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Phytochemical analysis and *in vitro* antimicrobial screenings of the methanolic stem bark extract and constituents of *Parkia bicolor* A. Chev. (Leguminosae)

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ABSTRACT

The phytochemical study of the methanolic extract of the stem bark of *Parkia bicolor* A. Chev. (Leguminosae) led to the isolation and characterization of ellagic acid (**1**), 3,3′-dimethoxyellagic acid (**2**), 3,3′,4,4′-tetramethoxyellagic acid (**3**), 3-glucopyranoside-3′-methoxyellagic acid (**4**), gallic acid (**5**), methylgallate (**6**), oleanolic acid (**7**), erythrodiol (**8**), β-amyrin (**9**), β-sitosterol (**10**), stigmasterol (**11**) and stigmasterol-3-*O*-β-D-glucopyranoside (**12**). The structures of these compounds were determined by spectroscopic analyses of 1D and 2D NMR and EI- and ESI-MS and comparison with the reported data. Compounds **1**-**4** are reported here from this species for the first time. The crude methanolic extract and isolated compounds **1**-**6** were submitted to micro dilution assay against the Gram negative bacteria *Escherichia coli* ATCC 8739 and *Salmonella typhi* clinical strain as well as the Gram positive bacterium *Staphylococcus aureus* clinical strain. While the methanolic extract showed moderate to significant antibacterial activity against above tests strain with MICs in the range of 64-128 µg/mL, compound **4** displayed the best activity against *Salmonella typhi* and *Staphylococcus aureus* clinical strains with an MIC of 33.4 µM against both, compared to gentamycin with 0.1-2.0 µM. Compounds **1**-**3** and **5**, **6** showed moderate to weak antibacterial activity with MICs in the range of 44.6 ± 0.9 to 376.4 ± 1.5 µM. MBCs were above the MICs in the range of 128-512 µg/mL for the methanolic extract resulting in MBCs/MICs in the range of 2 to 8. In antifungal assays, the methanolic extract when tested at 50 mg/mL gave growth inhibition of 34.3, 31.3, 28.3, 33.1, 30.6 and 22.4 % against *Aspergillus flavus* strain 1, *Aspergillus flavus* strain 2, *Aspergillus niger*, *Aspergillus gandidus*, *Absidia sp*. and *Penicillium* sp., respectively. When tested at 2 mg/mL, 4-glucopyranoside-4′-methoxyellagic acid (**4**) showed a moderate inhibition potency against all the fungal strains with growth inhibition of 24.50 to 40.40% compared to fluconazole with 100% at 2 mg/mL.

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1. Introduction

Infectious diseases such as typhoid fever, diarrhoea, urinary and pulmonary infections as well as wound infections are a global public health problem, caused by enterobacteria that have developed multiple resistances due to excessive use of synthetic antibiotics (WHO, 2017). Moreover, the daily intake of antibiotics places great selective pressure on bacteria to develop multidrug resistance (Michiels et al., 2016). Botanical therapies are a mixture of many active and non-active components from medicinal plants potentially having several modes of action, both direct and synergistically, which could help to decrease multiple resistances. Thus, the search for new antimicrobial agents for the management of infections is of increasing interest because of the intensely growing resistance of bacteria to antibiotics. The genus *Parkia* is in the family Leguminosae, which is a major plant group of Angiosperms or flowering plants, with *Parkia bicolor* A. Chev. as one of its species occurring in the Cameroonian rain forest as well as other parts of the Sub-Saharan forest belt (The Plant List, 2013). The genus has pantropical distribution with about 35 species (Hopkins, 1986). Being a tree usually found in the forest, it can also survive in the Savannah along marshy valleys and in gallery forests, always as solitary specimen and up to 30 m high (Hagos, 1962). It is a tree with an open, widely spreading crown. The cylindrical bole is usually straight but sometimes twisted

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or curved and can be up to 1.5 m in diameter. Its wood is exploited commercially, while bark, leaves and roots serve for medicinal preparations. The plant is usually not felled when rain forest is converted to agriculture land due to its usefulness as a shade tree for crops as well as its nutritious edible fruits and seeds. In the course of the Green Corridor Project dedicated to replant the tropical zone across the African continent, *P. bicolor* is among the nine tree species out of twenty-eight that survived in the Savannah until today (Matsuzawa, 2007).

In the Northern parts of Nigeria and Ivory Coast as well as in other parts of Western and Central Africa, pulverized stem bark of *P. bicolor* is applied in the treatment of wounds and treatment of dental disorders, while an infusion is used as tonic against diarrhoea and dysentery (Duker-Eshun et al., 2001; Ajaiyeoba, 2002; Lawal et al., 2010; Abioye et al., 2013). Bark maceration is also applied to treat eye complaints, a decoction to treat toothache, dried and powdered bark to enhance healing of wounds and sores, and a vapour bath of the bark to treat rheumatism (Tchinda, 2008). There is a close genetic relationship with *P. biglobosa* (Jacq.) G. Don and *P. filicoidea* Oliv., the latter two occurring in the same area and beyond, while *P. madagascariensis* is endemic to Madagascar.

Reported traditional medicinal use of the genus *Parkia* against infections motivated us to investigate the bark of *P. bicolor* closely for its antimicrobial potential. Previous phytochemical studies of this species revealed the presence of triterpenoids, xanthones and tannins from root extracts (Fotie et al., 2004). The present study focuses on the investigation of the chemical composition of the methanolic extract from the stem bark of *P. bicolor* and on the evaluation of its antibacterial and antifungal activities.

2. Experimental

2.1. General experimental procedures

Ultraviolet spectra were recorded on a Hitachi UV 3200 spectrophotometer in MeOH. Infrared spectra were recorded on a JASCO 302-A spectrophotometer. EI-MS (Electronic Impact-Mass Spectra) were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorokerosene as reference substance for HR-EI-MS (High Resolution-Electronic Impact-Mass Spectrometry) and HR-ESI-MS (High Resolution-Electrospray Ionization-Mass Spectra) were measured on Agilent Techn. 6220 TOF LCMS mass spectrometer (Agilent Technologies, Santa Clara, CA, USA).

The NMR (Nuclear Magnetic Resonance) spectroscopic analyses were performed in CD_3OD or $CDCl_3$ solution on a Bruker AMX 600 NMR spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C). Methyl, methylene and methane carbons were distinguished by DEPT (Distortionless Enhancement by Polarization Transfer) experiments. Homonuclear ^1H connectivities were determined by using the COSY (COrrelation SpectroscopY) experiment. ^1H-^{13}C one-bond connectivities were determined with HSQC (Heteronuclear Single Quantum Correlation) gradient pulse factor selection. Two- and three-bond connectivities were determined by HMBC (Heteronuclear Multiple Bond Connectivity) experiments. Chemical shifts are reported in δ (ppm) using TMS as internal standard and coupling constants (*J*) were measured in Hz. Column chromatography was carried out on silica gel (70-230 mesh, Merck). Thin Layer Chromatography (TLC) was performed on Merck pre-coated silica gel 60 F254 aluminium foil, and spots were detected using ceric sulphate spray reagent. The purity of compounds was investigated by the analytical HPLC on a Dionex UPLC 3000 (Thermoscientific, UK) HPLC coupled with a photo-diode-array (PDA) detector (Thermoscientific). The degree of purity of the positive control compound was ≥ 98%, while that of the isolated compounds was ≥ 95%. All reagents used were analytical grade.

2.2. Plant material

Stem bark of *Parkia bicolor* was collected in the West Region of Cameroon, locality of Foumban, Malantouen, GPS coordinates 5° 43′ 35.8″ North, 10° 53′ 55.1″ East, at an altitude of 1180 m, in March 2015 and identified by Mr. Victor Nana, botanist at the Cameroon National Herbarium, Yaoundé, where a voucher was deposited under reference number 50914/CNH.

2.3. Extraction and bioassay-guided isolation

The stem bark was cut, washed and dried in the shade for 14 days at 25 °C and powdered. The air-dried, powdered stem bark (2.5 kg) was extracted with MeOH at room temperature for 48 h and then concentrated under reduced pressure to yield 93.7 g of a solid dark black extract. The extract was subjected to column chromatography over silica gel using a mixture of hexane-EtOAc (2/5), hexane-EtOAc (1/1), EtOAc, and EtOAc-MeOH (9.5/0.5) in increasing polarity resulting in 4 major fractions A-D. Fraction A (15.5 g) was composed of sub-fractions 1-25 and eluted with *n*-hexane/EtOAc (5/1) to yield a mixture of β-sitosterol (**10**) and stigmasterol (**11**) (18.5 mg), while fraction B (8.6 g) was composed of sub-fractions 26-54 and eluted with *n*-hexane/EtOAc (5/3) leading to β-amyrin (**9**) (15.4 mg) and erythrodiol (**8**) (5.6 mg). Fraction C (23.6 g) resulting from sub-fractions 55-105 was eluted with 50% *n*-hexane/EtOAc gradually up to 100% EtOAc delivering 3,3′-dimethoxyellagic acid (**2**) (23.6 mg), 3,3′,4,4′-tetramethoxyellagic acid (**3**) (24.9 mg) and methylgallate (**6**) (12.9 mg).

Finally, fraction D (27.1 g) was composed of sub-fractions 106-254 followed by elution with 100% EtOAc and a mixture of EtOAc/MeOH (10/1) resulting in stigmasterol-3-*O*-β-D-glucopyranoside (**12**) (23.0 mg), oleanolic acid (**7**) (15.6 mg), 4-glucopyranoside-4′-methoxyellagic acid (**4**) (17.5 mg), gallic acid (**5**) (21.5 mg) and ellagic acid (**1**) (24.1 mg).

Ellagic acid **(1)**

Yellowish powder (DMSO-d_6); R_f = 0.21, silica gel 60

F254, EtOAc/MeOH (10.5/0.5); UV (MeOH) λmax (log ε) 257 (3.45), 283 (3.47), 359 (3.48) nm; IR (KBr) υmax 3425 (OH), 1750 (C=O), 1620 (C=C), 1090 (C-O) cm^{-1}; ^1H NMR data: δ*H* 7.18 (1H, s, H-5/H-5′) and ^{13}C NMR data : δ*C* 110.9 (C-1/C-1′), 136.1 (C-2/C-2′), 139.3 (C-3/C-3′), 149.0 (C-4/C-4′), 109.9 (C-5/C-5′), 113.8 (C-6/C-6′), 159.2 (C-7/C-7′); EI-MS *m/z* (%) = 302 $[M]^+$ (100); HR-EI-MS: *m/z* 302.1920 $[M]^+$ (calcd for $C_{14}H_6O_8$, 302.1926) (Ye et al., 2007).

3,3′-Dimethoxy ellagic acid **(2)**

Yellowish powder (DMSO-d_6); R_f = 0.30, silica gel 60 F254, EtOAc/MeOH (10.5/0.5); UV (MeOH) λmax (log ε) 260 (3.40), 290 (3.50), 356 (3.46) nm; IR (KBr) υmax 3430 (OH), 1750 (C=O), 1630 (C=C), 1060 (C-O) cm^{-1}; ^1H NMR data : δ*H* 7.53 (1H, s, H-5/H-5′), 4.02 (MeO-3/3′) and ^{13}C NMR data : δ*C* 112.0 (C-1/C-1′), 142.0 (C-2/C-2′), 141.1 (C-3/C-3′), 153.2 (C-4/C-4′), 112.0 (C-5/C-5′), 113.1 (C-6/C-6′), 160.0 (C-7/C-7′), 62.2 (MeO-3/3′); ESI-MS *m/z* (%) = 331 $[M + H]_+$ (89); HR-ESI-MS: *m/z* 331.0448 $[M+H]^+$ (calcd. $C_{16}H_{10}O_8$, 331.0454) (Ye et al., 2007).

3,3′,4,4′-tetramethoxyellagic acid **(3)**

Yellowish powder (DMSO-d_6); R_f = 0.35, silica gel 60 F254, EtOAc/MeOH (10.5/0.5); UV (MeOH) λmax (log ε) 262 (3.41), 291 (3.52), 356 (3.45) nm; IR (KBr) υmax 1748 (C=O), 1630 (C=C), 1062 (C-O) cm^{-1}; ^1H NMR data : δ*H* 7.63 (^1H, s, H-5/H-5′), 4.09 (MeO-3/3′), 4.02 (MeO-3/3′) and ^{13}C NMR data : δ*C* 112.6 (C-1/C-1′), 141.6 (C-2/C-2′), 141.3 (C-3/C-3′), 154.8 (C-4/C-4′), 110.0 (C-5/C-5′), 113.1 (C-6/C-6′), 158.8 (C-7/C-7′), 62.2 (MeO-3/3′), 57.3 (MeO-4/4′); ESI-MS *m/z* (%) = 359 [M + H]$^+$ (100); HR-ESI-MS: *m/z* 359.0753 $[M+H]^+$ (calcd. $C_{18}H_{14}O_8$, 359.0761) (Ye et al., 2007).

3-Glucopyranoside-3′-methoxy ellagic acid **(4)**

Yellowish powder (DMSO-d_6); R_f = 0.25, silica gel 60 F254, EtOAc/MeOH (10/1); UV (MeOH) λmax (log ε) 260 (3.43), 292 (3.51), 358 (3.40) nm; IR (KBr) υmax 3500 (OH), 1748 (C=O), 1630 (C=C), 1062 (C-O) cm^{-1}; ^1H NMR data: δ*H* 7.52 (1H, s, H-5/H-5′), 4.03 (MeO-3′), Glc. 5.14 (1H, d, 7.1 Hz, H-1″), 3.40 (1H, m, H-2″), 3.35 (1H, m, H-3″), 3.25 (1H, m, H-4″), 3.45 (1H, m, H-5″), 3.52 (1H, m, H-6a″), 3.75 (1H, m, H-6b″) and ^{13}C NMR data : δ*C* 110.0/114.1 (C-1/C-1′), 141.6/140.9 (C-2/C-2′), 140.2/141.6 (C-3/C-3′), 152.7/151.1 (C-4/C-4′), 111.3/111.8 (C-5/C-5′), 112.5/111.9 (C-6/C-6′), 158.3/158.2 (C-7/C-7′), Glc 101.4 (C-1″), 73.2 (C-2″), 76.4 (C-3″), 69.4 (C-4″), 77.2 (C-5″), 60.5 (C-6″); ESI-MS *m/z* (%) = 479 $[M + H]^+$ (100); HR-ESI-MS: *m/z* 479.0815 $[M+H]^+$ (calcd. $C_{21}H_{19}O_{13}$, 479.0820) (Ye et al., 2007).

Gallic acid ***(5)***

White powder (DMSO-d_6); R_f = 0.48, silica gel 60 F254, EtOAc/MeOH (10.5/0.5); UV (MeOH) λmax (log ε) 214 (2.90), 270 (3.50) nm; IR (KBr) υmax 3300-3500 (OH), 2700-3200 (COOH), 1705 (C=O), 1540-1618 (C=C) cm^{-1}; ^1H NMR data: δ*H* 7.22 (1H, s, H-2/H-6), 12.55 (OH, s) and ^{13}C NMR data: δ*C* 108.2 (C-2/C-6), 119.8 (C-1), 136.6 (C-4), 143.8 (C-3/C-5), 167.0 (C-7); EI-MS *m/z* (%) = 170 $[M]^+$ (60); HR-EI-MS: *m/z* 170.0220 $[M]^+$ (calcd. $C_7H_6O_5$, 170.0225) (Liu et al., 2014).

Methylgallate **(6)**

White powder (DMSO-d_6); R_f = 0.55, silica gel 60 F254, EtOAc/MeOH (10.5/0.5); UV (MeOH) λmax (log ε) 220 (2.95), 267 (3.35) nm; IR (KBr) υmax 3300-3500 (OH), 2700-3200 (COOH), 1700 (C=O), 1500-1600 (C=C) cm^{-1}; ^1H NMR data: δ*H* 4.01 (MeO), 7.26 (1H, s, H-2/H-6) and ^{13}C NMR data: δ*C* 50.4 (MeO), 107.8 (C-2/C-6), 118.8 (C-1), 136.9 (C-4), 144.1 (C-3/C-5), 165.6 (C-7); EI-MS *m/z* (%) = 184 $[M]^+$ (45); HR-EI-MS: *m/z* 184.0345 $[M]^+$ (calcd. $C_8H_8O_5$, 184.0375) (Liu et al., 2014).

2.4. Bioassays

2.4.1. Microbiologic materials

The potential bioactivity of the methanolic extract was studied against microorganisms including two Gram negative bacteria, namely *Escherichia coli* ATCC 8739 and *Salmonella typhi* clinical isolate - human stool and the Gram positive bacterium *Staphylococcus aureus* clinical isolate. Fungal strains used for bioassays were *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus gandidus*, *Asidia* spp. and *Penicillium* spp. Above microbes were received from the Pasteur Institute of Cameroon. Stock cultures were maintained at 4 °C on Mueller Hinton and Potato Dextrose Agar, respectively.

2.4.2. *In vitro* antibacterial assay

In vitro antibacterial activity of the extract and isolated compounds was performed by determining the minimum inhibitory concentration (MIC) using the microdilution method (CLSI, 2015). Briefly, the stock solution of the methanolic extract and isolated compounds were dissolved in 2.5% dimethyl sulfoxide (DMSO) and distilled water, respectively. Bacterial suspensions of 1.5×108 CFU/mL corresponding to 0.5 Mc Farland turbidity standard were diluted 1:100 in Mueller Hinton broth to give 1.5 × 106 CFU/mL as inoculum. For the antibacterial susceptibility tests, serial two-fold dilution lines of the plant extract in 96 wells microtiter plates were done to obtain a final concentration range of 8 to 1024 µg/mL for the crude extract and 2 to 256 µg/mL for both, isolated compounds and the positive control. The total volume was 200 µL/well, each containing 100 µL of the respective test substance at a particular concentration and 100 µL of the bacterial suspension in Mueller Hinton broth. The microtiter plates were covered with parafilm and incubated at 37 °C for 18 hours. Bacterial growth was monitored colorimetrically using a solution of *p*-iodotetrazolium chloride 2% (INT). It should be noted that viable bacteria change the yellow dye of *p*-iodonitrotetrazolium violet to a pink colour. Thereby, the MIC is regarded as significant for an MIC <

100 µg/mL, moderate for an MIC > 100 and ≤ 625 µg/mL and low for an MIC > 625 µg/mL (Mouokeu et al 2011; Djeussi et al., 2013; Mouokeu et al., 2014).

Bactericidal concentrations were determined by adding 40 µL from wells indicating no bacterial growth into 160 µL of Mueller Hinton broth, introducing the mixture into new plates, and adding INT after incubation at 37 °C or 48 hours. Extract/compound concentrations not leading to colour reactions were considered as bactericidal and the lowest concentration of the extract associated with no bacterial culture (Salie et al., 1996; Djeussi et al., 2013). The MBC/MIC ratio was calculated and evaluated according to Djeussi et al. (2013). Tests were carried out in triplicate. Gentamycin was used as positive control, and 2.5% DMSO solution as negative control.

2.4.3. *In vitro* antifungal assay

The determination of fungal inhibition was performed by the agar incorporation technique (Lahlou, 2004). Briefly, the methanolic extract and isolated compounds were adjusted to 2.5% in DMSO and distilled water to obtain 50 mg/mL of stock solution. Agar medium was supplemented with the extract/respective compound when still hand-warm at different concentrations prepared previously, poured into petri dishes and left at rest for solidification. Mycelial disks were each placed in the center of a plate and incubated in reversed position at 28 °C. Average diameter of growth and percentage fungal inhibition were determined according to Djeugap (2011).

Under a laminar-flow hood (LFM 8472S) near the flame of a Bunsen burner, 250 mg of the methanolic extract was dissolved in 2.5 mL of DMSO at 2.5% adjusted with 7.5 mL of distilled water to obtain a 25 mg/mL solution, while the positive control and compounds were used at 2 mg/mL. Above various solutions were supplemented by super fusion medium between 40-50 °C in order to obtain decreasing concentrations. After homogenization, media consisting of 1 mL of extract or compound and 9 mL of SDA-medium were poured into 90 mm petri dishes and left to rest for solidification.

Mycelial disks of 5 mm-diameter from one day cultured fungal isolates were cut with a cookie cutter, lifted with a scalpel and each of them was attached to the centre of an agar plate. The plates were sealed with paraffin and incubated in reverse position at 28 ± 2 °C. Negative control I plates contained SDA-medium and the fungal disk, positive control plates SDA-medium and fluconazole solution, and for negative control II the extract/compound solution was replaced by DMSO. All tests were carried out in triplicate.

Mycelial growth diameters were recorded after 1 to 6 days. At day 6, the negative control agar plate displayed complete invasion for all fungal test strains. Measurements by ruler following two perpendicular lines passing through the centre of the agar plates. The average diameter was determined According to Djeugap (2011), as (Eqn. 1):

$$D = [(D1 + D2)/2] - De \quad \text{(Eqn. 1)}$$

where D is the average diameter of mycelial growth, D1 and D2 are the perpendicular diameters of mycelial growth and De is the explant diameter. Furthermore, the effect of the extract/compounds on radial growth was expressed as percentage of inhibition and determined according to the formula reported by Leroux et al. (1978) (Eqn. 2):

$$I\% = [(Dt - De)/Dt] \times 100 \quad \text{(Eqn. 2)}$$

, where %I is the inhibition percentage, Dt the mean diameter of mycelial growth of the negative control in mm and De the mean diameter of mycelial growth under the influence of the extract, compound or positive control in mm. Extracts/compounds are considered as very active for 75 to 100% inhibition of the fungus indicating a very sensitive fungal test strain, active for 50 to 75% inhibition of the fungus, moderately active for 25 to 50% inhibition of the fungus indicating its limit-sensitivity and low or not active for 0 to 25% inhibition of the fungus characterizing the fungus as insensitive or resistant (Paranagama et al., 2003).

2.5. Statistical analysis

The statistical analyses were generated using SPSS software 20. Statistical comparison was made using the one-way ANOVA test with a significance threshold set at $\alpha = 0.05$.

3. Results and Discussion

3.1. Isolation and characterization of secondary metabolites

The stem bark of *P. bicolor* was extracted with distilled water and MeOH. The methanolic extract was subjected to column chromatography carried out on silica gel and preparative thin layer chromatography (pTLC) to afford twelve known compounds (Fig. 1). By comparison with the reported data, the known compounds were identified as ellagic acid (**1**), 3,3′-dimethoxyellagic acid (**2**), 3,3′,4,4′-tetramethoxyellagic acid (**3**), 4-glucopyranoside-4′-methoxyellagic acid (**4**), gallic acid (**5**), methylgallate (**6**), oleanolic acid (**7**), erythrodiol (**8**), β-amyrin (**9**), β-sitosterol (**10**), stigmasterol (**11**) and stigmasterol-3-*O*-β-D-glucopyranoside (**12**) (Ye et al., 2007; Lui et al., 2014) (Fig. 1). It should be noted that a previous investigation of the dicholoromethane-methanol extract of the roots had resulted in lichexanthone (1-hydroxy-8-methyl-3,6-dimethoxyxanthone), lupeol, 1′-monoglyceride of octacosanoic acid, methyl gallate and gallic acid (Fotie et al. 2004). Furthermore, arrays of chemical constituents were reported from this genus including phenolic acids, alkaloids, saponins, terpenoids, cyclic polysulfides and tannins. Most abundant phytochemicals are gallic acid followed by the catechin, ellagic acid and quercetin. The terpenoids, β-sterol, squalene, campesterol, stigmasterol and the cyclic polysulfides 1,2,4-trithiolane, 1,2,4,6-tetrathiepane and 1,2,3,5-tetrathiane and lenthionine were also identified (Ahmad et al., 2019). Interestingly, compounds **2-4** are reported here for the

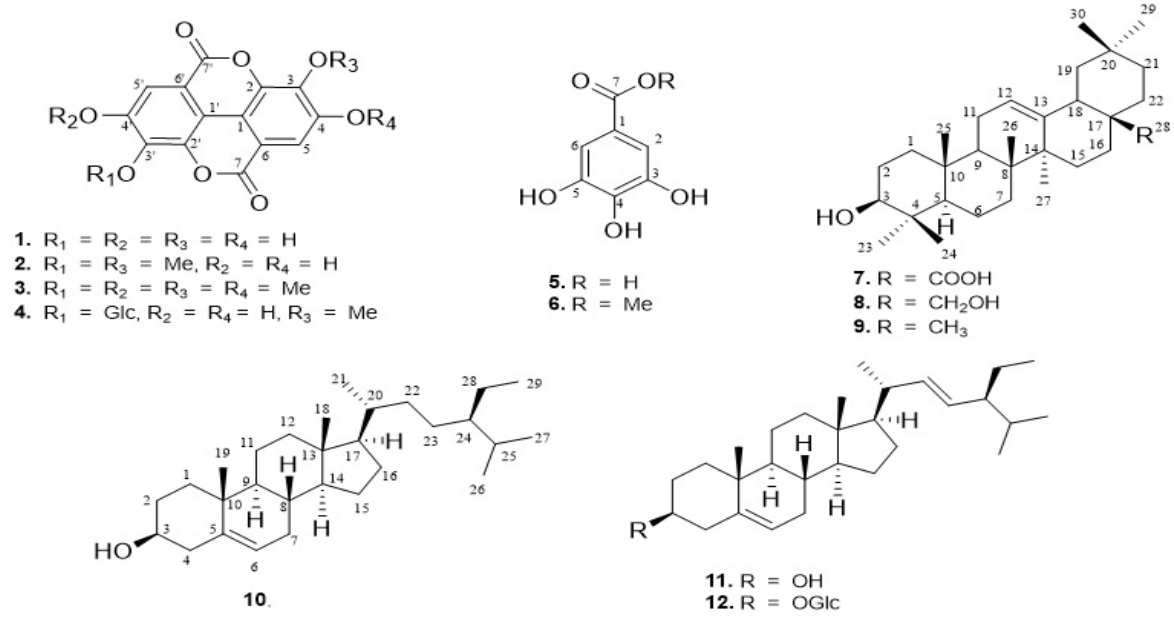

Fig. 1. Structures of isolated compounds from the stem bark of *Parkia bicolor*.

first time from the genus *Parkia* (Fotie et al. 2004). From a chemotaxonomic point of view, these compounds may help in the placement of species of the genus *Parkia* within the Leguminosae family.

3.2. Biologicals activities

The methanolic extract developed from the stem bark of the plant was tested for activity against the microbes *Escherichia coli* ATCC 8739 as well as *Salmonella typhi* and *Staphylococcus aureus* clinical strains by microdilution assay. Gentamycin was used as positive control. The methanolic extract gave moderate MICs of 64, 64 and 128 µg/mL, respectively. MBCs were above the MICs in the range of 256-512 µg/mL, compared to gentamycin with 0.1, 0.5 and 1 µM, respectively, resulting in MBC/MICs for extracts in the range of 4-8 and for the positive control of 2-8, as well (Table 1). For pure compounds, best activities against *Escherichia coli* ATCC 8739, *Salmonella typhi* and *Staphylococcus aureus* clinical strains were recorded with MICs of 44.6, 44.6 and 89.4 µM for 3,3′,4,4′-tetramethoxyellagic acid (**3**) and MICs of 133.4, 33.4 and 33.4 µg/mL for 3-glucopyranoside-3′-methoxyellagic acid (**4**). For 3,3′,4,4′-tetramethoxyellagic acid (**3**), MBCs values range from 89.4, 178.7 and 178.7 for *E. coli*, *S. thyphi* and *S. aureus* respectively with a MBC/MIC ranging from 2 to 4; and 3-glucopyranoside-3′-methoxyellagic acid (**4**) obtained MBCs values range from 66.9 to 133.4 respectively for *S. aureus*, *S. typhi* and *E. coli* with MBC/MIC ranging from 2 to 4. While MIC tests give here the lowest level of antimicrobial agent that inhibits growth, the MBC indicates the lowest level that results in microbial death: Even if an MIC shows inhibition, plating the bacteria onto agar can result in organism proliferation because the antimicrobial did not cause death. Antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC. It should be pointed out that though still routinely measured *in vitro* when determining the bioactive potential of crude extracts, fractions and pure compounds from plants, MBCs as well as MBC/MIC values have been advocated by some for treatment of serious infections such as endocarditis or for treatment of immunosuppressed patients. Their value has been controversial, and they are not widely performed in human or veterinary medicine (Sykes et al., 2014). Notably, activities kept close to those measured for the crude extracts indicating synergic processes between compounds, which means *per definitionem* that the effect of the compounds taken together is greater than the sum of their separate effect. It should be noted that the methanolic stem bark extracts of *P. biglobosa* and *P. bicolor* collected in Nigeria had been tested for activity against *Pseudomonas fluorescence* NCIB 3756, *Micrococcus luteus* NCIB 196 and *Proteus vulgaris* LIO, resulting in moderate to low MICs between 0.63 mg/mL and 5 mg/mL (Ajaiyeoba, 2002; Abioye et al., 2013). The methanolic stem bark extract of *P. bicolor* was as well tested *in vitro* against *Aspergillus flavus* strain 1 and 2, *Aspergillus niger*, *Aspergillus gandidus*, *Absidia* sp. and *Penicillium* sp. by nutrient poisoning method. Best activities were obtained for fluconazole with 100% at 2 mg/mL, while methanolic extract at 25 mg/mL showed moderate to weak fungal inhibition in the range of 22.4-34.3% compared to fluconazole. All tested compounds showed moderate to low antifungal effect with inhibition potency between 10.0 to 41.0 % compared to fluconazole with 100% at 2 mg/mL (Table 2). In general, the antifungal effect of the extracts of the stem

Table 1
In vitro antibacterial activity parameters of stem bark extracts of *Parkia bicolor*.

Compounds and extracts	MIC			MIC			MIC		
Test strain	EC	ST	SA	EC	ST	SA	EC	ST	SA
Methanolic	64	64	128	512	256	512	8	4	4
1	105.9 ± 0.5	216.8 ± 0.1	216.8 ± 0.3	216.8 ± 0.6	216.8 ± 0.3	433.6 ± 0.4	2	1	2
2	96.9 ± 0.2	96.9 ± 0.3	193.4 ± 0.6	193.4 ± 0.4	193.4 ± 0.1	193.4 ± 0.5	2	2	1
3	44.6 ± 0.9	44.6 ± 0.5	89.4 ± 0.8	89.4 ± 0.7	178.7 ± 0.4	178.7 ± 0.7	2	4	2
4	133.4 ± 1.2	33.4 ± 0.6	33.4 ± 0.9	133.4 ± 0.9	133.4 ± 0.5	66.9 ± 0.8	1	4	2
5	376.4 ± 1.5	376.4 ± 0.9	376.4 ± 0.9	376.4 ± 0.6	376.4 ± 0.6	753.0 ± 0.5	1	1	2
6	174.8 ± 0.1	174.8 ± 0.1	87.4 ± 0.2	379.0 ± 0.3	174.8 ± 0.2	174.8 ± 0.9	2	1	1
+ve control	0.1 ± 0.2	0.5 ± 0.1	1.0 ± 0.1	1.0 ± 0.3	2 .0 ± 0.4	2.0 ± 06	8	4	2

MIC = Minimum inhibitory concentration and MBC = Minimum bactericidal concentration in µg/mL for the extracts, positive control (+ve control) and in µM for the compounds; MBC/MIC = bactericidal or bacteriostatic effects; EC = *Escherichia coli* ATCC 8739; ST = *Salmonella typhi* clinical strain; SA = *Staphylococcus aureus* clinical strain; +ve control = gentamycine; -ve control = no antibiotic or extract added. MBC/MIC ≤ 4 bactericidal effect; MBC/MIC > 4 bacteriostatic effect (Djeussi et al., 2013).

Table 2
In vitro antifungal activity (% inhibition) of stem bark extracts of *Parkia bicolor*.

Compounds and extracts	Test strain					
	AF1	AF2	AN	AG	A sp.	P sp.
Methanolic	34.3 ± 1.1	31.3 ± 2.0	28.3 ± 3.1	33.1 ± 2.1	30.6 ± 3.0	22.4 ± 2.0
1	16.9 ± 1.5	17.1 ± 0.5	20.5 ± 0.8	21.3 ± 1.6	10.5 ± 1.3	15.2 ± 2.1
2	10.8 ± 1.1	12.0 ± 1.3	21.4 ± 0.8	21.5 ± 1.5	11.0 ± 0.1	16.5 ± 1.3
3	21.5 ± 1.2	10.7 ± 1.5	11.8 ± 1.8	14.4 ± 1.4	13.9 ± 0.9	15.4 ± 1.0
4	40.4 ± 1.1	25.1 ± 1.0	24.5 ± 0.9	30.1 ± 1.9	35.5 ± 1.5	38.9 ± 2.0
5	10.0 ± 1.0	11.5 ± 0.9	12.4 ± 1.0	19.8 ± 0.6	21.2 ± 1.2	20.4 ± 1.4
6	15.8 ± 0.2	16.5 ± 1.1	26.5 ± 1.2	28.2 ± 0.9	25.5 ± 1.1	21.6 ± 0.9
-ve control	90.0 ± 0.0	89.5 ± 0.3	88.0 ± 0.0	90.0 ± 0.0	89.0 ± 0.7	90.0 ± 0.0

AF1 = *Aspergillus flavus* strain 1, AF2 = *Aspergillus flavus* strain 2, AN = *Aspergillus niger*, AG = *Aspergillus gandidus*, A sp. = *Absidia sp.*, P sp. = *Penicillium sp.*, –ve control = negative control. All measurements were performed by antifungal test at 25 mg/mL for extracts and 2 mg/mL for fluconazole.

the growth of fungi following their adsorption on cell membranes, interaction with enzymes and effectors or deprivation in metal substrates and ions (Dhaouadi et al., 2010).

Finally, it should be noted that recent investigation had shown that ellagic acid (**1**) inhibits *in vitro* the growth of clinical *Helicobacter pylori* strains not responding well to or resistant to antibiotics (De et al., 2018); gallic acid (**5**) reduces membrane permeability of *Campylobacter jejuni* supporting antibiotic accumulation (Oh and Jeon, 2015; Shao et al., 2015) and can potentiate the antimicrobial activity of other antibiotics (Lee et al., 2014; Lima et al., 2016), while methyl gallate (**6**) exhibits significant biofilm inhibition and bacterial membrane damage in multi drug resistant *Vibrio cholerae*, showing its potential against severe secretory and inflammatory diarrhoeal disease as caused by this bacterium (Bag et al., 2019); oleanolic acid (**7**) affects multiple genes involved in the central metabolism of *Streptococcus mutans*, the main causative agent of human dental caries (Park et al., 2018); β-amyrin (**8**) is active against the plant pathogen fungus *Ascochyta rabiei*, the causative agent of chickpea blight (Jabeen et al., 2011), and erythrodiol (**12**) has antibacterial effects, reduces dental plaque and may be protective against tooth decay (de Cock, 2012). We suggest that antimicrobial activities of the methanolic stem bark extract of *Parkia bicolor* as reported here are based on the action of above secondary metabolites**1-6** and their synergistic action within the plant tissues. In fact, bioactivities received from various tropical plant sources indicate antibacterial and antifungal action of compounds **1**, **5-8** and **12** as compiled above, giving a ratio-

-nal for traditional anti-infectious use.

4. Concluding remarks

This study shows that the bark extract of *Parkia bicolor* is rich in some phytochemicals such ellagic acid, 3,3′-dimethoxyellagic acid, 3,3′,4,4′-tetramethoxyellagic acid, 4-glucopyranoside-4′-methoxyellagic acid, gallic acid, methylgallate, oleanolic acid, erythrodiol, β-amyrin, β-sitosterol, stigmasterol and stigmasterol-3-*O*-β-D-glucopyranoside which are of medical importance. The demonstration of the plant bark´s antimicrobial potential gives a rational for its use as agents against infections like tooth ache, caries, wound infection, diarrhoea and dysentery. It may also be useful in *Helicobacter pylori*, *Campylobacter jejuni* and *Vibrio cholerae* and multi-resistant *Staphylococcus aureus* infections. Moreover, the plant´s bark extract could be supportive in the treatment of dermatoses, as antifungal food additive and against chickpea blight. Further bioactivity assays may be carried out to investigate the complete spectrum of the bark´s medical/agricultural potential.

Conflict of interest

The authors declare that there is no conflict of interest.

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