ND	ND	ND	ND	ND
10	MIC (μg/mL)	250	500	500	250	500	500	500	250
	MBC (μg/mL)	500	>500	>500	500	>500	>500	>500	500

Continued on next page

Table 1 continued

	MBC/MIC	2	ND	ND	2	ND	ND	ND	2
13	MIC (μg/mL)	500	250	250	500	500	500	125	250
	MBC (μg/mL)	>500	500	500	>500	>500	>500	250	500
	MBC/MIC	ND	2	2	ND	ND	ND	2	2
14	MIC (μg/mL)	500	500	500	500	> 500	500	> 500	500
	MBC (μg/mL)	>500	>500	>500	>500	ND	>500	ND	>500
	MBC/MIC	ND	ND	ND	ND	ND	ND	ND	ND
17	MIC (μg/mL)	500	500	500	500	> 500	500	> 500	> 500
	MBC (μg/mL)	>500	>500	>500	>500	ND	>500	ND	ND
	MBC/MIC	ND	ND	ND	ND	ND	ND	ND	ND
18	MIC (μg/mL)	>125	>125	125	>125	>125	>125	31.25	125
	MBC (μg/mL)	ND	ND	250	ND	ND	ND	125	250
	MBC/MIC	ND	ND	2	ND	ND	ND	4	2
19	MIC (μg/mL)	500	500	500	250	500	500	500	500
	MBC (μg/mL)	>500	>500	>500	500	>500	>500	>500	>500
	MBC/MIC	ND	ND	ND	2	ND	ND	ND	ND
22	MIC (μg/mL)	250	500	>500	250	>500	500	500	500
	MBC (μg/mL)	500	>500	ND	500	ND	>500	>500	>500
	MBC/MIC	2	ND	ND	2	ND	ND	ND	ND
Ciprofloxacin	MIC (μg/mL)	2	1	0.5	1	1	0.5	0.5	0.5
	MBC (μg/mL)	4	2	1	2	2	1	2	2
	MBC/MIC	2	2	2	2	1	2	4	4

STM: *Salmonella typhimurium* cpc ; SE: *Salmonella enteritidis* cpc ; ST: *Salomonella typhi* cpc ; SAMR: *Staphylococcus aureus* MR ; SA: *Staphylococcus aureus* (ATCC25922); KP: *Klessiella pneumonae* (ATCC13883) ; EC: *Escherishia coli* (ATCC35218); PA: *Pseudomonas aeruginosa*. ND: not determined. MIC = Minimum inhibitory concentration; MBC = Minimum bactericidal concentration; MBC/MIC; The ratio MBC/MIC determine the bactericidal (MBC/MIC $\leq$ 4) or bacteriostatic (MBC/MIC > 4) effects of extracts. The antibacterial activity of a sample of plant-isolated compounds is strong, moderate, or weak if their MIC was $\leq$10, 10—100, or >100 μg/mL, respectively. However, the activity of plant extracts will be classified as significant (MIC < 100 μg/mL), moderate (100—625 μg/mL), or weak (MIC > 625 μg/mL). (Kuete et al., 2010).

Table 2
Minimum Inhibitory Concentrations (MIC) and Minimum Fungicidal Concentrations (MFC) of root extract, stem bark extract and isolated compounds.

Samples	Parameters	CA	CK	CP	CN	TM	MA	EF
Methanolic root crude extract	MIC (μg/mL)	500	500	500	500	500	500	500
	MFC (μg/mL)	>500	>500	>500	>500	>500	>500	>500
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND
Methanolic stem bark crude extract	MIC (μg/mL)	31.25	>250	>250	>250	>500	>500	>500
	MFC (μg/mL)	125	ND	ND	ND	ND	ND	ND
	MFC/MIC	4	ND	ND	ND	ND	ND	ND
Ethyl acetate fraction of stem bark	MIC (μg/mL)	250	500	500	250	> 500	> 500	> 500
	MFC (μg/mL)	500	>500	>500	500	ND	ND	ND
	MFC/MIC	2	ND	ND	2	ND	ND	ND
1	MIC (μg/mL)	>125	>125	125	>125	>500	>500	>500
	MFC (μg/mL)	ND	ND	>500	ND	ND	ND	ND
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND
2 + 4	MIC (μg/mL)	500	500	500	500	500	500	250
	MFC (μg/mL)	>500	>500	>500	>500	>500	>500	500
	MFC/MIC	ND	ND	ND	ND	ND	ND	2
3	MIC (μg/mL)	>125	>125	>125	>125	>500	>500	>500
	MFC (μg/mL)	ND	ND	ND	ND	ND	ND	ND
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND
10	MIC (μg/mL)	500	500	500	250	500	500	500
	MFC (μg/mL)	>500	>500	>500	500	>500	>500	>500
	MFC/MIC	ND	ND	ND	2	ND	ND	ND
13	MIC (μg/mL)	500	>500	500	500	>500	500	>500
	MFC (μg/mL)	>500	ND	>500	>500	ND	>500	ND
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND
14	MIC (μg/mL)	> 500	> 500	250	500	500	500	500
	MFC (μg/mL)	ND	ND	500	>500	>500	>500	>500
	MFC/MIC	ND	ND	2	ND	ND	ND	ND
17	MIC (μg/mL)	250	> 500	500	250	> 500	500	500
	MFC (μg/mL)	500	ND	>500	500	ND	>500	>500
	MFC/MIC	2	ND	ND	2	ND	ND	ND
18	MIC (μg/mL)	125	125	>125	>125	>500	>500	>500

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Table 2 continued

	MFC (μg/mL)	>500	>500	ND	ND	ND	ND	ND
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND
19	MIC (μg/mL)	500	125	500	125	500	250	500
	MFC (μg/mL)	>500	250	>500	250	>500	500	>500
	MFC/MIC	ND	2	ND	2	ND	2	ND
21	MIC (μg/mL)	500	500	>500	>500	>500	>500	>500
	MFC (μg/mL)	>500	>500	ND	ND	ND	ND	ND
	MFC/MIC	ND	ND	ND	ND	ND	ND	ND
22	MIC (μg/mL)	>500	250	500	250	500	250	>500
	MFC (μg/mL)	ND	500	>500	500	>500	500	ND
	MFC/MIC	ND	2	ND	2	ND	2	ND
Nystatin	MIC (μg/mL)	0.5	0.25	1	0.25			
	MFC (μg/mL)	2	1	2	1			
	MFC/MIC	4	0.25	2	0.25			
Griseofulvin	MIC (μg/mL)					0.25	1	0.5
	MFC (μg/mL)					1	2	2
	MFC/MIC					0.25	2	4

MIC : Minimum Inhibitory Concentrations, MFC : Minimum Fungicidal Concentrations, ND : Not determined, CA : *Candida albicans*, CK : *Candida krusei*, CP : *Candida parasilosis*, CN : *Cryptococcus neoformans*, TM : *Trichophyton mentagrophytes*, MA : *Microsporium audouinii*, EF : *Epidermophyton flocosum*.

Table 3
Cytotoxicity assay against vero cell line ATCC CRT-1586

Samples	CC_{50} (μg/mL)
1	>100
2	>100
3	>100
13	>100
14	>100
17	>100
18	52.60
19	69.10
Methanol crude extract of stem bark	>100
EtOAc fraction of the stem bark	>100
EtOAc/MeOH fraction the stem bark	>100
Methanol crude extract of root	>100
Podophyllotoxin	1.77

Values are expressed as means ± SEM. Each test was performed in triplicate

3.3. Chemophenetic significance of the study

In the present work, we have reported the identification of twenty-four known extractives from *F. sycomorus*, including ten pentacyclic triterpenoids (**1**–**10**), one diterpenoid (**11**), five sterols (**12**– **16**), four flavonoids (**17**–**20**), one benzoquinone (**21**), one phenyl ethanol derivative (**22**) and two peptide derivatives (**23**– **24**). Based on an extensive literature survey, and to the best of our knowledge, compounds **6**, **11** and **22** are reported herein for the first time from Moraceae family and compound **20** from *Ficus* genus. However, the four compounds have been already isolated from other plants species including *Hippophae rhamnoides* L. (Elaeagnaceae) (Yang et al., 2007), *Psiadia punctulata* (DC.) (Asteraceae) (Piaz et al., 2018) and *Buddleja cordata* (Scrophulariaceae) (Acevedo et al., 2000), respectively.

Pentacyclic triterpenes (**2**–**9**) constituted the main class of secondary metabolites herein reported, which were already obtained from several *Ficus* species. Thus, compounds **2**– **5** and **7** were reported from *F. aripuanensis* (Nascimento et al., 1999), compound **8** from *F. natalensis* (Mbougnia et al., 2021), *F. benjamina* (Simo et al., 2008), *F. pseudopalma* (Santiago & Mayor, 2014), *F. polita* (Kamga et al., 2010) and *F. auriculata* (El-Fishawy et al., 2011). Moreover, compound **9** was reported from *F. natalensis* (Mbougnia et al., 2021), *F. benjamina* (Simo et al., 2008), *F. polita* (Kamga et al., 2010), *F. auriculata* Lour (El-Fishawy et al., 2011) and *F. conraui* (Kengap et al., 2011), while compound **10** was reported from *F. racemosa* (Kosankar & Aher, 2018), *F. sansibarica* (Awolola et., 2014), *F. thonningii* (Ango et al., 2015) and *F. mucuso* (Bankeu et al., 2010). From this evidence, we can partially conclude that the genus *Ficus* is a rich source of different classes of triterpenoids including ursane-, lupane- and oleanane-type triterpenoids as the most prominent in the genus.

Steroids **12** and **14**– **16** are the main constituents of many species and were reported from many *Ficus* species (Awolola et al., 2018). They were also reported from another family including *Cola rostrata* (Malvaceae) (Dongmo et al., 2019). Flavonoids are also highly represented in *Ficus* genus. Compounds **17** and **18** were reported from many *Ficus* species e.g., *F. nymphaefolia* (Darbour et al., 2007) and *F. auriculata* (Qi et al., 2018) and compound **19** was reported from *F. tikoua* (Liao et al., 2015) and (Darbour et al., 2007). These isoflavones were also reported from *Erythrina* species (Fabaceae) e.g., *E. senegalensis* (Lee et al., 2009; Nkengfack et al., 2001) and *E. lysistemon* (Mvondo et al., 2011). Compound **20** was newly discovered from *Ficus* genus, it could be used to distinguish *F. sycomorus* from the other species. Compounds **23** and **25** were previously reported from *Ficus exasperata* (Popwo et al., 2019).

4. CONCLUDING REMARKS

In our search for antimicrobial and cytotoxic compounds from Cameroonian medicinal plants, we have isolated and characterized twenty-four compounds from the stem bark and roots of *F. sycomorus*, a medicinal plant used in folk medicine for the treatment of microbial illnesses. The results of the biological evaluations performed indicated that some compounds including lupeol acetate (**10**), stigmast-22-ene-3,6-dione (**13**), stigmast-7-en-3-one (**14**) and derrone (**18**) exhibited good to moderate activity ranging from MIC values 31.25 μg/ml to 125 μg/ml, while only derrone (**18**) and 3'-(3-methylbut-2enyl)-biochanin A (**19**) showed important inhibition of the VERO cell line ATCC CRT-1586 with CC_{50} values of 52.60 μg/ml and 69.10 μg/ml, respectively. From this evidence, derrone (**18**) was the most active compound from the extract and the other compounds in extract might play an antagonistic effect on its effectiveness. Additionnaly, although compound **18** display promising antimicrobial activity, it might have a risk for living cells due to its higher inhibition of VERO cells. These insights indicate that derrone (**18**) might be a lead compound that deserves further pharmaceutical investigations and chemical derivatization to reduce its toxicity and further increase its potency as much as possible. Moreover, it seemed obvious to the best of our knowledge that 2-O-trans-p-coumaroyl maslinic acid (**6**), ent-kauran-2β,3α,16α-triol (**11**) and 2-(4-hydroxyphenyl)-ethyldocyoctadecanoate (**22**) are reported for the first time from Moraceae family whereas atalantoflavone (**20**) is isolated herein for the first time from the genus *Ficus*. The results of this study extend the number of the chemical constituents of *F. sycomorus* and support its use in traditional medicine for the treatment of some microbial infections.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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A. APPENDIX. SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTIONS

Research concept and design : VSMM, ENH; Collection of data : WDTT; Data analysis and interpretation: MFN, GMH, AFKW, ENH; Writing the article : VSMM, MFN, GMH; Supervision, critical revision : GMH, AFKW ; Final approval of the article : ENH.

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