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Phytochemical screening, gossypol content and toxicological assessment of *Thespesia populnea* extracts

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Abstract
The cotton plant (*Gossypium*) is one of the most popular plants that contains gossypol, a yellow pigment described in the literature as a phytoalexin. *Thespesia populnea* (Family: Malvaceae) a neighboring plant, is closely related to *Gossypium* and other genera such as *Cienfuegosia* and *Thurberia* and is also described to contain gossypol as a natural product. Widely used in the Togolese pharmacopeia, *Thespesia populnea* is a medicinal plant not endemic but imported into Togo. The present work intends to study the phytochemical screening and toxicity of *T. populnea* leaves and bark. In addition to the phytochemical screening, free radical scavenging activity, gossypol extraction, and acute and sub-chronic toxicity were carried out. Results show that the bark contains more compounds than the leaves. Similarly, ethanolic extract of bark gave a much higher free radical scavenging activity (IC_{50} 77.45 mg EQ/g ES) than leaves (158.22 mg EQ/g ES). Gossypol's content evaluated from each organ was 0.12% and 0.42% for leaves and bark respectively. The LD_{50} of the extracts is greater than 5000 mg/kg. In addition, the ethanolic extract of bark at repeated doses of 500 mg/kg and 1000 mg/kg caused congestion lesions in the liver and kidneys of over 60% of the animals. This study shows that *T. populnea* should be used sparingly.

Keywords: *Thespesia populnea*, gossypol, free radical scavenging activity, acute toxicity, sub-chronic toxicity

1. Introduction
Also known as portia tree, *Thespesia populnea*, or pacific rosewood, is a medicinal plant that is originally from India. It is renowned for its biological potential among the seventeen species of the *Thespesia* genus [1], a member of the Gossypieae tribe. This plant has shown many biological activities and is frequently reported to possess properties such as anti-inflammatory, antibacterial [2], antidiabetic [3], and anticancer [4]. Traditionally, it has been used to treat skin diseases, dysentery, hemorrhoids and many other illnesses. Its bark and fruit have curative properties and the leaves have anti-psoriatic properties [5]. A decoction of the bark has been used in cancer therapy [4]. These properties are associated with the natural content of sesquiterpenoids, particularly gossypol, that is found in various parts of the plant. [6]. Gossypol is highly toxic [7] and is considered as phytoalexin. *T. populnea* is imported into Togo, and gardeners cultivate it, in order to make it widely available to most of the Togolese people. This present study aims to determine and compare the content of gossypol in the leaves and trunk bark extracts. Acute and sub-chronic intrinsic toxicities of extracts from both plant organs were also evaluated.

2. Materials and methods
2.1 Plant material
T. populnea plant was collected in August 2020 in the south-western port area of Togo, 500 m from the coast (GPS coordinates: N 6°8′12.5556″; E 1°16′6.186″). Botanical identification was carried out at the Department of Botany, University of Lomé, where a specimen was deposited (TOGO 15908) in the herbarium of the Togolese flora. The bark and leaves of this plant were collected and dried in the laboratory at room temperature.

2.2 Animals
Healthy Wistar rats of both sexes, aged 2 months and weighing between 100 and 160 g, were used in this study. Animals were supplied by the animal house of the Laboratory of Physiology, Pharmacology and Toxicology of the Faculty of Sciences, University of Lomé.

Animals were grouped by sex and placed in aerated metal cages (five rats per cage) at ambient temperature and humidity with a 12 h day-light cycle, with free access to food and water *ad libitum*. Animals were handled under the supervision of the national ethics committee (N° SBM/UL/15/ NS0009).

2.3 Method

2.3.1 Extraction of gossypol

The plant material was extracted with Soxhlet using EtOH and the gossypol was extracted using Carruth's method [8]. For the *in vitro* test, 2 g of dry extract was dissolved in 5 ml ethanol and 0.4 ml aniline was added.

2.3.2 Screening for major phytochemical groups

The major groups of natural products, namely alkaloids, tannins, flavonoids, anthocyanins and saponosides, from crude extract of *T. populnea* bark and leaves was screened by using Houghton's method [9].

2.3.2a Crude extracts preparation

Crude extracts of *T. populnea* bark and leaves were obtained by maceration**.** 100 g of each ground sample were mixed with 1 L of ethanol for 72 h, stirring at regular intervals. The resulting mixture was filtered and the filtrate concentrated at 40 °C using a BUCHI R-100 rotavapor. The extract yield was calculated in relation to the initial weight of the dried bark powder.

2.3.2 b Phytochemical screening

The phytochemical analyses from *T. populnea* ethanolic extracts were carried out using different qualitative test for alkaloids, tannins, gallic tannins, flavonoids, anthocyanins, saponosides, quinone derivatives, terpenes and mucilages.

2.3.2.1 Test for alkaloids

5 g of the plant powder was mixed with 25 ml of 5% hydrochloric acid. The mixture was macerated for 24 h. Then, 1 ml of filtrate was collected and 5 drops of Mayer's reagent was added. The formation of a pale yellow or squinty precipitate demonstrates the presence of alkaloids.

2.3.2.2 Test for tannins

1 ml of extract was treated with a few drops of 1% $FeCl_3$. A dark blue, green or black coloration indicates the presence of tannins.

2.3.2.3 Test for gallic tannins

1 ml of extract was saturated with sodium acetate and treated with a few drops of 1% $FeCl_3$. A blue or black blue reveals the presence of gallic tannins.

2.3.2.4 Test for flavonoids

5 ml of extract was treated with 5 ml of hydrochloric alcohol (SHINODA reagent) and a pinch of magnesium powder. The formation of orange colour indicates the presence of flavones, red colour indicates flavonols and violet colour indicates flavonones.

2.3.2.5 Test for anthocyanins

1 ml of extract was treated with a few drops of 5% HCl. The mixture was then alkalinized by adding a few drops of diluted ammonia. A deepening red color, turning violet-blue or greenish, indicates the presence of anthocyanins.

2.3.2.6 Test for saponosides

Saponosides are detected by the foam index, which is determined by the degree of dilution of an aqueous decoction of the drug which, under the given conditions, produces a persistent foam. A few volumes of plant extract were adjusted to 100 ml, is distributed into 10 test tubes in arithmetical series of 1/10 concentration. After 30 longitudinal shakes for 15 seconds, the tubes are left to stand for 15 minutes. The height of the foam is measured. If it is greater than 1 cm in one of the tubes, the dilution in these tubes is the foam index sought.

2.3.2.7 Test for quinone derivatives

2 g of plant powder was mixed with 2 ml of 5% HCl. 20 ml of chloroform was then added and the mixture was stirred continuously for 24 h. Then 5 ml of ammonia was added to the previous mixture. A pink or purplish-red color formation indicates a positive reaction.

2.3.2.8 Test for triterpenes

10 ml of EtOH at 70 °C to 1 g of powder and shake for 30 minutes. To this mixture, was added 10 ml of distilled water, then 2 ml of 10% lead acetate, equal volume V/V. After a 15-minute rest, 2 ml of 10% aqueous disodium phosphate solution was added to the filtrate. After 15 minutes' rest, the filtrate was collected in a separating funnel and extracted three times with 5 ml chloroform. The chloroform solutions were dried over anhydrous sodium sulfate and then evaporated. The first portion was solubilized with a few drops of acetic acid. To the resulting mixture was added 3 ml of a mixture of acetic anhydride-sulfuric acid. A purple, blue or green color indicates the presence of triperpenoids.

2.3.2.9 Test for mucilages

1 ml of 10% diluted extract was introduced into a tube and 5 ml of absolute alcohol was added. The appearance of a flaky precipitate indicates the presence of mucilages after around ten minutes.

2.3.3 DPPH radical test

The antioxidant activity of *T. populnea* bark and leaf was measured using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) test, a relatively stable free radical according to the method described by McCune [10; 11] and applied by Saloufou [12]. Quercetin (1mg/ml) diluted in EtOH was used as standard. Extract samples and standard (0-60 µg/ml) were added to the freshly prepared DPPH ethanolic solution. The mixture was then quickly vortexed and incubated in the dark at room temperature for 30 min The absorbance of the test mixture was read at 517 nm using UV-vis spectrophotometer (UV-1800PC, Macylab instruments inc) against DPPH control containing EtOH in the place of extracts. EtOH was used as a blank. All experiments were performed in triplicate and the result were averaged. The antioxidant capacities of diverse concentrations of bark and leaves extracts were determined based on the reduction in absorbance and expressed as percentage inhibition. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The IC_{50} value (mg EQ/g ES) was calculated and it denoted the concentration of extract required to scavenge 50% of DPPH radicals.

2.3.4 Acute toxicity

Female Wistar rats were used according to the toxicity limit test protocol for the acute toxicity study. Prior to the 14-day

test, rats were divided into three groups of 3 females with body weights ranging from 100 g to 140 g. The animals were deprived of food for 12 hours. The first group (control) received distilled water at 10 ml/kg, the second group received the same dose of *T. populnea* leaf extract at 5000 mg/kg, while the third group received *T. populnea* bark extract at the same above-mentioned dose. Extracts were dissolved in DMSO 0.05%. After oral administration, the animals were returned to their cages, where they had access to food and water. Rats were regularly observed every 15 minutes for the first four (4) hours on the first day and once a day for 14 days to record any changes in their behavior and deaths. Behavioral observations included mobility, agitation, respiration, asthenia, tremor, convulsion, appearance of feces, lethargy, sleep and coma. On day 15, all surviving animals were sacrificed and organs such as liver, kidneys, lungs and heart were removed for macroscopic observation.

2.3.5 Sub-chronic toxicity

As in the case of acute toxicity, sub-chronic toxicity was studied orally using the OECD (2008) protocol [13]. A total of 20 rats weighing 100 to 160 g were used. Animals were divided into 4 groups of 5 (3 males and 2 females), with each group having a very similar average weight (121 g; 126 g; 126.5 g and 128 g). Two groups received daily doses of 500 mg/kg and 1000 mg/kg body weight (bw) of ethanolic extracts of *Thespesia populnea* trunk bark for 28 days. The other groups received distilled water and distilled water + DMSO (0.05%). Rats were observed for 28 days. During this period their weight was recorded every two (2) days and any changes in behavior were also recorded. At the end of the experiment, the rats were fasted for 12 hours, but had free access to water. Blood was drawn from each animal on day 29 using a capillary hematocrit tube. All animals were then sacrificed and organs such as liver, spleen, kidneys, heart and lungs were removed, weighed and macroscopically examined for lesions and signs of toxicity. Relative organ weights were determined according to the following formula:

Rw = (Organ weight/Body weight per rat) x 100

2.3.6 Measurement of haematological and biochemical parameters

Haematological analysis was carried out using an automatic haematological system (Mindray BC 6000). Biochemical analysis of blood was performed after centrifugation at 3000 rotations per minute (rpm) for 10 min. Serum was separated and collected, then stored at -20°C for analysis. Biochemical parameters were determined on an automated biochemical analyzer (Mindray BC 240 Pro) using standard kits available from Human GmbH. D-65205 (Wiesbaden, Germany) for spectrophotometric determination of biochemical parameters.

Table 1: Heamatological and biochemical examination

Hematological tests	Biochemical tests
Levels of white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), platelets and hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCHC), mean corpuscular hemoglobin concentration (MCHC**).**	Determination of enzymes such as transaminases (alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT)), alkaline phosphatase (PAL), total protein (TP), total bilirubin (BT), total cholesterol and triglycerides

2.3.7 Histopathological section of liver and kidney

Organs such as liver and kidney from all exposed rats were fixed in 10% formalin followed by a series of dehydration in ethanol baths and kerosene embedding. These organs were cut at 5 µm under a microscope, then stained with Hematoxylin and Eosin and observed under a light microscope (Leica DM1000, Germany). These sections were photographed using a camera linked to software (Microssystem leica LAS EZ Framework, Germany).

2.3.8 Statistical analysis

Graph Pad Prim 5.00 (Software Inc., USA) was used for statistical analysis. Toxicity results are expressed as mean±mean standard error (±SEM). Statistical analysis was performed by comparison of means and variances using the ANOVA test followed by the Tukey test.

3. Results

Since *T. populnea* was already describe to contain gossypol and gossypol-based derivatives, [14] it was decided to demonstrate the presence of this natural compound in the Togolese specie. For this end, Carruth's method [8] that was largely revisited in the semisynthesis of gossypol Schiff base derivatives through SECheM concept, [15] was used.

3.1 Gossypol content of extracts

Carruth's method is usually used to prove the presence of gossypol in plant extract. This method was developed for the first time on *Gossypium* seed extract in order to isolate gossypol from cottonseed. It consists in adding aniline to the plant extract that possibly contains gossypol as gossypol precipitating agent. The formation of dianilinogossypol (DAG), a gossypol Schiff base as yellow microcrystals, prove the presence of Gossypol in the plant ethanolic extract. Thus, this method was used here to prove the presence of gossypol in our extract. Indeed, after the aniline addition to the *T. populnea* extract, we were delighted to observe the formation of a yellow precipitate. The latter was then filtered under vacuum, and dried before full characterization.

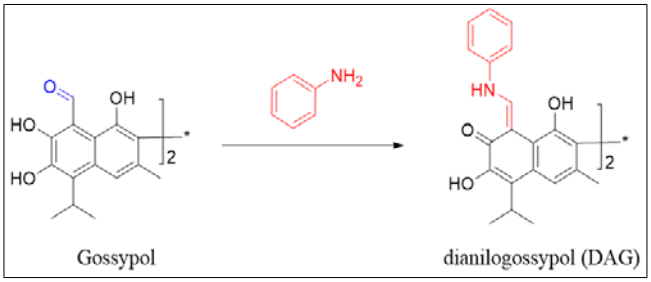


Fig 1: Transformation of gossypol into DAG

The characterization of the formed precipitate, especially by NMR 1D and 2D, shows the formation of dianilogossypol (DAG), a Schiff base of gossypol. [15, 16]. Since the yield of DAG formation is quantitative for longer reaction time, Carruth method or slightly modified one, has been frequently used for gossypol amount estimation in different matrices.

Table 2: Amount of gossypol in biomass (Carruth method)

T. *populnea*	Sample (g)	DAG (mg)	Gossypol (mg)	Estimation of Percentage of gossypol: m/m $_{sample}$
Bark	29	160	124.07	0.42%
Leaf	29	46	35.67	0.12%

3.2 Phytochemical screening and free radical scavenging of ethanolic extract of *T. populnea* bark and leaves

Some chemical tests were realized to point out the major phytochemical groups in the two organs of the *T. populnea*. The results of the screening are displayed in Table 2. These showed that the bark contains alkaloids, tannins, flavonoids, antocyanins, saponosides, quinone derivatives, terpenes, and mucilages, while the leaves contain only tannins, saponosides, terpenes and mucilages. Free radical scavenging test results are shown in Figure 1. Quercetin was used as the reference molecule, and all results relate to a standard curve whose equation is: Y= 0.6503x +4.3004 with a correlation coefficient r^2 = 0.9973. Equations obtained from linear regression for bark and leaf extracts are respectively Y= 0.562x + 6.4712 (r^2 = 0, 9771) and Y= 0.2589x + 9.0362 (r^2 = 0.9106). From these equations we determined the IC_{50} values of bark and leaf extracts and quercetin on the reduction of the DPPH radical. The IC_{50} of these samples taken in this order are respectively 77.45 mg EQ/g ES; 158.22 mg EQ/g ES; 70.27 mg EQ/g ES.

Table 3: Phytochemical screening

Chemical group	Test	*T. populnea* bark ethanolic extract	*T. populnea* leaf ethanolic extract
Alkaloids	Mayer's reagent	+	-
Tannins	$FeCl_3$	+	+
Gallic tannins	$FeCl_3$	+	+
Flavonoids	Shinoda reagent	+	-
Anthocyanins	HCl and NH_3	+	-
Saponosides	Foam index	+	+
Quinone derivatives	Born-Trager's reaction	+	-
Triterpenes	Lead acetate	+	+
Mucilages	Alcoholic precipitation	+	+

(+) present; (-) absent

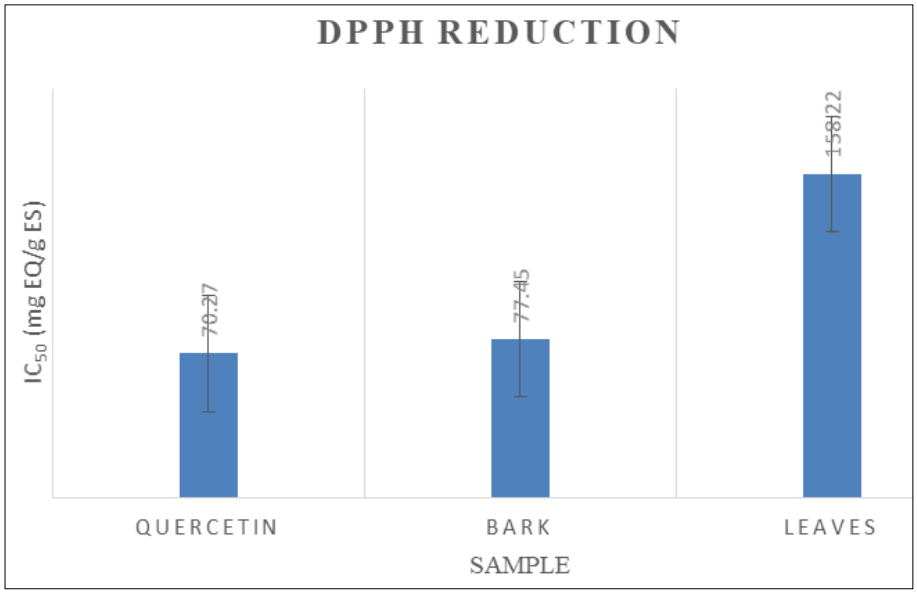


Fig 2: IC_{50} of ethanolic extracts from the two organs of *T. populnea*

3.3 Acute oral toxicity

The dose of 5000 mg/kg administered orally to rats did not cause death of any animal during treatment. However, in the first 15 minutes after oral administration, there was a lack of appetite and a decrease in agitation. Observations revealed no signs of mobility, respiration, asthenia, changes (in hair, eyes and mucous membranes), trembling, convulsion, appearance of faeces, lethargy, sleep or coma. No organ abnormalities were observed at necropsy.

3.4. 28-day Sub-chronic oral toxicity

3.4.1 Symptomatic signs and mortality

During 28 days of oral administration of *T. Populnea* bark extract, no obvious symptoms were observed in rats at either dose level. Similarly, no changes were observed in general behaviour, appetite or water consumption. No deaths were recorded during the 28 days of extract administration at the two doses used.

3.4.2 Effect of ethanolic extract of *Thespesia populnea* bark on rat body weight

Figure 3 below displays the evolution of rat body weights during the study. As shown on the figure, the body weight of control rats increased progressively throughout the study. In contrast, the body weight curve for treated rats shows four different phases. Firstly, an increase of the body weight in the first six days was observed followed by a decrease between days 6 and 10. Then, a quasi-stagnation phase occurred between days 10 and 16, and finally a slight increase was again observed from day 16 to the end of treatment. In general, both extract doses (500 mg/kg and 1000 mg/kg) markedly slowed down the weight gain of the rats. In the case of rats given the 1000 mg/kg dose, the difference in body weight was significant (p<0.05) compared with control rats from days 16 to 28.

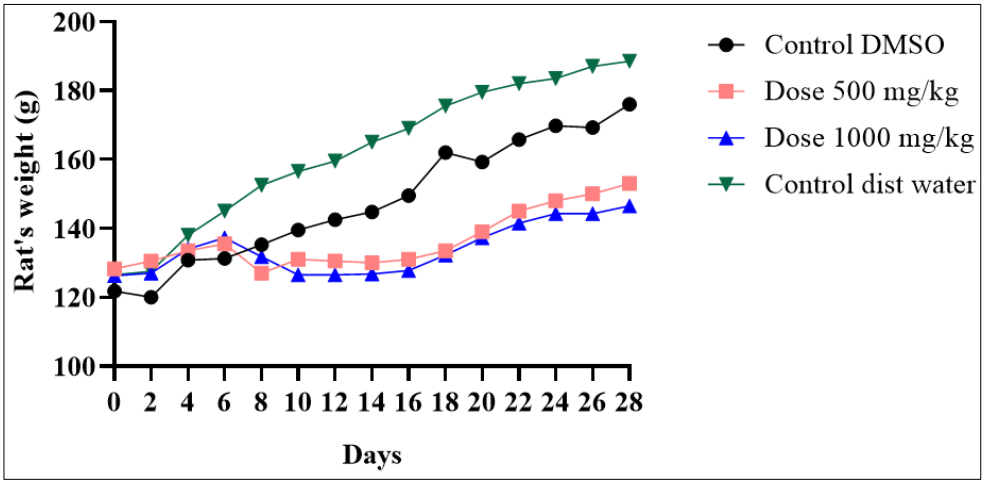


Fig 3: Effet of *T. populnea* bark extract on rat weight development

3.4.3 Macroscopic observation and statistical analysis of relative rat weights

It should be noted that the batch of rats receiving distilled water is not the authentic control, but only provides information on whether DMSO induces toxicity at 0.05% or not. Macroscopic observation of the organs showed slight changes in the lungs of rats treated at doses of 500 mg/kg and 1000 mg/kg. There were no significant effects on relative heart, spleen and kidney weights in treated rats compared with untreated ones (Table 4). No treatment-related gross pathology was observed in any of these organs. However, in the 1000 mg/kg dose group, there was a significant effect on lung weight. Indeed, the weight of the 1000 mg/kg dose group was largely increased (0.87±0.07 g) when compared to the 500 mg/kg dose group (0.61±0.09 g). Similarly, a significant decrease in liver weight was observed in rats receiving the extract at a dose of 1000 mg/kg (Table 4).

Table 4: Effect of ethanolic extract of *T. populnea* trunk bark on relative organ weights in rats

Organs	Relative organ weights			
	Control water +DMSO	Control water distilled	Bark extract dose (mg/kg)	
			500	1000
Heart	0.33±0.005	0.35±0.02	0.39±0.02	0.37±0.01
Lung	0.61±0.05	0.59±0.01	0.61±0.09	0.87±0.07*
Spleen	0.30±0.03	0.39±0.02	0.35±0.06	0.34±0.03
liver	3.68±0.15	3.65±0.01	3.64±0.15	3.40±0.10**
kidney	0.56±0.02	0.59±0.05	0.57±0.02	0.55±0.02

Values are expressed as mean ± MSE (n=5); no significant difference for heart, spleen and kidney of control group and treated rats (p> 0.05). On the other hand, there was a significant difference (p<0.05) between the lung weights of the control group and the 1000 mg/kg dose group. Similarly, in the liver, there was a significant difference between the weight of the control group and that of the 1000 mg/kg group; (ANOVA one way followed by tuley's multiple comparison test).

3.4.4 Effect of extract on biochemical and haematological parameters in rats

3.4.4.1 Effect of extract on biochemical parameters

Results of biochemical analyses in rats were shown in Table 5. The extract produced no significant changes in blood glucose, triglycerides, cholesterol or total proteins contents. Furthermore, this study revealed that oral administration of *T. populnea* bark extract for 28 days showed significant increase (p<0.0001) on alkaline phosphatase (PAL) for the 1000 mg/kg dose, and a non-significant increase for the 500 mg/kg dose. Similarly, over the 28-day period, aspartate aminotransferase (AST) was non-significantly increased at both doses. For alanine aminotransferase (ALT), statistical analyses revealed a non-significant increase for the 500 mg/kg dose and a significant increase (p<0.0001) for the 1000 mg/kg dose.

Results also show that total bilirubin and direct bilirubin underwent a non-significantly increase in ethanolic extract-exposed rats bark (Table 5).

In summary, results show that the biochemical parameters of the rats after administration of the extract were not altered overall.

Table 5: Effect of *T. populnea* trunk bark extract on biochemical parameters

Biochemical parameters	Control DMSO	Control distilled water	Bark extract dose (mg/kg)	
			500	1000
Urea	0.31±0.02	0.33±0,03	0.29±0.03	0.28±0,04
Blood glucose (g/dl)	0.61±0.07	0.7±0.02	0.545±0.01	0.64±0.01
Creatinine (mg/l)	9.71±0.15	3.91±0.20	9.62±0.13	8,17±1.49
AST (U/l)	145.90±7.68	131.85±5.72	147.95±6.66	159.20±6.33
ALT (U/l)	45.67±5.56	46±5.80	52.95±5.91	87.65±5.47**
GGT	3.10±1.47	2.35±0.14	1.95±0.02	1.97±0.43
PAL (UI/l)	160.67±10.30	159.45±9.40	173.42±4.93	200.10±4.20****

Total Bilirubine	1.11±0.13	1.11±0.19	1.13±0.12	1.94±0.42
Bilirubine Direct	0.82±0.11	0.82±0.13	0.86±0.06	1.11±0.22
Total Cholesterol	0.53±0.03	0.47±0.04	0.51±0.04	0.55±0.06
Triglycerides (g/l)	1.21±0.20	1.33±0.29	0.72±0.04	0.74±0.23
Total Protein (g/l)	69.10±2.18	69.40±0.63	65.70±0.92	67.85±2.27

Values are expressed as mean ± MSE, n=5; $p>0.05$ no significant difference from controls for all organs except platelets. In fact, a difference ($p<0.0001$****) was observed for alkaline phosphatase (PAL) and ALT ($p<0.001$**) between the control group and the 1000 mg/kg dose group, and a non-significant increase for the 500 mg/kg dose (one-way ANOVA followed by tukey's multiple comparison test.

3.4.4.2 Effect of extract on hematological parameters

Heamatological analysis showed no significant changes in white and red blood cell counts, hemoglobin levels, mean corpuscular volume (MGV), mean corpuscular hemoglobin content (MCH) or mean corpuscular hemoglobin concentration (MCHC). On the other hand, the platelet count was significantly ($p<0.0001$) reduced at the 1000 mg/kg dose.

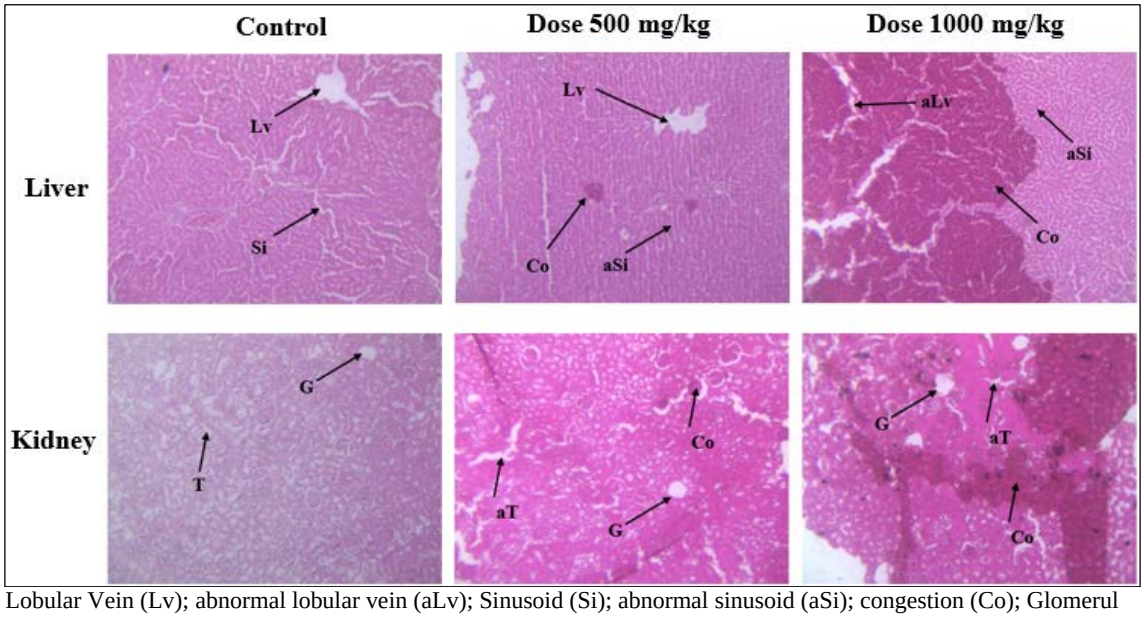
Table 6: Effect of extracts on haematological parameters

Haematological Parameters	Control DMSO	Control distilled water	Dose of bark extract (mg/kg)	
			500	1000
White blood cell (10^9/l)	17.77±2.49	15.37±2.17	14.03±1.92	15.15±3.57
Red blood cell (10^{12}/l)	8.40±0.20	8.12±0.33	8.75±0.03	8.18±0.24
hemoglobin level (g/dl)	15±0.27	15.05±0.43	15.25±0.31	14.27±0.19
Hematocrit (%)	43.80±1.10	44.75±1.52	44.85±0.89	42.15±0.71
MGV (fl)	52.22±1.03	55.15±0.43	51.25±0.77	51.65±2.09
MCH (pg)	17.87±0.33	18.55±0.20	17.40±0.28	17.45±0.67
MCHC (g/dl)	34.20±0.34	33.65±0.14	33.95±0.02	33.82±0.24
PLT (10^3/µl)	915.50±44.97	829±38.10	930.75±36.92	1056.5±33.19****

Values are expressed as mean ± MSE, n=5; $p>0.05$ no significant difference from controls for all organs except platelets. In fact a difference ($p<0.0001$****) is observed for platelets between the control group and the 1000 mg/kg dose group; (ANOVA one way followed by tukey multiple comparison test); MGV: mean corpuscular volume; MCH: mean corpuscular hemoglobin content; MCHC: mean corpuscular hemoglobin concentration; PLT: platelet.

3.2.5 Histopathological analysis of liver and kidneys

Histopathological examination of the liver and kidneys revealed no cellular necrosis or atrophy of these organs. The livers of all animals showed no oedema, but congestion lesions were observed in the kidneys and livers of over 60% of rats treated with both doses (Figure 4).



Lobular Vein (Lv); abnormal lobular vein (aLv); Sinusoid (Si); abnormal sinusoid (aSi); congestion (Co); Glomerul (G); Tubule (T); abnormal tubule (aT)

Fig 4: Histological sections of liver and kidneys

4. Discussion

The present study has first assessed the quantity of gossypol in the leaves and trunk bark of *T populnea* and then studied the plant's toxicity. Results of comparative phytochemical screening of bark and leaves (Table 2) show that the bark has a richer compound profile than the leaves. According to Table 2, all the major targeted groups of natural compounds are present in the bark extract, whereas the leaf extract contains neither alkaloids nor quinone derivatives. Among phenolic compounds, only tannins are present in the leaf extract. This difference may be caused by the low content of these compounds in the leaves, or the method used to identify them. Regarding the absence of alkaloids, flavonoids and anthocyanins in the leaf extract, this result is different from

those reported in the literature. Indeed, a study was carried out in 2011 in India to assess the cell morphology and phytochemical compounds of *T populnea* leaves [17]. The phytochemical compounds found were alkaloids, flavonoids, carbohydrates, phytosterols, tannins, saponins, proteins and amino acids, terpenes, phenols, gums and mucilages. Similarly, a phytochemical test carried out on a Cameroonian species of *T. populnea* gave similar results [18]. These results were confirmed in 2018 by Rangani and colleagues [19], who profiled *T. populnea* leaf extract to show that this plant organ contains high levels of polyphenols. In Togo, the phytochemical composition of leaves does not reveal the same compound profiles as that studied in India and Cameroon. This variability in the composition of secondary metabolite groups could be due to factors such as: the nature of the extraction solvent, soil type, climate, harvesting period and storage conditions [20]. In view of our phytochemical results, we can admit that the defense arsenal of this Togolese plant, based on secondary metabolites, seems to be more concentrated in the bark. Correlatively the results of the free radical scavenging test show that bark has good free radical scavenging activity (IC_{50} =77.45 mg EQ/g ES) compared with leaves (IC_{50} =158.22 mg EQ/gES). The following antioxidant IC_{50} value of bark is close to that of quercetin used as a reference molecule (70.27 mg EQ/g ES). This result is similar to that found by Ilavarasan *and al* in their antioxidant activity study of the *T. populnea* bark extract against liver damage induced in rats [21]. Undoubtedly, the antioxidant capacity of *T. populnea* bark could only be explained by the presence of high levels of flavonoids, or polyphenols in general, known for their powerful antioxidant properties [22]. In Rangani's work [19], for example, this interdependence was noted. Indeed, 18 polyphenolic compounds have been identified and, through correlation analysis, are the main contributors to the plant's antioxidant capacity. The phytochemical screening results also show a correlation with those of the free radical scavenging test. The low proportion of phenolic compounds in the leaves used in this study would explain the low antioxidant capacity of the leaf extract, and vice versa in the case of bark extracts.

It should be remembered that gossypol has been identified in *T. populnea* [14] and its toxicity has been reported in the literature [23], but the content of this molecule in *Thespesia* organs has not been reported. This work shows that bark contains a significant amount of gossypol compared to leaves. Acute oral toxicity of leaves and bark studied at 5000 mg/kg revealed no obvious sign of toxicity. There were no deaths or significant changes in behavior, except a slight lack of agitation during the first 15 minutes after administration. After 15 days, the organs were necropsied, and after careful examination, no abnormalities were noted. These results show that the lethal dose (LD_{50}) of leaf and bark extracts is greater than 5000 mg/kg. Extracts might be considered non-toxic by the oral route. Consequently, the oral intake of *T. populnea* bark and leaf extracts at a single high dose can be considered safe in humans, justifying their use in traditional pharmacopoeia [2, 4, 24].

With regard to the sub-chronic toxicity of *Thespesia populnea* bark, oral administration over 28 days generally showed no clinical signs. In addition, a reduction in body weight was observed in rats treated with doses of 500 mg/kg and 1000 mg/kg. This reduction in body weight was significant in the case of the 1000 mg/kg dose (p<0.05). This result could be due to the lack of appetite induced by the extracts in these rats, leading to a relaxation in daily food consumption. Relative organ weight measurements are reported in table 4. Analysis of these results shows that the extracts had no significant effect on kidney, heart and spleen weights. On the other hand, a significant reduction and increase (p<0.05) was noted respectively in liver and lung weight at the 1000 mg/kg dose level. This result indicates that *T. populnea* bark extract exerts a toxic effect on the liver and lung. Organ weight changes often correlate with changes in haematological and biochemical parameters [25]. With this in mind, clinical hematological and biochemical serum analyses were carried out to assess possible alterations in liver and kidney functions influenced by the extracts. The extract produced no significant changes in blood sugar, triglyceride, cholesterol or total protein levels. In addition, liver enzymes such as phosphate transferase (PAL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly elevated at both doses (Table 5). These results suggest that the extract is toxic to liver cells, as these three enzymes are common markers of liver toxicity [26].

The results also highlighted the fact that total bilirubin and direct bilirubin are not significantly increased by the ethanolic extract of *T. populnea* trunk bark (Table 5).

With regard to haematological parameters, analysis reveals no significant effect of the extract on white and red blood cell counts, haemoglobin levels, mean corpuscular volume (MCV), mean corpuscular haemoglobin content (MCH) and mean corpuscular haemoglobin concentration (MCHC). On the other hand, the number of blood platelets increased in rats treated with both doses. This increase was significant (p<0.0001) at 1000 mg/kg. So, the extract could cause thrombocytosis which would lead to possible blood clotting.

First and foremost, the organs were examined macroscopically, and results showed no change in color compared to control group. Organ hypertrophy is a direct indication of toxicity of a chemical or biological substance. However, no organ hypertrophy was observed in this study. In addition, microscopic examination of the liver and kidneys revealed no edema, cellular necrosis or atrophy. However, congestive lesions were observed in the kidneys and livers of over 60% in exposed rats (Figure 4). In general, any damage to parenchymal liver cells leads to elevation of both transaminases in the blood [27]. Thus, for the 1000 mg/kg dose, the significant increase observed in ALT and PAL activities and non-significant increase in AST activities strongly suggest that repeated administration of *T. populnea* bark extract causes toxicity for this dose. We suspect that this toxic effect of *T. populnea* bark is mainly due to the presence of gossypol.

5. Conclusion

The phytochemical study, determination of gossypol content and toxicological study of ethanolic extracts from the leaves and trunk bark of *Thespesia populnea* were carried out in order to contribute to the traditional use of this plant in Togo. The bark of the trunk is richer in chemical compounds than the leaves. Similarly, its antiradical power and gossypol content are much higher than those of the leaves. In terms of toxicology, extracts from both plant organs were non-toxic at a single dose of 5000 mg/kg. However, congestion lesions were observed in the liver and kidneys of several rats treated at doses of 500 mg/kg and 1000 mg/kg. *Thespesia populnea* should therefore be used sparingly.

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7. Conflicts of Interest: Nil

8. References

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