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Comparative study of the *in vivo* acute toxicity, phytochemical analysis, *in vitro* antimicrobial, antiplasmodial and cytotoxic activities of leaves, stem bark and roots of *Guibourtia tessmannii*

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Abstract
Guibourtia tessmannii (Harms) J. Leonard's is use traditionally in the treatment of sexually transmitted diseases (gonorrhea), hypertension, typhoid fever, female infertility and malaria. This study aimed to assess the comparative antibacterial, antifungal, antiplasmodial and cytotoxic activities of *G. tessmannii* methanolic leaves, root bark, root wood, stem bark extracts. The antimicrobial activity was evaluated by the method of microdilution. The antimalarial activity was carried out on the 3D7 strain of *Plasmodium falciparum*, the cytotoxic activity on human HeLa cells, the acute toxicity on Wistar rats according to the modified OECD 423 guidelines. The stem bark extract proved to be the most active with good antibacterial activity with MIC values equal to 256 µg/mL for *S. aureus* and *K. pneumonia* strains, average antibacterial and fungal activities with an MIC equal to 512 µg/mL on bacterial and fungal strains/isolates namely *N. gonorrhoeae*, *S. typhi* and *C. albicans*, thereby justifying the use of *G. tessmannii* in the traditional pharmacopoeia to treat certain conditions such as gonorrhea, typhoid fever, female infertility linked to infections. This extract was had bactericidal properties (MMC/MIC < 4). None of the 4 extracts showed a significant inhibition of *P. falciparum* 3D7 cells growth, and thus no antimalarial activity was observed at 50 µg/ml. The HeLa cells viability was reduced by 2% (leaves), 6% (root wood), 34% (root bark) and 44% (stem bark) at 50 µg/ml and therefore no IC_{50} was determined. All four extracts presented an LD_{50} greater than 5000 mg/kg. Phytochemical analysis revealed the presence of triterpenes including saponins, and phenolic compounds such as flavonoids and anthraquinones. Anthocyanins were only found in the leaves extract. Coumarins and alkaloids were absent.

Keywords: *Guibourtia tessmannii*, acute toxicity, phytochemical analysis, antimicrobial, antiplasmodial, cytotoxicity, medicinal plant

1. Introduction
There has been an increased interest in the use of traditional medicine to control various pathologies. For millennia, health conditions and events all over the world have been managed with drugs extracted from plant and animal materials. Knowledge on the techniques used has been passed down from one generation to another. To date, 80% of the population in Africa and Asia refers to traditional medicines rather than modern medicines for primary health care [1] despite the rise in medications of synthetic and semi-synthetic origin. These drugs constitute an emergency exit because of the appearance of various side effects and the development of resistances. In Cameroon, traditional medicine is an increasingly popular sector and collaborating researchers have made it possible to isolate molecules with various activities including antibacterial [2], gastroprotective [3], cytotoxic [4] and antiplasmodial [5, 6]. The concern for a repetitive use of plant extracts also led to the unraveling of multiple toxicological parameters [2, 6]. In the quest to contribute to the enhancement of Cameroonian floristic biodiversity, a focus has been placed on *Guibourtia tessmannii* (Harms) J. Leonard. In African traditional medicine, *G. tessmannii* is widely used in Cameroon in the treatment of sexually transmitted diseases (gonorrhea), hypertension, hemorrhoids, cancer, female infertility and malaria [7, 8]. The barks and leaves of *G. tessmannii*are used to treat typhoid fever and to manufacture biological pesticides for the control of rotting cocoa pods. In pharmacological studies carried out by different researchers, the stem bark, roots and leaves of *G. tessmannii* showed anti-apoptotic and antioxidant [9-11], pro-ejaculatory [12], hypotensive [13] and anticancer properties [14]. These properties are due to the presence of secondary metabolites in the plant. Compounds' structures including stilbenes [15], chalconoids [16], flavonols [17] and tannins [18] have been elucidated.

The acute and sub-chronic toxicity of the stem bark have already been the subject of studies [19]. Nevertheless, to our knowledge, there is no report of antimicrobial, antiplasmodial and cytotoxic activities shown by *G. tessmannii*'s extracts. The present work aims to assess the antibacterial, antifungal, antiplasmodial and cytotoxic activities of *G. tessmannii*'s leaves, root bark, root wood and stem bark extract, in order to further validate the species' traditional use and compare the effectiveness shown by the different parts.

2. Materials and methods

2.1 Sample collection and Extraction

The plant materials were obtained from the forest in the Centre Region, Cameroon and identified at the Cameroon National Herbarium, by comparison to specimen number N°2684/HNC. The stem bark, leaves, roots bark and roots wood of *Guibourtia tessmannii* were harvested, air-dried away from light for 8 weeks and pulverized. Exactly 8 kg, 7 kg, 0.82 kg and 0.37 kg of the pulverized stem bark (Gtsb), leaves (Gtlv), roots bark (Gtrb) and roots wood (Gtrw), respectively, were cold extracted in 96° methanol for 48 hours (h) with occasional shaking. Each extract was then filtered through Whatman's N°1 filter paper and the methanol filtrates were separately concentrated to dryness *in vacuo* using a rotary evaporator at 60 rpm [20]. The extraction yield was then calculated according to the following formula:

$$Yield = \frac{\text{weight of the dried crude extract}}{\text{weight of the powder}} \times 100$$

2.2 Phytochemical Analysis of Plant Extracts

The four extracts were tested for the presence of secondary metabolites (alkaloids, triterpenoids, saponins, anthraquinones, coumarins, flavonoids, anthocyanins, tannins) following common procedures described by Ronchetti et Russo (1971), Hegnauer (1973), Wagner (1983), Békro*et al*. (2007), with slight modifications [21-24].

2.3 Acute toxicity study

The acute toxicity study was conducted following the Organization for Economic Co-operation and Development (OECD) protocol directives N° 423, with minor changes applied for the chemical products assay and acute oral toxicity [25]. Briefly, 9-week-old female, non pregnant, well fed and hydrated Wistar rats weighing between 115 and167 g were randomly chosen and kept in laboratory conditions for 10 days. A total of 9 batches of 3 rats each were formed. The rats were fasted for 16 h prior to the experimentation. One of the substances (Gtsb) was administered to a batch at increasing doses (5, 50, 300 then 2000 mg per kg of body weight (bw)). Since no deaths were observed, all the extracts (Gtsb, Gtrb, Gtrw and Gtlv) were administered at a dose of 2000 mg/kg bw to 4 batches (B1-4, respectively), and 5000 mg/kg bw to 4 others (B5-8, respectively). The control batch (B0) received 10 ml/kg bw of distilled water. Physical parameters such as eyes and urine color, feces appearance, sensitivity to stimuli, social behavior, fur and motion were observed minutes after administration (D0), then after 2 h, 4 h and 8 h. A daily visual observation was carried out every subsequent day, for 14 days (D1-D14). Moreover, the rats were weighed every second day, then humanly sacrificed on D14 and an autopsy on a macroscopic level was performed.

2.4 Assessment of antibacterial activity

2.4.1 Sterility test

A sterility test was carried out to ensure the absence of bacteria in the extract. To do this, the different extracts were sown on specific culture environments, namely Chapman and Mac Conkey, and incubated 24 hours.

2.4.2 Test microorganisms

The antibacterial properties of Gtsb, Gtlv, Gtrb and Gtrw were assessed against the following 5 bacterial strains: *Neisseria gonorrhoeae*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella pneumoniae*.

2.4.3 Inoculum preparation

The different bacterial strains and isolates were streaked on Petri dishes, and then incubated at 37°C for 24 hours, in order to obtain young and well-isolated colonies. The suspension method was used directly from those colonies. A bacterial suspension in saline solution was made to achieve a turbidity equivalent to that of the 0.5 standard from the McFarland range, corresponding to an inoculum of about 1 to 2 $x10^8$ CFU/ml.

2.4.4 Determining the MIC

The antibacterial properties of Gtsb, Gtlv, Gtrb and Gtrw were then assessed using the National Committee of Clinical Laboratory Standards'broth microdilution method described elsewhere [26]. Briefly, for each extract, a 4096 µg/ml mother solution was obtained by dissolving 40.96 mg of extract into 5 ml of 5% dimethylsulfoxide (DMSO) and completed to 10 ml with Mueller-Hinton broth (MHB). Each of the 4 extracts was tested against 100 µl of the 5 bacterial strains in labelled microplates at 8 different concentrations, starting at 1024 µg/ml and followed by serial two-fold dilutions (512 µg/ml, 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml and 8 µg/ml). The final concentrations were for 200 µl of mixture and the wells were covered with aluminum foil for incubation at 37 °C for 24 h. After incubation, bacterial growths were revealed using a 2% *p*-iodonitrotetrazolium (INT) solution (40 µl per well) and incubation at 37 °C for 30 minutes. The MIC was retained as the lowest concentration of extract for which no change in INT coloration was observed i.e. the change from colorless INT to a pink color in the presence of bacteria. The antibiotic ciprofloxacin was used as reference substance for the positive control, at the following concentrations: 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, 2 µg/ml and 1 µg/ml. The negative control was made of the culture medium, the inoculum, and 5% DMSO for each test. The tests were carried out in triplicate.

2.4.5 Determining the MBC

To determine the minimum bactericidal concentrations (MBC), 150 µl of MHB were introduced into each well. In the plates of the columns that did not receive the INT, 50 µl were taken from the contents of the wells of concentrations greater than or equal to the MICs and introduced into the wells of the new plate. These plates were carefully covered and incubated for 24 h at 37°C then revealed with INT. The wells in which no color change was observed were considered and the one corresponding to the lowest concentration of extract was retained as the MBC. The calculation of the MBC/MIC ratio made it possible to determine the bactericidal (MBC/MIC < 4) or bacteriostatic (MBC/MIC ≥ 4) character of the substances tested.

2.5 Assessment of antifungal activity

The method described above was used to assess the antifungal activity on *Candida albicans* strain, with a slight difference.

The mother solution was prepared as 40.96 mg of extract into 5 ml of 5% dimethylsulfoxide (DMSO) and completed to 10 ml with Sabouraud dextrose broth (SDB). The labelled plates with extracts solutions at 8 different concentrations (1024 µg/ml, 512 µg/ml, 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml and 8 µg/ml) and 100 µl of inoculum (total of 200 µl) were covered with aluminum foil and incubated at 35°C for 48 h. The MIC was determined after revealing with *p*-Iodonitrotetrazolium (40 µl per well). Fluconazol served as positive control, at the following concentrations: 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, 2 µg/ml and 1 µg/ml. To determine the minimum fungicidal concentration (MFC), the covered plates were incubated for 48 h at 35°C. The calculation of the MBC/MIC ratio made it possible to determine whether the tested substances had fungicidal (MFC/MIC < 4) or fungistatic (MFC/MIC ≥ 4) effects.

2.6 Assessment of antiplasmodial activity

Activity against *Plasmodium falciparum* chloroquine-sensitive 3D7 strains was assessed following the procedure described by Desjardins *et al*. 1979 [27] with slight modifications as per Mbosso*et al*. (2018), Fouokeng*et al*. (2019), Mbosso*et al*. (2020) [28-30]. Briefly, a 4-8% parasitemia was used. Malaria parasites were maintained in RPMI 1640 medium containing 2 mM L-glutamine and 25 mM hepes (Lonza). The medium was further supplemented with 5% Albumax II, 20 mM glucose, 0.65 mM hypoxanthine, 60 µg/mL gentamycin and 2-4% hematocrit human RBC. The parasites were cultured at 37ºC under an atmosphere of 5% CO_2, 5% O_2, 90% N_2 in sealed T25 or T75 culture flasks. The natural extracts Gtsb, Gtlv, Gtrb and Gtrw solutions (60 µl) were added to the parasite cultures in 96-well plates and incubated in a 37 ºC CO_2 incubator. After 48 h, the plates were removed from the incubator. A volume of culture (20 µl) was removed from each well and mixed with 125 µl of a mixture of Malstat solution and NBT/PES solution in a fresh 96-well plate. The parasite lactate dehydrogenase (pLDH) activity was then measured in these solutions. A purple product forms when pLDH is present, and this product could be quantified by absorbance at 620 nm (Abs620). The Abs620 reading in each well was thus an indication of the pLDH activity and thereby of the number of parasites in the well. For each concentration of the extracts, the percentage of parasite viability and the activity of the pLDH in the wells of the well-treated compounds compared to the untreated controls were calculated. The test was performed in triplicate and the standard deviations were determined. Products that significantly reduced parasite viability at fixed concentration were used to determine the IC_{50}.

2.7 Cytotoxicity study

To assess the overt cytotoxicity, samples were incubated for 24 h, at a concentration of 50 µg/mL in 96-well plates containing HeLa cells (human cervix adenocarcinoma), maintained in a culture medium made of Dulbecco's Modified Eagle's Medium (DMEM) with 5 mM L-glutamine (Lonza), supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin/streptomycin/fungizone - PSF) cells for 24 h. HeLa cells were plated in 96-well plates at 2x104 cell per well. After an overnight incubation in a 5% CO_2 humidified incubator, 10-fold serial dilutions of compounds were added to the cultures (triplicate wells; 200 µl final culture volume) and incubation continued for an additional 48 hours. The numbers of cells surviving drug exposure were quantified using the resazurin based reagent and resorufin fluorescence quantified ($Excitation_{560}/Emission_{590}$) in a multiwell plate reader [28, 29, 31].

3. Results

3.1 Extraction

Extraction of the different parts of *Guibourtia tessmannii*allowed us to have the concentrations of the crude extracts, their color and appearance (Table 1).

Table 1: Extraction yield of the methanolic extracts of the different parts of *Guibourtia tessmannii*

Vegetal material	Powder (g)	Crude extract (g)	Yield (%)
Root wood	6000	45	0.75
Root bark	600	82	13.66
Stem bark	8000	98	1.23
Leaves	350	30	8.57

The percentage yield of *G. tessmannii* methanolic extracts increases from the roots wood (Gtrw: 6000:45 g; 0.75%) to the stem bark (Gtsb: 8000:98 g; 1.23%), to the leaves (Gtlv: 350:30 g; 8.57%) and finally to the roots bark (Gtrb: 600:82 g; 13,66%).

3.2 Qualitative phytochemical screening

The 4 extracts were subjected to qualitative phytochemical screening using procedures described in the literature. The results are shown in the following table (Table 2).

Table 2: Phytochemical content of extracts from *Guibourtia tessmannii* parts

Phytochemicals	*Guibourtia tessmannii* parts			
	Gtsb	Gtrb	Gtrw	Gtlv
Alkaloids	-	-	-	-
Triterpenes	+	+	+	+
Saponins	+	+	+	+
Phenolics	+	+	+	+
Coumarins	-	-	-	-
Flavonoids	+	+	+	+
Anthraquinones	+	+	+	+
Anthocyanins	-	-	-	+

Gtsb = *G. tessmannii* stem bark, Gtrb = *G. tessmannii* root bark; Gtrw = *G. tessmannii* root wood and Gtlv = *G. tessmannii* leaves.

The presence (+) or absence (-) of secondary metabolites in the extracts from different parts of *G. tessmannii* was revealed. All extracts showed almost the same phytochemical composition in terms of secondary metabolites groups despite the difference in the number of compounds. No extract contained alkaloids, while they all contained triterpenes, including the glycosidic class of saponins. As for the phenolic compounds, coumarins were not found in the extracts, while flavonoids and anthraquinones were presents in all extracts. As expected, the natural pigments anthocyanins were found only in the Gtlv extracts.

3.3 Acute toxicity

Four batches (B1-4; n=3) of test rats were administered 2000 mg/kg.bw of the 4 extracts Gtsb, Gtrb, Gtrw and Gtlv, respectively; while 4 other (B5-8; n=3) were administered the extracts at a concentration of 5000 mg/kg.bw. The control batch (B0; n=3) was administered 10 ml/kg.bw of distilled water. Physical parameters were observed for 14 days and body weight was measured every 2 days. At single doses of 2000 and 5000 mg/kg.bw, the overall monitoring of toxicity-related parameters did not show any major change in the usual

behavior of treated animals. However, excessive agitation and aggressiveness were observed on D0 (Table 3) during the first 8 hours following administration, in all batches treated with the extract, compared to the control.

Table 3: Observed toxicity parameters at D0

Parameters	B0	B1	B2	B3	B4	B5	B6	B7	B8
Eye colour	N	N	N	N	N	N	N	N	N
Urine colour	N	N	N	N	N	N	N	N	N
Faeces aspect	N	N	N	N	N	N	N	N	N
Noise sensitivity	N	N	N	N	N	N	N	N	N
Pain sensitivity	N	N	N	N	N	N	N	N	N
Agitation	N	E	E	E	E	E	E	E	E
Aggressiveness	N	E	E	E	E	E	E	E	E
Grouping	N	N	N	N	N	N	N	N	N
Grooming	N	N	N	N	N	N	N	N	N
Fur aspect	N	N	N	N	N	N	N	N	N
Motion	N	N	N	N	N	N	N	N	N
Mortality	0	0	0	0	0	0	0	0	0

N: normal; E: excessive.

The parameters were mostly normal (N) on D0, except for the excessive (E) agitation and aggressiveness observed in all batches treated with the 4 extracts, compared to the control batch, up to 8 h after administration of the extracts (Table 3). Nonetheless, the aggressive behavior subsided within the 14 following days of observation (Table 4).

Table 4: Overall observed toxicity parameters after 14 days

Parameters	B0	B1	B2	B3	B4	B5	B6	B7	B8
Eye color	N	N	N	N	N	N	N	N	N
Urine color	N	N	N	N	N	N	N	N	N
Feces aspect	N	N	N	N	N	N	N	N	N
Noise sensitivity	N	N	N	N	N	N	N	N	N
Pain sensitivity	N	N	N	N	N	N	N	N	N
Agitation	N	N	N	N	N	N	N	N	E
Aggressiveness	N	N	N	N	N	N	N	N	E
Grouping	N	N	N	N	N	N	N	N	N
Grooming	N	N	N	N	N	N	N	N	N
Fur aspect	N	N	N	N	N	N	N	N	N
Motion	N	N	N	N	N	N	N	N	N
Mortality	0	0	0	0	0	0	0	0	0

N: normal; E: excessive.

From D1 to D14, the parameters were observed and appeared mostly normal (N). A particularity was noted in B8, which showed excessive (E) agitation and aggressiveness compared to all other batches. B8 was treated with Gtlv at a dose of 5000 mg/kg.bw, an extract with anthocyanins as sole difference when compared to other extracts. Furthermore, no deaths were observed in the different batches (Tables 3 & 4), making the LD_{50} greater than 5000 mg/kg.bw. Also, the weight of the rats was recorded on D0, then subsequently every 2 days for 2 weeks (Figure 1), after which the rats were sacrificed and an autopsy was performed on a macroscopic level.

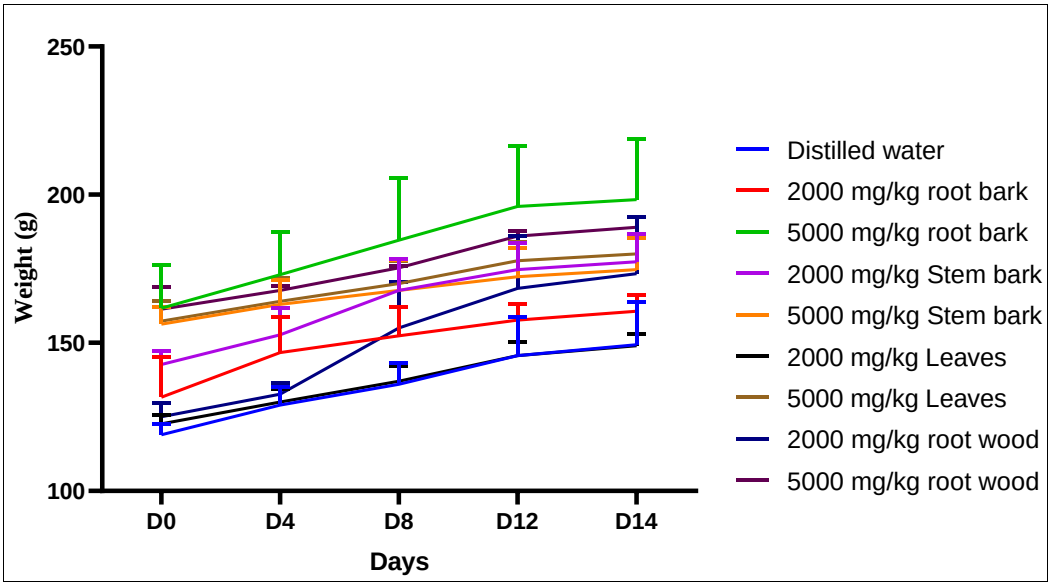

Fig 1: Evolution of rats' weight gain after administration of *Guibourtia tessmannii* extracts.

Despite the difference in size between the rats that received the 4 extracts at a dose of 2000 and 5000 mg/kg.bw, the average weight of the rats generally increased over the 14-day span, regardless of the batch.

3.4 Antibacterial activity

The sterility test showed no bacteria or fungi present in the different extracts: Gtsb, Gtlv, Gtrb and Gtrw. The extracts were assessed for their antibacterial properties against 5 bacterial strains, namely *Neisseria gonorrhoeae*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella pneumoniae*, with ciprofloxacin used as the control. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined (Table 5) using the broth microdilution method.

Table 5: MIC and MBC of *Guibourtia tessmannii* methanolic extracts on five bacterial strains and isolates

Administered substance	Assessed parameters	Bacterial strains and isolates				
		EC	NG	ST	SA	KP
Ciprofloxacin	MIC (µg/ml)	2	0.25	0.5	4	2
	MBC (µg/ml)	2	0.25	1	8	4
	MBC/MIC	1	1	2	2	2
Gtsb	MIC (µg/ml)	1024	512	512	256	256
	MBC (µg/ml)	1024	512	1024	512	1024
	MIC/MBC	1	1	2	2	4
Gtrb	MIC (µg/ml)	1024	512	1024	512	512
	MBC (µg/ml)	1024	1024	1024	1024	1024
	MIC/MBC	1	2	1	2	2
Gtrw	MIC (µg/ml)	1024	1024	512	512	512
	MBC (µg/ml)	> 1024	> 1024	1024	1024	1024
	MIC/MBC	ND	ND	2	2	2
Gtlv	MIC (µg/ml)	> 1024	1024	1024	1024	1024
	MBC (µg/ml)	ND	> 1024	> 1024	> 1024	1024
	MIC/MBC	ND	ND	ND	ND	1

EC = *Escherichia coli*; NG = *Neisseria gonorrhoeae*; ST = *Salmonella typhi*; SA = *Staphylococcus aureus* and KP = *Klebsiella pneumoniae* KP1; Gtsb = *G. tessmannii* stem bark, Gtrb = *G. tessmannii* root bark; Gtrw = *G. tessmannii* root wood and Gtlv = *G. tessmannii*leaves; MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration and ND = Not determined.

The lowest MIC was observed with *S. aureus* and *K. pneumoniae* requiring Gtsb at a concentration of 256 µg/ml. This was followed by *S. aureus* being inhibited by 512 µg/ml of Gtrb and Gtrw, *K. pneumoniae* inhibited by 512 µg/ml of Gtrb and Gtrw, *N. gonorrhoeae* inhibited by 512 µg/ml of Gtsb and Gtrb, and *S. typhi* inhibited by 512 µg/ml of Gtsb and Gtrw. All other activity was observed at a dose of 1024 µg/ml, except for Gtlv which showed no inhibition of *E. coli* up to that dose. Hence, the MBC could not be determined. It was worth noting that Gtsb had the lowest MBCs (512 µg/ml against *N. gonorrhoeae* and *S. aureus* and 1024 µg/ml against all other 3 bacteria), followed by Gtrb (1024 µg/ml against all 5 bacteria), Gtrw (1024 µg/ml against 3 bacteria and >1024 µg/ml against 2 bacteria), and Gtlv (1024 µg/ml against only 1 bacteria and 1024 µg/ml against 3 bacteria with 1 MBC not determined due to an MIC >1024 µg/ml). These results suggest a higher antibacterial activity in the bark of the plant and a lower activity in the leaves. Using the MBC/MIC ratio, the overall effect was determined. The Gtsb extract exhibited a bactericidal effect (MBC/MIC < 4) against all bacterial strains, except *K. pneumoniae* against which it showed a bacteriostatic effect (MBC/MIC ≥ 4). The Gtrb extract had a bactericidal effect (MBC/MIC < 4) against all 5 bacterial strains. The Gtrw and Gtlv extracts had a bactericidal effect (MBC/MIC < 4) against 3 (*S. typhi*, *S. aureus* and *K. pneumoniae*) and 1 (*K. pneumoniae*) bacterial strains, respectively, while the ratio could not be determined for the remaining strains, due to a higher MBC or MIC than the maximum dose used (1024 µg/ml).

3.5 Antifungal activity

The 4 extracts were simultaneously assessed for their antifungal properties against *Candica albicans*, following the same method as for the antibacterial properties. The MIC and minimal fungicidal concentration (MFC) were determined (Table 6).

Table 6: MIC and MBC of *Guibourtia tessmannii*methanolic extracts on *Candida albicans*

Fungal isolate	Assessed parameters	Administered substances				
		Gtsb	Gtrb	Gtrw	Gtlv	Fluconazole
CA	MIC (µg/ml)	512	1024	1024	>1024	256
	MFC (µg/ml)	1024	1024	>1024	ND	256
	MFC/MIC	2	1	ND	ND	1

CA = *Candida albicans*; Gtsb = *G. tessmannii* stem bark; Gtrb = *G. tessmannii* root bark; Gtrw = *G. tessmannii* root wood and Gtlv = *G. tessmannii* leaves; MIC = Minimum Inhibitory Concentration; MFC = Minimum Fungicidal Concentration and ND = Not determined.

The Gtsb extract had the lowest MIC (512 µg/ml), followed by Gtrb and Gtrw(1024 µg/ml). The Gtlv extract did not inhibit the growth of *C. albicans* at the tested doses, thus an MFC could not be determined.

The Gtrw extract had an MFC >1024 µg/ml. Only Gtsb and Gtrb extract showed a measurable MFC (1024 µg/ml) and a ratio could be calculated and the overall effect inferred. The 2 extracts had a fungicidal effect (MFC/MIC < 4).

3.6 Antiplasmodial activity

Each of the 4 extracts was tested at a concentration of 50 µg/ml, to determine the parasite viability and the activity of the (parasite lactate dehydrogenase) pLDH in the wells. The

Gtlv extract reduced the viability of *P. falciparum* 3D7 cells by approximately 1%, while the Gtrw, Gtrb and Gtsb all presented a 100% viability (Figure 2). Determining the IC_{50} was not made possible, since the extracts subjected to the antiplasmodial activities did not demonstrate any significant inhibition of the growth of the *P. falciparum* 3D7 cells.

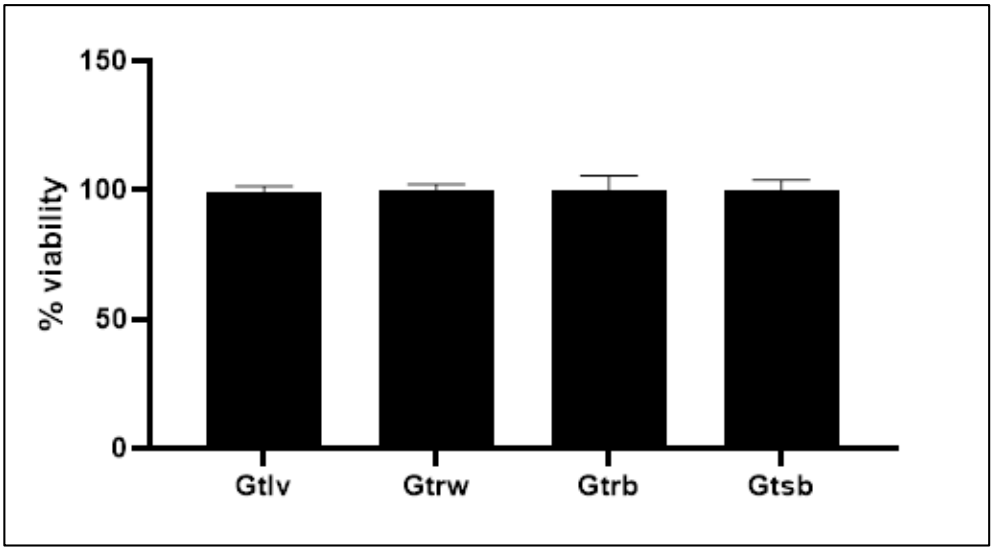

Fig 2: Percentage of *Plasmodium falciparum* 3D7 viability on *Guibourtia tessmannii* methanolic extracts% viability = Percentage viability; Gtsb = *G. tessmannii* stem bark, Gtrb = *G. tessmannii* root bark; Gtrw = *G. tessmannii* root wood and Gtlv = *G. tessmannii* leaves

3.7 Cytotoxicity test.

The 4 methanolic extracts of *G. tessmanni* were subjected to cytotoxicity tests. The microplates were read and the results for percent viability and standard deviation are shown in the bar graph below (Figure 3).

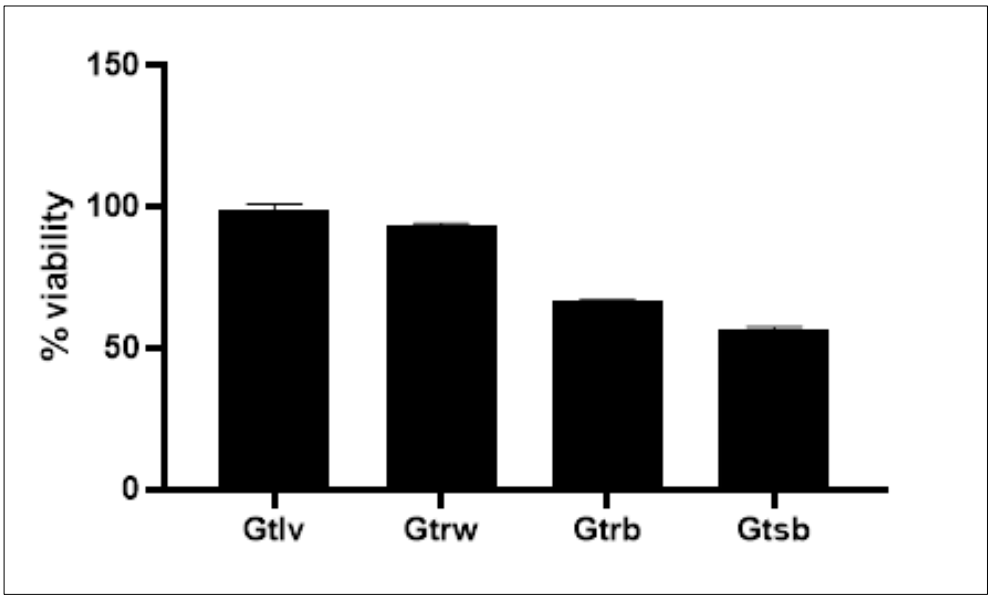

Fig 3: Percentage of HeLa cells viabilityon *Guibourtia tessmannii* methanolic extracts% viability = Percentage viability; Gtsb = *G. tessmannii* stem bark, Gtrb = *G. tessmannii* root bark; Gtrw = *G. tessmannii* root wood and Gtlv = *G. tessmannii* leaves

This diagram illustrates the *in vitro* cytotoxic activity of the methanolic extracts Gtsb, Gtrb, Gtrw and Gtlv on HeLa cells, tested at a concentration of 50 µg/ml. The Gtlvextract reduced cell viability by a relative 2%, followed by the Gtrw extract (6%), then the Gtrb extract (34%) and finally, the Gtsb extract (44%). The extracts did not significantly reduce HeLa cell viability (> 50%) and therefore the IC_{50} was not determined for any of the extracts.

4. Discussion

The extraction by maceration of *Guibourtia tessmannii* extracts was carried out in methanol, which is a protic polar solvent chosen in our study on the one hand for its low boiling temperature of around 65 °C (temperature which allows to minimize the risk of damage to secondary metabolites during the concentration of the macerate) and on the other hand for its ability to dissolve a large proportion of polar and non-polar compounds [32]. The percentage yield of *G. tessmannii* methanolic extracts increases from the roots wood (Gtrw: 6000:45 g; 0.75%) to the stem bark (Gtsb: 8000:98 g; 1.23%), to the leaves (Gtlv: 350:30 g; 8.57%) and finally to the roots bark (Gtrb: 600:82 g; 13,66%). In this plant the secondary metabolites are mostly stored in the roots of the plants, probably to help fight against invader from the soil. These yields are closely comparable to those obtained by Fuendjie *et al*. in 2002 on the stem bark of *G. tessmannii*, extract with EtOAc to yield 6.67% [33].

Table 2 presented to us the different groups of secondary metabolites present in the extracts of the different parts of *G. tessmannii*. Our results agree with those from Nyemba *et al*. in 1995 on the stem bark of *G. tessmannii* which showed the presence of stilbenes, compounds isolated from the stem bark of *G. tessmannii* after successive extraction with acetone and methanol [15]. Our study confirms the presence of phenolic compounds from the stem bark of *G. tesmannii*, several of which have been isolated from the stem bark (stilbenes and flavonoids) by Nkengfack *et al*. in 2001 in addition to reducing sugars [16], which we were unable to test in this study. The same applies to the stilbene glycosides isolated from the stem bark of *G. tesmannii*, by Fuendjie *et al*. in 2002 which are phenolic compounds, in addition to the chalconoids [33] that we were unable to test in this study. Mbaveng*et al*. in 2011 isolated compounds such as phenols, flavonoids, tannins, anthraquinones and terpenes from leaves, roots and stem bark of methanolic extracts of *G. tesmannii* in accordance with our work [14]. Similar results were obtained by Madingou*et al*. in 2012 which showed the presence of secondary metabolites such as tannins, triterpenoids, polyphenols and sterols in methanolic extract of the stem bark of *G. tessmannii* [34]. Roux in 1959 isolated compounds such as flavonoids from the stem bark of *G. tessmannii*, in agreement with our studies, as well as leucoanthocyanins for which we did not observe any positive result [17].

Acute toxicity studies undertaken on the female Wistar-type rats at limit doses of 2000 mg/kg and 5000 mg/kg of body weight of the methanolic extracts Gtlv, Gtrw, Gtrb and Gtsb of *G. tessmannii* showed that behavioral monitoring (physical parameters) revealed no related signs of oral toxicity. This fact denotes an LD_{50} of plant extracts strictly greater than 5000 mg/kg bw per day, suggesting a bio-tolerance on the whole organism over a wide range of doses of the extracts. These results are in agreement with those of Madingou*et al*. in 2016 who demonstrated the safety of the aqueous extract of *G. tessmannii* stem bark in rats at doses ranging from 3000 to 5000 mg/kg bw[19]. Similar results were also obtained by Tjeck*et al*. in 2020, showing that the aqueous extract of *G. tessmannii* stem bark is non-toxic to mice at a dose of 5000 mg/kg body weight [35]. Thus, by sticking to the criteria for classification of toxic substances of the revised OECD guideline N° 423, the methanolic extracts Gtlv, Gtrw, Gtrb and Gtsb of *G. tessmannii* could be classified in on range 5 as being relatively little toxic products [25].

Microdilution compared to diffusion agar is a method perfectly suited to assess the *in vitro* antibacterial/antifungal activities of plant extracts because of its high reproducibility [36]. The Gtsb extract showed a moderate inhibitory activity on the germs: *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae* and *Candida albicans* and a weak inhibitory activity on *Escherichiacoli*. Gtrb presented a moderate inhibitory activity on the germs *S. typhi*, *K. pneumoniae* and *N. gonorrhoeae*, a weak inhibition on the germs; *E. coli*, *C. albicans* and *S. aureus*. Gtrw also exhibited moderate inhibitory activity against *S. aureus*, *S. typhimurium* and *K. pneumonia*, weak inhibition against *N. gonorrhoeae*, *C. albicans* and *E. coli* strains/isolates. Gtlv showed weak inhibition only on *K. pneumoniae*. The antimicrobial potential of *G. tessmannii* extracts would be due to the presence of its secondary metabolites identified during phytochemical screening. Indeed, several authors have reported that phenols are endowed with antibacterial and fungal activities [37]. Other metabolites such as triterpenoids and steroids characterized in this plant would also be responsible for the antimicrobial activity. These results are in agreement with the work of Tene*et al*. in 2009 which showed that buteline and 12-oxohardwickic acid, isolated from *Croton macrotaschys*, both belonging to this family, possessed antimicrobial activities [38]. Also, the work of Kporou*et al*. in 2009 revealed that the extract containing only sterols and triterpenes had better antifungal activity [39]. Finally, many authors such as Yaya *et al*. in 2016 showed that the antimicrobial activity of *Eryngium foetidium* leaves would be due to the presence of secondary metabolites such as phenols, phytosterols and triterpenes as well as the Mbem Song studies in 2017 on the roots of *Terminalia mantaly* [40,41]. Gtrb and Gtsb extracts showed activity against all the germs tested, unlike the Gtlv and Gtrw extracts. This could be due to the content of these groups of secondary metabolites, which would *a priori* be more concentrated in the extracts Gtrb and Gtsb compared to Gtlv and Gtrw extracts. Various authors have given standard graduation values for the antimicrobial activities of plant extracts. Nevertheless, the most recent scale is that of Kuete*et al*. in 2023 which considers that's the antimicrobial activity for plant extracts can have outstanding activity when MIC ≤ 8 µg/mL; excellent activity when $8 < \text{MIC} \leq 64$ µg/mL; very good activity when $64 < \text{MIC} \leq 128$ µg/mL; good activity when $128 < \text{MIC} \leq 256$ µg/mL, average activity when $256 < \text{MIC} \leq 512$ µg/mL, weak activity when $512 < \text{MIC} \leq 1024$ µg/mL, and not active MIC > 1024 µg/mL[42]. Given this classification, the methanolic extract Gtsb proves to be the most active on all bacterial and fungal strains/isolates with MIC values equal to 256 µg/mL for *S. aureus* and *K. pneumonia* strains/isolates with good activity. Average activity with an MIC equal to 512 µg/mL on bacterial and fungal strains/isolates *N. gonorrhoeae*, *S. typhi* and *C. albicans*. A weak activity with an MIC equal to 1024 µg/mL on *E. coli*. In the same vein, according to Marmonier*et al*. in 1990 and Tabouguia*et al*. in 2017, the CMM/CMI ratio when it is less than 4 the extract or the compound has a microbicidal activity and when it is greater than or equal to 4 the activity is microstatic [36,43]. From the above, MMC/MIC ratios less than or equal to 4 for each sample (Gtlv, Gtrw, Gtrb and Gtsb) tested against strains/isolates (*N. gonorrhoeae*, *S. aureus*, *S. typhi*, *E. coli* and *C. albicans*) can be considered as bactericidal and fungicidal. The antimicrobial activity shown by of *G. tessmannii*'s extracts, although moderate, could justify the use of its stem bark in traditional medicine to treat affections such as: typhoid fever, gonorrhea, STDs, staphylococcal skin infections and staphylococcal gastroenteritis [7].

The evaluation of the *in vitro* antiplasmodial activity of the methanolic extracts of *G. tessmannii* at a concentration of 50 µg/mL, on the 3D7 *Plasmodium falciparum* strain (Figure 2) showed that all the extracts would not present any antiplasmodial activity, therefore did not significantly reduce parasite viability. According to the results of the phytochemical screening, the absence of the antiplasmodial activity could be due to the absence of alkaloids on different plant extracts. However, according to the ethnobotanical survey and the traditional use of the plant, the stem bark would be used in synergy with *Carica papaya* leaves already known for its antiplasmodial properties [44]. The presence of secondary metabolites from the stem bark such as anthraquinones and terpenes that may be responsible for the antimalarial activity, could justify this synergistic effect. In addition, the work of Iwanette in 2016 showed a moderate *in vitro* antiplasmodial activity with an IC_{50} at 50 µg/mL of aqueous extracts of a plant of the same genus, namely

Guibourtia coleosperma [45]; This justifies the presence of antimalarial activity in the genus *Guibourtia*. These results are contrary to those of Titanji*et al*. in 2008, on the antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine, which revealed that the decoction of aqueous extracts of *G. tessmannii* stem bark would have remarkably inhibited the viability of *Plasmodium falciparum* with an MIC of 2.4 µg/L using Giemsa and 3.4 µg/L with the hypoxanthine technique [46]. We can also speculate that the absence of antiplasmodial activity could be due to the fact that the solvent used during the extraction (methanol) is different from that used during the previous studies and traditional practices (water). Another potential reason would be the synergetic use of *G. tessmannii* in traditional medicine *C. papaya* leaves, which would potentiate its activity. The antimalarial activity could also result from a synergy of action due to the association with other plants as indicated by traditional use.

The diagram in Figure 3 shows the results of the *in vitro* cytotoxicity of methanolic extracts of *G. tessmannii*, tested at a concentration of 50 µg/mL on HeLa cells (from the human cervix). These results showed that the treatment of these cells with extracts of *G. tessmannii* would have affected cell viability. However, the cytotoxic effect is revealed by a mortality rate of 1.7 to 20%, which suggests that the toxicity of *G. tessmannii* extracts towards these human cells remains very low. The percentages of cell viability remained high (> 50%) for all tested extracts, their IC_{50} values were not assessed. Hence, extracts of leaves, stem bark and roots of *G. tessmannii* were considered non-cytotoxic.

5. Conclusion

The phytochemical screening of the methanolic extracts of the stem bark, the root bark, the root wood and the leaves of *Guibourtia tessmannii* (respectively Gtsb, Gtrb, Gtrw and Gtlv) enabled us to identify on the one hand the presence of polyphenols, triterpenoids, saponins, flavonoids and anthraquinones, and to note on the other hand, the absence of alkaloids, coumarins and anthocyanins in these extracts. The study of the toxicity of different methanolic extracts of *G. tessmannii*at limit doses of 2000 mg/kg and 5000 mg/kg did not reveal any acute oral toxicity, thus allowing us to consider the evaluation of the biological activities on these different extracts. Antimicrobial tests by the microdilution method reveled that only the stem bark extract proved to be the most active with good antibacterial activity with MIC values equal to 256 µg/mL for *S. aureus* and *K. pneumonia* strains, average antibacterial and fungal activities with an MIC equal to 512 µg/mL on bacterial and fungal strains/isolates *N. gonorrhoeae*, *S. typhi* and *C. albicans*. The MMC/MIC ratio allowing to be classified as bactericidal/fungicidal or bacteriostatic/fungistatic. The antimicrobial activities of *G. tessmannii* stem bark could justify its use in traditional medicine in the treatment of gonorrhea and other bacterial STDs, as well as in the treatment of female fertility linked to bacterial infections. Extracts from stem barks, root barks, root wood and leaves had almost no effect at a concentration of 50 µg/mL on the *Plasmodium falciparum* 3D7 strain and therefore does not present any antiplasmodial activity and were also considered non-cytotoxic. This observation might be an indicator of their selectivity as drugs used in pharmacopeia. The absence of the antiplasmodial property thus could justify the use of this plant in association with other plants for the treatment of malaria in the traditional pharmacopoeia.

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7. Conflicts of interest

There is no conflict of interest among the authors.

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