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Phytochemical, Antioxidant and Antiproliferative Activities of Aqueous and Methanolic Extracts of *Aucoumea klaineana, Macaranga monandra* and *Uvaria klainei* from Gabon

Marlaine Boukandou Mounanga [a], Reine Raissa Rolande Aworet Samseny [a*], Line Edwige Mengome [a] and Sophie Aboughe Angone [a]

[a] Institute of Pharmacopoeia and Traditional Medicine (IPHAMETRA), Libreville, Gabon.

Authors' contributions

This work was carried out in collaboration among all authors. Authors RRRAS, LEM and SAA performed the experimental studies and drafted the manuscript. Authors MBM and RRRAS played roles in the writing and editing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Plant-based medicine is utilized as an alternative therapy against various ailments and diseases, especially in developing countries. *Aucoumea klaineana, Macaranga monandra* and *Uvaria Klainei* are used to alleviate many ailments in Gabon and few data on the potential biological activities are available.

**Corresponding author: E-mail: awsams@yahoo.fr;*

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Study Design: The aim of the present study is to evaluate the phytochemical constituents, the *in vitro* antioxidant and cytotoxic effects of these plants.
Place and Duration of Study: Department of Phytochemistry (Institute of Pharmacopoeia and Traditional Medicine), between March 2022 and February 2023.
Methodology: The plant dried powders were extracted using distilled water and absolute methanol. Qualitative phytochemical screening was performed, followed by the determination of the total phenolic and flavonoid contents. The antioxidant activity was evaluated using DPPH method and reducing power assay. The cytotoxicity was assessed using dual staining and fluorescence microscopy technique.
Results: The qualitative phytochemical screening revealed the presence of phenols, tannins, flavonoids and steroids in all the extracts. The highest TFC and TPC were determined in *M. monandra* methanolic extracts (2.26±0.06 mg QE/g and 12.04±0.4mg GAE/g). *M. monandra* and *A. Klaineana* methanol extracts (115.00±0.67 µg/ml and 131.9±1.31 µg/ml respectively) showed a statistically ($p<0.05$) higher reducing power than the ascorbic acid. *A. klaineana* methanolic extract IC_{50} (15.21±2.11 µg/ml) was significantly greater ($p<0.05$) than the standard. All the plant extracts lowered the number of HeLa cells of more than 50%.
Conclusion: *Okoume* and *U. klainei* showed promising antioxidant capacity. The antiproliferative activity and selective cytotoxicity of *M. monandra* towards HeLa cell have to be extendedly explored and could lead to the isolation of anti-cancerous agents.

Keywords: Antioxidant; antiproliferative; cytotoxicity; medicinal plants; TPC; TFC; Gabon.

1. INTRODUCTION

Worldwide, plant-based medicine is utilized as an alternative therapy against various ailments and diseases, especially in developing countries. “The World Health Organization (WHO) reported that about 80% of these countries relay on plants for socio-economic and cultural reasons” (WHO, 2003). “Among these countries, Gabon, located in Central Africa and crossed by the equator line, is covered with more than 80% dense equatorial forest, making plant-based medicine a major component of the local population’s healthcare” (Vliet, 2012; Mengome et al., 2009). “Studies have mentioned that more than 1600 medicinal plants are reported in Gabon” (Vliet, 2012). However, few data on the biological potential of these plants are available in the literature. In Gabon, as in many other sub-Saharan African countries, indigenous knowledge is monopolized and mastered by traditional healers who empirically manage a large range of pathologies with good results using plant-based recipes. Unfortunately, little research is undertaken to evaluate the safety of these plants and to validate their use. Among the plants entering in traditional remedy composition, several are endemic to the country such as *Aucoumea klaineana, Uvaria klainei* and *Macaranga monandra* (not endemic).

Aucoumea klaineana Pierre, known as Okoume in Gabon, belongs to Burseraceae family. *A. klaineana* is endemic to Gabon, Equatorial Guinea and Congo but can also be found in the South of Cameroon. Okoume is a dioecious, medium-sized to large evergreen tree, up to 50 (-60) m tall, with a cylindrical bole, often contorted and bent, up to 110 (-240) cm in diameter. “The bark is lightly fibrous and when cut, exudes a strong smelling resin which becomes opaque during coagulation” (Prota, 2013). “The resin is widely used for traditional torches, incense, oil lamps and also for cosmetic applications in skin care products and nail polish. Bark resin is exported from Gabon to France and USA for cosmetic applications” (Prota, 2013). “*A. klaineana* barks are used to treat abscesses, menstrual disorders, diarrhea and dysentery, while the resin is applied to superficial wounds, sores and as a water disinfectant” (Medzegue et al., 2013). “The roots and leaves are used to treat fever, constipation, malaria, diarrhea and jaundice” (Obame et al., 2014). Studies on the pharmacological potential of this species mainly focused on the volatile components extracted from the resin. “Essential oils contained *p*-cymene, terpinolene, terpineol, α-pinene, β-pinene, limonene, α-phellandrene, β-phellandrene, 3-carene, α-terpineol and eucalyptol” (Medzegue et al., 2013; Koudou et al., 2009). “The essential oil displayed some antioxidant activity with a weak DPPH radical scavenging activity and lipid peroxidation inhibition” (Koudou et al., 2009). Obame et al. (2014) also demonstrated the antimicrobial activity of essential oil from the resin against several bacterial and fungal strains.

Uvaria klainei Pierre ex Engl. & Diels. (Annonaceae) is a 3-4 meter scrambling shrub with descending branches, endemic to Gabon (Prota, 2013). "The leaves are used by the population as antiseptic but also to treat venereal diseases and diarrhea. Studies demonstrated that polysaccharides isolated from this plant showed some immunostimulant, hemostatic and antioxidant capacities" (Mengome et al, 2014 a, b, c).

Macaranga monandra Müll. Arg. is a shrub or a tree that can reach 25 m tall, belonging to Euphorbiaceae family. "It is distributed in Angola, Burundi, Cameroon, Central African Republic, Congo, DR Congo, Equatorial Guinea, Gabon, Nigeria, Tanzania and Uganda" (Prota, 2008). A decoction of the stem bark is taken as a galactagogue, to treat sterility, dyspnea, in case of threatened abortion. "A Bioassay-guided fractionation led to the isolation of two clerodane-type diterpenes" (Salah et al., 2003). "*M. monandra* displayed an antifungal activity against *Colletotrichum acutatum*, *C. fragariae, C. gloeosporioides, Fusarium oxysporum, Botrytis cinerea*, *Phomopsis obscurans*, and *P. viticola"* (Salah et al., 2003). Boukandou et al. (2021) also demonstrated antibacterial activity of some extracts against Gram-positive bacteria including *S. aureus, B. cereus, E. faecalis* and *S. pneumoniae.*

To date, few data on the potential biological activities of *A. klaineana, M. monandra* and *U. klaineana* from Gabon are available. Therefore, to contribute to the knowledge of the biological properties of these plants, the phytochemical constituents, the *in vitro* antioxidant and cytotoxic activities of the aqueous and methanol extracts from these plants were evaluated in the present study.

2. MATERIALS AND METHODS

2.1 Plant Collection

Okoume (barks), *M. monandra* (barks) and *U. klainei* (aerial parts) were identified by a botanist from the Institute of Pharmacopoeia and Traditional Medicines (IPHAMETRA) and collected in Libreville. The fresh samples were air-dried under the sun, crushed into powder and stored for further utilization.

2.2 Plant Extraction and Phytochemical Screening

The extraction was performed using 100 g of sample powder macerated into 1000 ml of distilled water or absolute methanol for 24 hours with constant shaking. After filtration, the aqueous filtrates were frozen and freeze dried by lyophilisation, while the methanol filtrates were evaporated using a rotary evaporator at 50 °C. Qualitative Chemical tests were carried out on both the methanol and aqueous crude extracts obtained using standard colorimetric methods (Purena and Bhatt, 2018; Adil et al, 2024). The constituents investigated included cardiac glycosides, saponins, phenols, tannins, terpenoids, steroids, flavonoids and alkaloids:

- **Cardiac glycosides (Keller- Kilani test):** In test tubes, crude extracts (1 ml) were mixed with 0.5 mL of glacial acetic acid (96%), followed by a few drops of a 2% solution of $FeCl_3$ and 1 ml of concentrated sulfuric acid (H_2SO_4). A brown ring at the interphase or a brown coloration was an indication of the presence of this group of compounds.
- **Saponins:** In test tubes, 1 ml of crude extracts was added 5 mL of distilled water. The test tubes were vigorously shaken for 5 minutes. The formation of a persistent foam indicated the presence of saponins.
- **Phenols and Tannins**: One (1) ml of crude extract was mixed with a few drops of 2 % ferric chloride ($FeCl_3$) in a glass tube. Any black, brown, green or dark blue coloration confirmed for the presence of both secondary metabolites.
- **Terpenoids:** Crude extracts (1 ml) were dissolved in 0.5 ml of chloroform and 1 ml of concentrated sulfuric acid. A brownish, reddish or dark coloration indicated the presence of the compounds.
- **Steroids:** A volume of crude extracts (1 ml) was mixed with a few drops of concentrated acetic acid and a few drops of concentrated sulfuric acid. A violet or green coloration indicated the presence of steroids.
- **Flavonoids:** In a test tube, a few drops of a 4 mg/ml sodium hydroxide (NaOH) solution were added to 1 ml of crude extracts, followed by the addition of a few drops of a 32% hydrochloric acid (HCl) solution. A yellowish coloration or a colorless mixture indicated the presence of flavonoids.
- **Alkaloids (Wagner's test):** Wagner's reagent was prepared by mixing of 2 g of iodine, 6 g of potassium iodide and 100 ml of distilled water. A few drops of the prepared Wagner's reagent were added to

1 ml crude extract. Observed turbidity or precipitation was considered evidence of the presence of alkaloids.

2.2.1 Quantification of total phenolic contents (TPC)

Folin-Ciocalteu method was used to quantify the total phenolic content of the extracts according to Anokwuru et al. (2017). In brief, each well of a 96 well plates was seeded with 80 µl of distilled water followed by 100 µl of extracts (1 mg/ml). A volume of 60 µl of sodium carbonate (3.5 g dissolved in 50 mL distilled water) and 60 µl of Folin Ciocalteu solution (10 ml dissolved in 90 ml distilled water) were added to the plates. The experiment was carried out in triplicate. Serial dilution of the standard Gallic acid was performed for the realization of the standard curve. Absorbance was measured at 420 nm using a micro-plate reader after 30 min of incubation at room temperature in the dark. The total phenolic content of the extracts was calculated as gallic acid equivalents (mgGAE/g).

2.2.2 Quantification of total flavonoid contents (TFC)

Quercetin was used as standard for the evaluation of the total flavonoid content as described by Anokwuru et al. (2017) with slight modifications. Briefly, 100 µl of Aluminium chloride solution (1 g dissolved in 50 ml of methanol) were seeded in each well of a 96 well plates following by the addition of 100 µl of plant extracts (1 mg/ml). Serial dilutions of a solution of quercetin (1 mg/ml) were used for the standard curve. The plates were allowed to stand for 30 min at room temperature and absorbance was measured at 760 nm by spectrophotometer. The TFC was expressed in mg of Quercetin. The experiment was carried out in triplicate

2.3 Antioxidant Assays

2.3.1 Reducing power assay

The reducing power assay was performed according to Ramli et al. (2011) with a few modifications. In each well of 96 well plates, 50 µl of sodium phosphate buffer (6.6 pH;0.2M) was added followed by 50 µl of the extracts (1 mg/ml) and control (1 mg/ml). Two-fold serial dilutions of the extracts and the control were performed, giving the following concentrations in µg/mL: 500, 250, 125, 62.5, 31.25, 15.63, 7.81 and 3.9. and 50 µl of potassium hexacyanoferrate solution (0.1 g in 10 ml of distilled water) were added into each well. The plates were then incubated 30 min at 50°C. Afterwards, 50 µl of trichloroacetic acid solution (1 g in 10 ml of distilled water) was introduced into each well and a volume of 80 µl of the mixture was transferred into new 96 well plate. Distilled water (80 µl) was added into the wells of the new plate, followed by 15 µl of ferric chloride solution (0.01 g in 10 ml of distilled water). The plates were then read at 690 nm using a microplate reader (Versa max). The assay was done in triplicate. Ascorbic Acid was used as control. The reducing effect was calculated by using the following equation:

$$\% \text{ Reducing effect} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

A = measured absorbance

The EC_{50} express the amount or concentration of extracts needed to scavenge 50% of the free radicals. The EC_{50} values were calculated graphically.

2.3.2 DPPH assay

DPPH assay was ran as described by Mohd and Wan, (2018) with slight modifications. Briefly, 100 µl of distilled water was seeded into each well of a 96 well plates, then 100 µl of each extract (1 mg/ml) and ascorbic acid (1 mg/ml) were added in the first row of the plate in triplicate. Two-fold serial dilutions of the samples were realized, giving the following concentrations in µg/mL: 500, 250, 125, 62.5, 31.25, 15.63, 7.81 and 3.9. A solution of 0.125 mM of DPPH (200 µl) diluted in ethanol was added into each well and the plates were incubated 30 min in the dark. After incubation, the absorbance of each well was read by a microplate reader (Versa max) at 517 nm. The inhibitory concentration 50 (IC_{50}) was determine using the percentage of inhibition of the DPPH.

$$\%\text{Inhibition} = [(A_{DPPH} - A_{sample})/A_{DPPH}] \times 100$$

A = measured absorbance

The percentage of inhibition allowed to determine graphically the inhibitory concentration 50 (IC_{50}) which is the concentration of extract/control that causes 50% reduction in the DPPH color.

2.4 Cytotoxicity

2.4.1 Cell lines

A cervical cancer cell line (HeLa ATTC # CCL-2; obtained from Cellonex, South Africa) and a control cell line from African Green monkey kidney cells (Vero ATTC # CCL-81; obtained from the ATCC) were used in this study to evaluate the cytotoxic potential of the plant extracts. Vero cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified environment with 5% CO_2 (Thermo Fisher, Waltham, MA USA). HeLa cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified environment with 5% CO_2.

2.4.2 Dual staining coupled to fluorescence microscopy technique

For the cytotoxic screening assay the cells (HeLa and Vero) were seeded into 96 well microtiter plates at a density of 6000 cells/well using a volume of 200 µl in each well. The microtiter plates were incubated at 37°C, 5% CO_2 (Thermo-Fisher, Waltham, MA USA), and 100% relative humidity for 24 hours prior to addition of test compounds or extracts to allow for cell attachment. Two hundred microliter aliquots of diluted extracts (50 and 100 µg/ml) in fresh medium was used to treat cells after aspiration of seeding medium. Treated cells were incubated at 37°C in a humidified 5% CO_2 incubator (Thermo-Fisher, Waltham, MA USA) for 48 hours. Treatment medium was aspirated from all wells and replaced with 100 µl of Hoechst 33342 nuclear dye (5 µg/ml) and incubated for 10 minutes at 37°C. Thereafter, cells were stained with propidium iodide (PI) at 100 µg/ml in order to enumerate the proportion of dead cells within the population. Cells were imaged immediately after addition of PI using the Image-Xpress Micro XLS Widefield Microscope (Molecular Devices; San Jose, CA; USA). Melphalan (40 µM) was used as a positive control in all experiments.

2.5 Statistical Analysis

The results are presented in mean ± standard deviation. Quantification of live and dead cells for the cytotoxic screening assay was performed using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices) and acquired data was compiled using the MetaXpress software and Multi-Wavelength Cell Scoring Application Module. The data were analysed using Graph pad prism 6.0 and Microsoft Excel 2010. The IC_{50} was obtained by nonlinear regression followed by dose response equation. The EC_{50} was determined using linear regression. The significance was evaluated using 2-way ANOVA.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

In Table 1, the results revealed that all the compounds (saponins, alkaloids, phenols, tannins, terpenoids, flavonoids, steroids and cardiac glycosides) were detected in *U. klainei* both aqueous and methanolic extracts. Alkaloids, phenols, tannins, terpenoids, flavonoids, steroids and cardiac glycosides were detected in *A. klaineana* aqueous and methanol, while saponins were not detected in the methanol extract. Only phenols, tannins, flavonoids and steroids were present in both *M. monandra* extracts. Alkaloids and terpenoids were absent from the aqueous extracts, and glycosides was not detected in methanol extracts. Saponins were not found in both aqueous and methanol extracts.

In this study, Table 2 presents the TPC and TFC values of the different extracts. Methanol extracts showed the highest amount of TFC and TPC in *M. monandra* (2.26±0.06 mg QE/g and 12.04±0.4 mg GAE/g), *A. klainei* (1.71±0.02 mg QE/g and 10.95±0.23 mg GAE/g) and *U. klainei* (1.93±0.05 mg QE/g and 5.07±0.24 mg GAE/g) compared to aqueous extracts where TFC and TPC values were respectively 0.89±0 mg QE/g and 4.53±0.44; 0.90±0 mg QE/g and 2.63±0.13 mg GAE/g; 1.29±0.01 mg QE/g and 3.06±0.30 mg GAE/g.

3.2 Antioxidant

3.2.1 Reducing power assay

The result of the reducing power activity is presented in Table 3. *M. monandra* aqueous extract (156.08±1.73 µg/ml) displayed a reducing power not statistically different from the standard ascorbic acid (152,16±1,09 µg/ml), while *M. monandra* and *A. klaineana* methanol extracts (115.00±0.67 µg/ml and 131,9±1,31 µg/ml respectively) showed a statistically ($p<0.05$) higher reducing power than the standard. The remaining extracts displayed a reducing power significantly lower than the standard with EC_{50} values ranged between 286.74±3.06 µg/ml and 348,46±4,24 µg/ml.

Table 1. Results of phytochemical screening of methanol and aqueous extracts

Extracts		Saponins	Alkaloids	Phenols	Tannins	Terpenoids	Flavonoids	Steroids	Glycosides
Aucoumea klaineana	Methanol extract	Abs	Pres	Pres	Pres	Pres	Pres	Pres	Pres
	Aqueous extract	Pres	Pres	Pres	Pres	Pres	Pres	Pres	Pres
Macaranga monandra	Methanol extract	Abs	Pres	Pres	Pres	Pres	Pres	Pres	Abs
	Aqueous extract	Abs	Abs	Pres	Pres	Abs	Pres	Pres	Pres
Uvaria klainei	Methanol extract	Pres	Pres	Pres	Pres	Pres	Pres	Pres	Pres
	Aqueous extract	Pres	Pres	Pres	Pres	Pres	Pres	Pres	Pres

Abs=absent; Pres=present

Table 2. Total phenolic and flavonoid content values of methanol and aqueous extracts

	TFC (mg QE/g)		TPC (mg GAE/g)	
	Methanol	**Aqueous**	**Methanol**	**Aqueous**
Aucoumea klaineana	1.71±0.02^{a}	0.90±0^{b}	10.95±0.23^{a}	2.63±0.13^{b}
Macaranga monandra	2.26±0.06^{c}	0.89±0^{b}	12.04±0.4^{c}	4.53±0.44^{d}
Uvaria klainei	1.93±0.05^{a}	1.29±0.01^{b}	5.07±0.24^{d}	3.06±0.30^{b}

The numbers with the same index were not significantly different.

Table 3. Antioxidant activity of the selected plant extracts

		DPPH IC_{50} values (µg/ml)	Reducing power EC_{50} values (µg/ml)
A. klaineana	Aqueous extract	38.6±2.41	348.46±4.24*****
	Methanol extract	15.21±2.11*****	131.9±1.31*****
M. monandra	Aqueous extract	184.73±1.57*****	156.08±1.73
	Methanol extract	79.97±0.86*****	115.00±0.67*****
U. klainei	Aqueous extract	36.46±0.13	339.24±3.15*****
	Methanol extract	49.23±4.43	286.74±3.06*****
Ascorbic Acid		38.87±1.54	152.16±1.09

*Significant differences are indicated by * (P<0.05) compared to ascorbic acid*

3.2.2 DPPH assay

The DPPH assay is a method assessing the free radical scavenging capacity of an antioxidant substance. The results of this assay is presented in Table 3. Free radical scavenging effect of the aqueous and methanol extracts was compared with standard ascorbic acid. Both aqueous extract and the standard displayed a similar dose dependent activity. However, the effect of *A. klaineana* methanolic extract (IC_{50}= 15.21±2.11 µg/ml) was significantly greater ($p<0.05$) in comparison to ascorbic acid (IC_{50}=38.87±1.54 µg/ml). *U. klainei* extracts displayed a free radical scavenging effect statistically close to the standard with IC_{50} values of 36.46±0.13 µg/ml and 49.23±4.43 µg/ml for the aqueous and the methanolic extracts respectively. *M. monandra* extracts (184.73±1.57 µg/ml for aqueous extract and 79.97±0.86 µg/ml for methanolic extract) demonstrated an activity significantly lower than the standard.

3.3 Cytotoxicity

High-content imaging-based in vitro toxicity assays such as dual staining coupled with fluorescence microscopy technique show promise for safety and efficacy testing as with this technique the actual number of cells can be measured and living cells can be differentiated from dead cells. These assays are accurate because they can be performed using high throughput systems for simultaneous screening of many compounds.

For the present study, the results of the cytotoxic potential of the plant extracts on normal cells (Vero) and cancerous cells (HeLa) are depicted in Figs. 1 and 2 respectively. The graphs present the average of living cells and dead cells for each extracts and controls. The results depicted in Fig. 1 show the activity of the plant extracts on Vero cells. The results indicated that the number of cells per site were found to be less than 1000 in *A. klaineana* (both extracts and concentrations) and *U. klainei* (methanol at both concentrations) (diminution of 43% to 78%) when compared to the untreated cell (1500 cells per site). *M. monandra* (both extracts and concentrations) and *U. klainei* (aqueous extract at both concentrations) showed a number of cells per site higher than 1000 (diminution of 8% to 22%).

The results of the anticancer effects of the plant extracts are presented in Fig. 2. All the plant extracts were able to significantly ($p<0.05$) lowering the number of cells in the sites of more than 50% (diminution of 63% to 90%) compared to the untreated cells except *M. monandra* methanol extract at 50 µg/ml (diminution of 28%). The number of dead cells was higher (70% to 247%) after treatment with *A. klaineana* (both extracts at 50 µg/ml) and *M. monandra* (both extracts at all concentrations) than the number of dead cells in the untreated site.

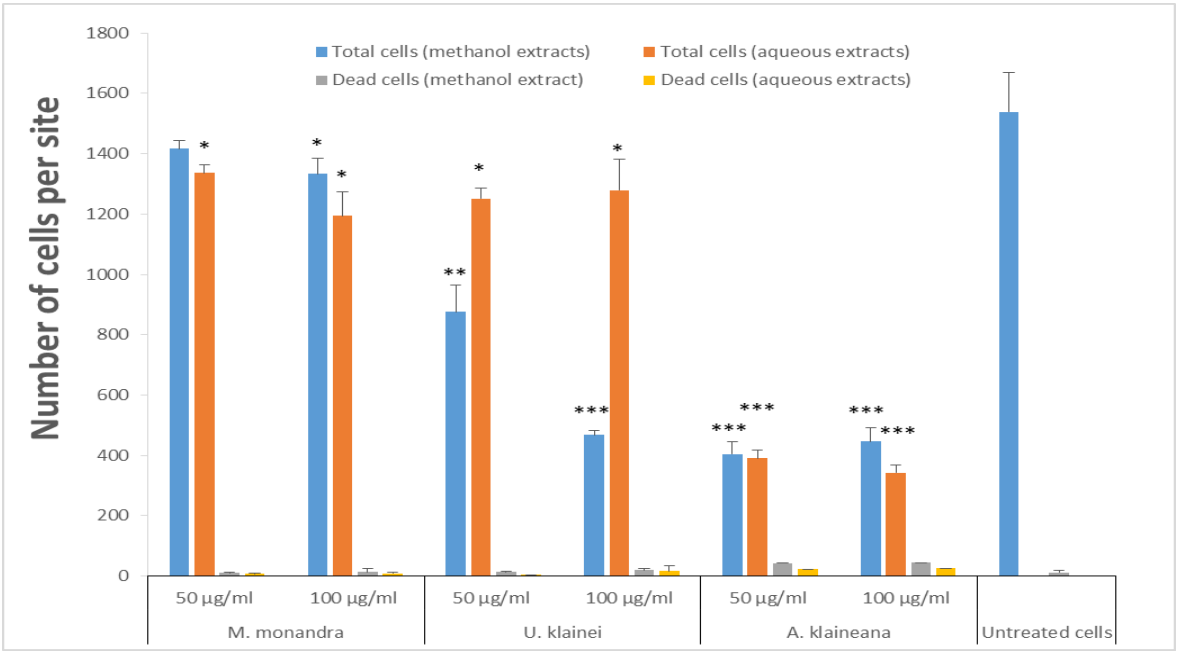

Fig. 1. Antiproliferative effects of *A. klaineana* extracts on Vero cells. Results are indicated as number of total and dead cells per site. Errors bars denote standard deviation. Significant differences are indicated by * (P<0.05) compared to the untreated control (UC)

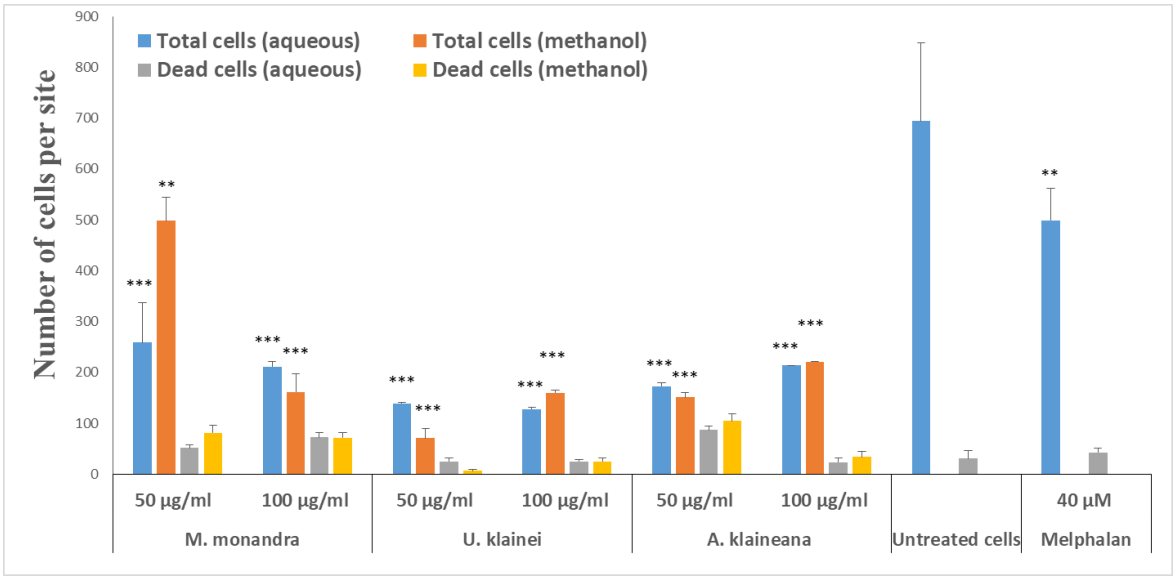

Fig. 2. Anti-cancer activity of the plant extracts on HeLa cells. Results are indicated as number of total and dead cells per site. UC (untreated cells); Errors bars denote standard deviation of one experiment performed as quadruplicate. Significant differences are indicated by * (P<0.05) compared to the untreated control (UC)

4. DISCUSSION

The purpose of the present study was to highlight the family compounds present in *A. Klaineana*, *M. monandra* and *U. klainei* extracts and also to evaluate their antioxidant and their potential anticancer activity. The phytochemical screening of the plant extracts showed the presence of various chemical compounds in both aqueous and methanol extracts. According to Koudou et al. (2009), *A. klaineana* essential oil from the bark resin is rich in terpenoids. This finding suggests that terpenoids found in both aqueous and methanolic extracts of the bark in the present study might be similar to those described in the resin. Interestingly, saponins

were found only in the aqueous extract Okoume. Ismail et al. (2016) also showed the presence of saponins only in aqueous extract when compared to the methanolic extract. Limited phytochemical investigation was done on the genus *Macaranga* and very few on *M. monandra*. However, on overview of the previous phytochemical investigation from the genus *Macaranga* indicated the presence of coumarins, tannins, stilbenes, flavonoids, steroids and other compounds such as Ferulic acid, macarangioside and abscisic acid what sustains the findings in the present study. "A bioassay-guided fractionation of *M. monandra* led to the isolation of two clerodane-type diterpenes" (Salah et al, 2003). To date, only polysaccharides were isolated from *U. klainei* (Mengome et al, 2014, c). Phytochemical compounds are responsible for the biological activities of medicinal plants. "Terpenoids have displayed activity against malaria, cancer, inflammation and various infectious diseases" (Wang et al, 2005). Compounds such as alkaloids, steroids, glycosides and tannins possess a wide broad of biological capacities justifying the use of these plants in traditional medicine in Gabon for the treatment of several ailments.

Polyphenols are compounds including tannins, coumarins, flavonoids and phenolic acids. Their quantification gives an idea on the amount of these compounds in plant extracts. Total phenolic content (TPC) and TFC are generally extrapolated from calibration curves of standards (quercetin, gallic acid or caffeic acid).

A study by Hatano et al. (1989) assessing the effect of extraction solvent on TPC and TFC revealed that in a system methanol/water (100%, 75%, 50%) the TPC and TFC of the extracts were decreasing with increased water content. This finding is in accordance with the results in the present study where TPC and TFC were higher in methanol extracts compared to aqueous extracts. "An explanation of this result could be that methanol has the ability to inhibit the action of polyphenol oxidase" (Yao et al, 2004). The present study has demonstrated that methanol is a better solvent compared to water for the extraction of polyphenolic compounds from the studied plants.

In the present study, more electrons are donated by methanol extracts compared to aqueous extracts. The methanol extracts displayed higher reducing power activity compared to aqueous extracts. The reducing power reaction occurring between plants and free radicals corresponds to a redox reaction in which free radical is reduced at the expense of the oxidation of the plant. The presence of reductants in the antioxidant samples tested causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. "The appearance of Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm" (Chung et al, 2002). The presence of reductants in the plants of the study is undoubtedly responsible for the reducing capacity demonstrated by the extracts. "Reductants in plants show antioxidant properties via prevention of chain initiation, binding of metal ions, decomposition of peroxides and radical scavenging" (Yildirim et al, 2001).

DPPH (1,1-diphenyl-2-picrylhydrazyl) has an odd electron which gives a strong absorption maximum at 517 nm. The purple color of DPPH turns into yellow when the odd electron of DPPH radical becomes paired with hydrogen from scavenging substance to form a reduced DPPH-H. Medicinal plants such as the plants in the study which possess the capacity of scavenging free radicals are of great importance for fighting oxidative stress.

In the last few years the search for natural antioxidant agents able to reduce or to control oxidative stress in the body has been emphasized. Oxidative stress creates free radicals responsible of damaging DNA, proteins and lipids leading to cancer, inflammation, aging or cardiovascular diseases. Antioxidants such as ascorbic acid or certain medicinal plants have demonstrated encouraging protective and preventive effects from cell damages. Several studies have successfully demonstrated the antioxidant activities of medicinal plant extracts by different mechanisms. "Phenolic compounds and flavonoids are usually highly correlated with antioxidant activity of medicinal plants because of their ability to donate a hydrogen or an electron due to their hydroxyl groups" (Hatano et al, 1989). "Several studies on antioxidant activity of plants showed that phenols are responsible of this activity" (Mello et al, 2014; Aggarwal et al, 2017). In the present study, TPC and TFC were better extracted with methanol and the results of the study indicated that the highest antioxidant activity (reducing power and DPPH) was also displayed by methanolic extracts. The compounds isolated by methanol may contribute for a large part to the antioxidant activity of *A.*

Klaineana, *M. monandra* and *U. klainei*. “Several studies have also demonstrated the role of methanol in extracting antioxidant compounds from medicinal plant” (Anokwuru et al, 2011). A study by Koudou et al. (2009) showed that *A. klaineana* essential oil extracted from the resin have a weak DPPH free radical scavenging effect. The results in the present study indicate that *A. klaineana* barks have a free radical scavenging capacity similar to the one of ascorbic acid as well as a promising reducing property.

“Amongst the non-communicable diseases, cancer is the second leading cause of death” (Bajpai et al, 2024). “Despite the large collection of useful chemotherapeutic agents, various kinds of toxicities may occur as a result of the treatments” (Remesh, 2027; Gao et al, 2024; Yan et al., 2024). “Plant-derived drugs are preferred for anticancer treatment as they are efficient and well-tolerated by the body, being non-toxic to human cells” (Al-Rimawi et al, 2024; Omara et al, 2020). In the present study, we screened plant extracts for safety and a potential anticancer activity. Under in vitro assay conditions, exposure to an antitumor agent may decrease the number of viable cells by direct cell killing (cytotoxicity) or by simply decreasing the rate at which the cells proliferate (cytostasis/antiproliferative). The evaluation of the cytotoxicity on Vero cells suggests that *M. monandra* and *U. klainei* exerted a weak toxicity characterized by an antiproliferative action rather than inducing the death of the cells. *Aucoumea klaineana* showed higher antiproliferative effects suggesting a probable toxicity of the bark. Regarding the anticancer activity, all the tested extracts seemed to possess an anticancer effect. These results indicate that *A. klaineana* (lowest concentration) and *M. monandra* display both antiproliferative and cytotoxic activity against the cancerous cells, while *U. klainei* showed an antiproliferative effect. These results imply that the plants under study especially *M. monandra* could be a promising source of anticancer molecules. The identification of compounds that induce cytotoxicity in cancer cells is an important initial step in the development of anti-cancer drugs, these compounds need to kill cancer cells at a concentration that does not harm normal cells. Unfortunately, this in vitro screening merely highlights the potential of the studied plants as anticancer agents. Further studies are required to fully characterize the mechanism of cell death and to determine the therapeutic selectivity of promising lead compounds.

5. CONCLUSION

The present study has demonstrated the presence of various chemical compounds such as phenols, tannins, flavonoids and steroids in all the extracts of the studied plants. The quantification of polyphenol compounds reveals that the plant extracts were rich in phenolics which were well extracted with methanol. In addition, *Okoume* and *U. klainei* showed a high antioxidant capacity useful against aging and oxidative stress what constitutes an important key to fight diseases. The antiproliferative activity and the interesting selective cytotoxicity of *A. klaineana*, *M. monandra* and *U. klainei* towards HeLa cells have to be extendedly explored and could lead to the isolation of anti-cancerous molecules.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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