Botanical from the Fruits Mesocarp of *Raphia vinifera* Displays Antiproliferative Activity and Is Harmless as Evidenced by Toxicological Assessments

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Abstract

*Raphia vinifera* is widely used to treat several diseases including digestive disorders, dysentery, and genitourinary infections. In this study, the mineral contents, the cytotoxicity, and the toxicological effect of the crude CHCl3/MeOH extract (RVM) from the mesocarp of *Raphia vinifera* were evaluated. The mineral contents were evaluated using the method described by the Association of Official Analytical Chemists (AOAC). The cytotoxicity of both extract and chemical compounds from the plants was determined by a resazurin reduction assay (RRA). The toxicological studies were carried out using the experimental procedure of the Organization for Economic Cooperation and Development (OECD). After killing the rats, biochemical, histopathological, and hematological studies were performed. The result indicated that RVM is rich in zinc (6.52 mg/100 g of DM) and sodium (194.5 mg/100 g of DM). RVM had a cytotoxicity effect with IC50 values lower than 30 μg/mL in 18/18 cancer cell lines tested. These recorded IC50 values were between 12.35 μg/mL (toward CCRF-CEM leukemia cells) and 26.66 μg/mL (toward SKMel-505 BRAF wild-type melanoma cells). Raphvin 4 displayed good cytotoxicity against MaMel-80aBRAF-V600E homozygous mutant with the IC50 of 10.42 μM. RVM was relatively nontoxic to rats, the median lethal dose (DL50) being above 5000 mg/kg body weight. However, during the oral administration period extending for 28 days, precautions should be taken due to the increase in urinary creatinine level and decrease in spleen weight in the male rats given the highest dose (1000 mg/kg) of extract. Conclusively, the extract of *Raphia vinifera* is weakly toxic in rats and could be further used in the development of anticancer phytomedicines.

1. Introduction

Despite the numerous means to fight against cancer, the number of deaths caused by this disease is increasing significantly in many countries [1]. In 2018, WHO (World Health Organization) recorded 9.6 million deaths and 18.1 million new cases because of cancer [2]. Due to morbidity and mortality that it generates, cancer represents a major health problem both nationally and globally. This pathology becomes increasingly difficult to diagnose and to treat when cancer cells develop resistance mechanisms against the usual chemotherapeutic agents [3]. Regarding the increasing resistance developed by these cells, research for alternative treatments should be performed. The varieties of secondary metabolites contained in medicinal plants are responsible for the pharmacological effects including cytotoxic activity [4]. *Raphia vinifera* (Arecaceae) is a plant from the genus *Raphia*; medicinal properties of different parts of the plants have been demonstrated. Raffia wine from *Raphia vinifera* is rich in lactic acid bacteria [5], which prevents the incidence of diarrhea and promotes the course of the immune response in rats; these probiotic isolates could strengthen the immune system in children [6]. Also, many medicinal plants used in Africa have shown interesting antiproliferative properties against the sensitive and multi-drug-resistant (MDR) cancer cells linked to their secondary metabolites [4, 7]. The boiled
solution of apical bud of Raphia vinifera is used to treat some diseases like genitourinary infections and gonorrhea in West Cameroon. The leaf is used to fight against poison and for various sexually transmitted diseases and witchcraft [8]. To solve liver problems, the young leaves of this plant are used, and the crushed fruits are poured into water to capture fish easily [9, 10].

Palm has been proven to have minerals like calcium [11]. The Raphia vinifera fruit pulp and pericarps were found to contain a high concentration of saponins, alkaloids, and oxalate; a moderate concentration of tannin, flavonoid, and steroid; and a low concentration of phytate, phenol, and glucoside, which are responsible for its therapeutic activity [12]. This plant has provided steroidal saponins [13], which are beneficial in preventing tumors and treating many cancers with high efficiency associated with weak toxicological effect [13]. In addition, saponins are also cytotoxic and act by blocking the cell cycle and could significantly disrupt the mitochondrial membrane potential and selectively upregulate the protein levels of Bax, cytochrome C, and cleaved caspase 3/9 and downregulate the levels of Bcl-2 [14]. The pulp of Raphia vinifera contains oil that was extracted and characterized physically and chemically by Igwenyi et al. [15].

Many investigations have demonstrated the ability of medicinal plants in the prevention and treatment of many diseases [4, 16]. However, little information is provided on the toxicological effect of plants on consumers. The research on toxicological effects of medicinal plants and their extract is crucial in the development of drugs and to rise human safety [17]. Many toxicological studies have been carried out using Raphia vinifera on fish [11], but studies hardly describe the biochemical toxicity of this plant on rats. This investigation was therefore carried out to evaluate antiproliferative potential of Raphia vinifera extract and its constituents, as well as the toxicity of the crude extract.

2. Material and Methods

2.1. Chemicals and Preparation of the Extract. The phytochemicals used were (25R)-spirost-5-ene-3β, 22β-3-O-β-D-glucopyranosyl(1→2)-O-α-L-rhamnopyranoside (1); (25S)-26-O-(β-D-galactopyranosyl)-furost-5-ene-3β, 22α, 26-trihydroxy-3-O-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside or raphvinin 1 (2); (25R)-26-O-(β-D-galactopyranosyl)-furost-5-ene-3β, 22α, 26-trihydroxy-3-O-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside or raphvinin 2 (3); (25R)-26-O-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranosyl]-furost-5-ene-3β, 26-dihydroxy-22α-methoxy-3-O-β-D-glucopyranosyl - (1→2) -[α-L-rhamnopyranosyl-(1→2)]-β-D-lucopyranosyl (1→4]-β-D-glucopyranoside or raphvin 3 (4); diosgenin (5); diosgenin-3-O-β-D-glucopyranoside or (22R, 25R)-3β-spirost-5-ene-3-O-β-D-glucopyranoside or trillin (6); deltonin (7); 26-O-β-D-glucopyranosyl-(22R,25R)-3β, 22, 26-trihydroxyfurost-5-ene-3-O-β-D-glucopyranoside (8); and sitosterol (9). The NMR spectra and the chemical shifts of compounds 1–9 are provided in the Supplementary file (S1 to S18). Raphia vinifera fruits collected in Bambili, Northwest Region of Cameroon on April 2016, and identified at the Cameroon National Herbarium (voucher number: 38374/HNC) as previously reported [18]. Doxorubicin 98.0% (Sigma-Aldrich) (Munich, Germany) comes from the Medical Center of the Johannes Gutenberg University (Mainz, Germany) and is dissolved in phosphate buffer saline (PBS; Invitrogen, Egggenstein, Germany) at 10 mM. The fruits of Raphia vinifera were dried and powdered. This powder (1 kg) was thereafter macerated in CHCl3/MeOH (5 L) in the proportions 1:1 at room temperature. After 2 days, the extract obtained was filtered with Whatman filter paper (No. 1) and rotary evaporator (Buchi R-200) was used to concentrate the filtrate at 40°C. The crude extract was assembled in sterile flask and dried by oven (40°C) until the solvent completely evaporated.

2.2. Cell Cultures and Origins. 18 cancer cell lines and normal hepatocyte AML12 were used in the present study. Cancer cell lines such as drug-sensitive leukemia CCRF-CEM and multidrug-resistant P-glycoprotein-overexpressing subline CEM/ADR5000 cells [19–21], breast cancer MDA-MB-231-pCDNA cells and its resistant subline MDA-MB-231-BCRP clone 23 cells [22], colon cancer HCT116 p53+/− cells and its knockout clone HCT116 p53−/−, glioblastoma U87MG cells, and its resistant subline U87MG.ΔEGFR [7, 23, 24]. The maintenance of HepG2 cells and AML12 hepatocytes was also published [25]. The CC531 rat colon carcinoma cells, B16-F1, B16-F10, A2058, SK-Mel505, MaMel-80a, MV3, SkMel-28, and Mel-2A, were previously reported [26–30].

2.3. Experimental Animals. For the toxicological studies, adult Wistar rats (8 to 9 weeks old) of the 2 sexes were selected. To ensure their growth, the animals engaged in the animal house received food daily and tap water. They were maintained at standard laboratory conditions of regular 12 h light/12 h dark cycle. This work was carried out with respect to the well-being of rats like the Institutional Ethical Review Committee of the University of Dschang Cameroon recommended.

2.4. Determination of the Mineral Contents. Mineral contents (Ca, P, Mg, Fe, Na, Zn, and K) were determined by the extract using the AOAC method [31]. Raphia vinifera fruit extract was introduced into a porcelain crucible and calcinated at 450°C for 2 hrs. The contents of potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe), zinc (Zn), and phosphorus (P) were determined colorimetrically by UV-visible spectrophotometer (Technel 752 P), according to AOAC procedure. Mineral contents of the sample were determined from calibration curves of standard minerals. All minerals were analyzed in duplicate.

2.5. Cytotoxicity Assay. Different types of human cancer cell lines were used in this study. The resazurin reduction assay
(RRA) as previously described [24, 32] with similar experimental conditions to those reported earlier [26–30] was used to measure the cell cytotoxicity. Fluorescence was measured on an Infinite M2000 Pro™ plate reader (Tecan, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. The viability was determined based on a comparison with untreated cells. The values representing the sample’s concentrations required to inhibit 50% of cell proliferation (IC50) were calculated from a calibration curve by linear regression using Microsoft Excel 2013 [33, 34].

2.6. Acute Toxicity Study in Rats. This test was realized under the OECD guidelines [35]. We followed the methods described by Nguenang [36]. Three adult female rats (8–9 weeks) were treated orally with one dose of extract (5000 mg/kg), after 12 hrs of fasting. These rats were individually and frequently observed to check any signs of toxicity during the first day; observation was continued daily for a total of 14 days of the experiment. The body weight of animals on the 15th day was measured. Subsequently, they were anesthetized through intraperitoneal injection with a solution containing diazepam and ketamine (0.2/0.1 ml per 100 grams of the animal), the vital organs such as lung, spleen, heart, kidneys, and liver were removed and weighed, and the macroscopic examinations were performed on those organs. The relative organ weight was determined.

2.7. Subchronic Toxicological Study

2.7.1. Treatments. This study was performed under the protocol of the OECD Guidelines [37]. We followed the methods described by Nguenang [36]. Thirty-two Wistar rats (16 males and 16 females) aged from 08 to 09 weeks were distributed in 4 groups of 4 rats per group. The groups received the doses of 250, 500, and 1000 mg/kg b.w. of extract, while the control group received only distilled water during at least 24h, dehydrated in a graded series of ethanol (80–100°), and enclosed (embedded) in paraffin. Thereafter, 5-μm sections were prepared using a microtome and stained with hematoxylin-eosin before the microscopic examination. The microscopic features of the animal’s (male and female) organs-treated groups were compared with the control group [36, 38].

2.7.2. Evaluation of Hematological Parameters. To determine these parameters, the blood sample of rats was collected in the EDTA tubes after the kill. The hematological analysis was performed using an automated analyzer hematology (QBC Autoread plus, United Kingdom). The hematological parameters analyzed included white blood cells (WBCs), hemoglobin (Hb), red blood cells (RBCs), hematocrit (HCT), mean corpuscular volume (MCV), lymphocytes (LYMs), mean corpuscular hemoglobin concentration (MCHC), platelets (PLTs), monocytes, granulocytes, mean corpuscular hemoglobin (MCH), and mean platelet volume (MPV).

2.7.3. Evaluation of Biochemical Parameters. The blood collected in dry tubes was centrifuged at 3000 rpm for 15 min to obtain the serum. The biochemical parameters measured were as follows: total serum protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine (CREA), alkaline phosphatase (ALP), high-density lipoprotein-cholesterol (HDL-C), total cholesterol (TC), serum urea (UREA), low-density lipoprotein-cholesterol (LDL-C), and triglycerides (TG).

2.7.4. Histopathological Examination. After killing the rats, kidneys and liver were removed and cleaned in saline solution. The parts of these organs were collected for histological studies. These tissues were fixed in formalin (10%) during at least 24h, dehydrated in a graded series of ethanol (80–100°), and enclosed (embedded) in paraffin. Thereafter, 5-μm sections were prepared using a microtome and stained with hematoxylin-eosin before the microscopic examination. The microscopic features of the animal’s organs were compared with the control group [36, 38].

2.8. Statistical Analysis. The data are expressed as mean ± standard deviation (SD). These results have been submitted to the analysis of variance (ANOVA) at one factor according to the general linear model. Statistical analysis was done using version 21 of the IBM-SPSS statistical program, and statistical comparisons were made using the test of Waller Duncan for the subchronic toxicity at the 5% probability level.

3. Results

3.1. Mineral Content in Extract. The mineral composition of RVM is presented in Table 1. The data obtained from the mineral levels showed that RVM contains more zinc (6.52 mg/100 g of DM) and sodium (194.5 mg/100 g of DM) and less potassium (575.4 mg/100 g of DM), calcium

| Mineral (mg/100 g) | RVM |
|-------------------|-----|
| Calcium | 536.5 ± 0.5 |
| Iron | 5.28 ± 0.02 |
| Potassium | 575.4 ± 0.4 |
| Magnesium | 133.67 ± 0.02 |
| Sodium | 194.5 ± 0.5 |
| Phosphorus | 277.49 ± 0.52 |
| Zinc | 6.52 ± 0.02 |

The table values are presented as mean ± standard deviation.

Table 1: Mineral composition of RVM.
β-glucopyranosyl-(22,25β-D-glucopyranoside (8), and sitosterol (9).

R-glucopyranosyl(1-RVM resistance (D.R.) was calculated as the ratio of the IC50 value of hepatocytes (Tables 2 and 3; Figure 1). The degree of proliferation of 18 cancer cell lines and normal AML12 determined by RRA.

3.2. Cytotoxicity of Extract. The RRA was used to evaluate the effects of RVM, compounds 1–9, and doxorubicin on the proliferation of 18 cancer cell lines and normal AML12 hepatocytes (Tables 2 and 3; Figure 1). The degree of resistance (D. R.) was calculated as the ratio of the IC50 value of the resistant cell line divided by that of the corresponding parental sensitive cell line. The D. R. lower than 0.9 was defined as hypersensitivity or collateral sensitivity; D. R. around 1 was interpreted as normal sensitivity, while D. R. greater than 1.2 was signified as cross-resistance. The botanical RVM and doxorubicin revealed antiproliferative effects against the 18 cancer cell lines (Tables 2 and 3). The IC50 values obtained were from 12.35 μg/mL (towards CCRF-CEM leukemia cells) to 26.66 μg/mL (against SKMel-505 melanoma cells) for RVM and from 0.02 μM (against CCRF-CEM cells) to 26.66 μg/mL (against SKMel-505 melanoma cells) for doxorubicin. Normal sensitivity was achieved with MDA-MB-231-BCRP cells (D.R. of 0.96), with HCT116 (p53−/−) (D.R. of 1.05) and CEM/ADR5000 (D.R. of 1.05) were cross-resistant to extract compared with their respective sensitive counterpart U87MG cells. RVM (selectivity index (S.I.): 2.76) displayed acceptable selectivity to respective sensitive counterpart U87MG cells. RVM (selectivity index (S.I.): 2.76) displayed acceptable selectivity to

### Table 2: Cytotoxicity of RVM and doxorubicin against drug-sensitive cell lines, their resistant counterparts, and normal hepatocytes as determined by RRA.

| Cell lines | RVM IC50 values (μg/mL) and degrees of resistance * or selectivity index** |
|------------|--------------------------------------------------------------------------------|
| CCRF-CEM   | 12.35 ± 1.03                                                                      |
| CEM/ADR5000| 14.22 ± 0.98                                                                      |
| MDA-MB-231-pcDNA | 17.67 ± 2.01                                                                          |
| MDA-MB-231-BCRP | 16.92 ± 0.86                                                                         |
| HCT116 (p53+/−) | 14.56 ± 1.65                                                                          |
| HCT116 (p53−/−) | 15.28 ± 0.67                                                                          |
| U87MG      | 13.93 ± 1.16                                                                      |
| U87MG.AEGFR| 18.76 ± 1.64                                                                      |
| AML12      | 56.12 ± 3.77                                                                      |
| Selectivity index** | (2.79)                                                                                       |

(*): The degree of resistance was determined as the ratio of IC50 value in the resistant divided by the IC50 in the sensitive cell line; CEM/ADR5000, MDA-MB-231-BCRP, HCT116 p53−/−, and U87MG.AEGFR were used as the corresponding resistant counterpart for CCRF-CEM, MDA-MB-231-pc DNA, HCT116 p53+/+, and U87MG cell lines, respectively. (**) The selectivity index was determined as the ratio of IC50 value in the normal AML12 hepatocytes divided by the IC50 in HepG2 hepatocarcinoma cells; nd: not determined.

### Table 3: Cytotoxicity of RVM, compounds, and doxorubicin against animal cancer cell lines as determined by RRA.

| Features and cell lines | Samples, IC50 values in μg/mL (extract) or μM (compounds) ± SD | Doxorubicin |
|-------------------------|---------------------------------------------------------------|-------------|
| BRAF-V600E homzygous mutant MaMel-80a | 18.92 ± 1.23 33.47 ± 2.06 65.45 ± 7.66 10.42 ± 1.26 — — 8.66 ± 0.56 |             |
| BRAF-V600E heterozygous mutant A2058 | 18.56 ± 2.43 19.46 56.12 ± 4.29 48.18 ± 5.18 27.06 ± 1.18 38.91 ± 2.78 0.29 ± 0.04 |             |
| BRAF wild type SKMel-505 | 26.66 ± 2.19 — — 43.19 ± 3.72 — — 9.39 ± 1.01 |             |
| Rat colon adenocarcinoma CC531 | 16.39 ± 0.96 — — 58.90 ± 5.25 17.35 ± 3.1 — — 0.44 ± 0.23 |             |
| Murine melanoma B16-F1 | 14.98 ± 0.76 — — 84.29 ± 6.63 23.19 ± 2.81 — — 79.18 ± 5.39 0.22 ± 0.01 |             |
| B16-F10 | 16.55 ± 2.05 — — 19.89 ± 1.06 — — 0.24 ± 0.03 |             |

(−): IC50 values above 100 μM; the IC50 values were above 100 μM on all cell lines tested with compounds 3, 7, 8, and 9; * the selectivity index was determined as the ratio of IC50 value in the normal AML12 hepatocytes divided by the IC50 in other cell lines; (25R)-spirost-5-ene-3β, 22β-3-O-β-D-glucopyranosyl(1→2)-O-a-L-rhamnopyranoside (1), raphvinin (2), raphvinin 2 (3), raphvinin 3 (4), diosgenin (5), trillin (6), deltonin (7), 26-O-β-D-glucopyranosyl(22R,25R)-3β, 22β-26-trihydroxyfurost-5-ene-3-O-β-D-glucopyranoside (8), and sitosterol (9).
values, extract displayed good antiproliferative activity with IC\textsubscript{50} values below 20 \(\mu\)g/mL and 30 \(\mu\)g/mL in 15/18 and 18/18 cancer cell lines, respectively. Compound 4 (IC\textsubscript{50}: 10.42 \(\mu\)M) displayed good cytotoxicity against MaMel-80aBRAF-V600E homozygous mutant; compounds 4 and 1 displayed moderate antiproliferative activity against 8/9 and 3/9 cell lines, respectively. Compounds 3, 7, 8, and 9 were not active (IC\textsubscript{50} > 100 \(\mu\)M) towards the tested cell lines.

3.3. Acute Toxicity of RVM. During this experiment, no animals died among the female rats receiving 5000 mg/kg of RVM. The signs of toxicities were not detected based on the behavior of rats during the observation period (14 days). Therefore, lethal dose (LD\textsubscript{50}) of this extract was estimated greater than 5000 mg/kg in female rats. Tables 4 and 5 represent the body weights (g) and relative organ weights in the female rats during acute toxicity, respectively.

3.4. Subchronic Toxicity of RVM

3.4.1. Food Consumption. The food consumption changes in both female and male rats treated with different doses (250, 500, and 1000 mg/kg b.w.) of extract are presented in Figures 2(a) and 2(b). During the treatment period, both sexes of rats showed reduction in the food intake compared with the control group. However, the reduction of food consumption was significant from the 16\textsuperscript{th} day of treatment in male rats treated at highest with respect to controls.

3.4.2. Body Weight. The body weight gain changes in both female and male rats treated with different doses (250, 500, and 1000 mg/kg b.w.) of extract are represented in Figures 3(a) and 3(b). At all doses during the treatment period, the female and male rats showed a decrease in their body weight gain compared to the control group.
3.4.3. Organ Weights. Table 6 represents the effect of RVM on organ weights (g) of both female and male rats during subchronic toxicity. The results show that no significant difference ($p < 0.05$) was remarked in organ weights of treated rats with respect to those of the controls. Nevertheless, the spleen weight of male rat was significantly decreased at the dose 1000 mg/kg compared with the control group.

3.4.4. Biochemical Parameters

(1) Effect of Extract on ALT, AST, ALP Activity, and Total Serum Protein Levels. The effect of different doses of extract on the activity of transaminases (ALT and AST), total serum protein levels, and alkaline phosphatase levels is shown in Table 7. After repeated administration doses of extract, the results showed that, in female and male rats, the activity of serum total proteins and alkaline phosphatase was significantly reduced at doses 250 and
pared with the control group. These parameters was observed at dose 250mg/kg compared with the control group. However, in male rats, the significant reduction of transaminases at doses 500 and 1000mg/kg in the male rats, the activity of ALT and AST was significantly decreased (p < 0.05) at all doses compared with controls. No significant difference was observed in the activity of transaminases at doses 500 and 1000mg/kg in the male rats. However, in male rats, the significant reduction of these parameters was observed at dose 250mg/kg compared with the control group.

**Table 6: Effect of RVM on organ weights (g) of the rats during subchronic toxicity study.**

| Sexes | Organs (g) | Control | 250 | 500 | 1000 |
|-------|-----------|---------|-----|-----|------|
| Female | Liver     | 3.12 ± 0.16a | 2.91 ± 0.17a | 2.93 ± 0.16a | 3.06 ± 0.28a |
|        | Kidneys   | 0.65 ± 0.02ab | 0.68 ± 0.04a | 0.61 ± 0.05b | 0.69 ± 0.03a |
|        | Lung      | 0.57 ± 0.04a | 0.54 ± 0.04a | 0.54 ± 0.03a | 0.66 ± 0.26a |
|        | Heart     | 0.30 ± 0.01ab | 0.29 ± 0.01ab | 0.27 ± 0.01a | 0.32 ± 0.01a |
|        | Spleen    | 0.20 ± 0.02a | 0.36 ± 0.15a | 0.26 ± 0.12a | 0.30 ± 0.08a |
| Male   | Liver     | 3.39 ± 0.44a | 3.16 ± 0.57a | 3.23 ± 0.31a | 3.06 ± 0.15a |
|        | Kidneys   | 0.65 ± 0.05a | 0.65 ± 0.05a | 0.65 ± 0.10a | 0.58 ± 0.04a |
|        | Lung      | 0.59 ± 0.12a | 0.65 ± 0.05a | 0.52 ± 0.04a | 0.53 ± 0.06a |
|        | Heart     | 0.32 ± 0.04a | 0.31 ± 0.01a | 0.30 ± 0.01a | 0.31 ± 0.01a |
|        | Spleen    | 0.35 ± 0.09b | 0.40 ± 0.09b | 0.30 ± 0.10b | 0.20 ± 0.01a |

The table values are presented as mean ± standard deviation of 4 repetitions. In the same line and by sex, the values bearing the different letters are significantly different according to Waller Duncan’s multiple comparison test (p < 0.05).

**Table 7: Effect of RVM on biochemical parameters (ALT, AST, total proteins, and alkaline phosphatase) of the rats during subchronic toxicity study.**

| Sexes | Parameters | Control | 250 | 500 | 1000 |
|-------|------------|---------|-----|-----|------|
| Female | ALT        | 69.69 ± 1.13c | 57.88 ± 0.71a | 66.63 ± 1.43b | 65.53 ± 0.84b |
|        | AST        | 98.88 ± 2.14c | 85.31 ± 3.39b | 74.38 ± 3.27a | 86.63 ± 1.89b |
|        | T. proteins| 11.14 ± 0.31c | 8.80 ± 0.29a | 9.29 ± 0.46ab | 9.90 ± 0.36b |
|        | PAL        | 358.42 ± 7.52d | 342.46 ± 2.30c | 324.67 ± 3.94b | 281.35 ± 4.80b |
| Male   | ALT        | 67.72 ± 1.31b | 62.47 ± 1.31a | 69.03 ± 1.80b | 69.25 ± 2.47b |
|        | AST        | 103.69 ± 5.37bc | 87.94 ± 1.52a | 101.50 ± 5.72b | 109.38 ± 1.24c |
|        | T. proteins| 11.31 ± 1.07b | 9.31 ± 0.82a | 9.59 ± 1.09a | 9.95 ± 0.52ab |
|        | PAL        | 438.22 ± 5.65d | 417.70 ± 2.98c | 396.26 ± 4.80b | 317.83 ± 5.65a |

The table values are presented as mean ± standard deviation of 4 repetitions. In the same line and by sex, the values bearing the different letters are significantly different according to Waller Duncan’s multiple comparison test (p < 0.05). Indicators: ALT: alanine aminotransferase; AST: aspartate aminotransaminase; T. proteins: total proteins, ALP: alkaline phosphatase.

**Table 8: Effect of RVM on the level of serum creatinine, serum urea, and urinary protein.**

| Sexes | Parameters (mg/dL) | Control | 250 | 500 | 1000 |
|-------|-------------------|---------|-----|-----|------|
| Females | Serum urea     | 30.55 ± 1.15b | 27.38 ± 1.10a | 27.15 ± 0.84a | 25.33 ± 1.78a |
|        | Urinary urea    | 1522.85 ± 12.30c | 1280.32 ± 8.08b | 1259.83 ± 5.57a | 1268.66 ± 6.12ab |
|        | Serum creatinine| 0.83 ± 0.05ab | 0.83 ± 0.02ab | 0.88 ± 0.05b | 0.80 ± 0.01a |
|        | Urinary creatinine| 94.51 ± 5.40c | 55.49 ± 1.22a | 79.27 ± 1.41b | 82.93 ± 3.96b |
|        | Urinary protein | 12.73 ± 1.84a | 10.74 ± 1.52a | 12.33 ± 2.72a | 10.34 ± 0.92a |
| Males  | Serum urea     | 26.07 ± 1.82a | 24.67 ± 1.40b | 23.65 ± 1.15c | 24.58 ± 1.04c |
|        | Urinary urea    | 1318.40 ± 9.24c | 1235.84 ± 6.10b | 1184.87 ± 4.80a | 1231.34 ± 4.45b |
|        | Serum creatinine| 1.07 ± 0.07c | 0.83 ± 0.04b | 0.81 ± 0.02ab | 0.73 ± 0.04a |
|        | Urinary creatinine| 112.20 ± 3.45b | 95.12 ± 1.99a | 96.95 ± 4.17a | 119.51 ± 1.99c |
|        | Urinary protein | 15.51 ± 1.52a | 14.72 ± 1.52a | 14.32 ± 1.84a | 15.51 ± 0.80a |

The table values are presented as mean ± standard deviation of 4 repetitions. In the same line and by sex, the values bearing the different letters are significantly different according to Waller Duncan’s multiple comparison test (p < 0.05).

500 mg/kg compared with the control group. In female rats, the activity of ALT and AST was significantly decreased (p < 0.05) at all doses compared with controls. No significant difference was observed in the activity of transaminases at doses 500 and 1000 mg/kg in the male rats. However, in male rats, the significant reduction of these parameters was observed at dose 250 mg/kg compared with the control group.

(2) Effect of RVM on Level of Urea, Creatinine, and Urinary Protein. The effects of RVM on the level of serum creatinine, serum urea, and urinary protein are represented in Table 8. Serum and urinary urea level and urinary creatinine level showed a significant reduction in female rats compared with their control group. Urinary urea and serum creatinine levels were significantly reduced in male rats treated at all doses with respect to control groups. However, urinary
(3) Effect of RVM on Serum Lipid Profile. The effect of administration of extract on lipid profile in both female and male rats is represented in Table 9. The HDL cholesterol levels reduced in males treated at three doses compared with the control group. An increment in triglyceride levels (TG) was observed in male rats treated at three doses of extract compared with controls. As compared to the control groups, other parameters measured did not show significant differences.

Other parameters measured did not show significant differences.

### 3.4.5. Hematological Parameters

Table 10 presents the effect of RVM on hematological parameters of the rats treated with RVM during subchronic toxicity study.

| Sexes | Parameters (mg/dL) | Control | Extract doses (mg/kg) |
|-------|-------------------|---------|----------------------|
|       | TC                | 80.41 ± 2.31  | 82.44 ± 3.81  | 84.73 ± 3.54  | 84.22 ± 1.47  |
|        | HDL               | 50.95 ± 0.41  | 52.08 ± 1.67  | 53.22 ± 1.86  | 52.46 ± 1.09  |
|        | TG                | 52.00 ± 5.80  | 50.71 ± 3.83  | 55.51 ± 6.28  | 50.26 ± 2.90  |
|        | LDL               | 19.46 ± 1.14  | 20.62 ± 3.73  | 20.41 ± 1.90  | 21.71 ± 2.45  |
| Females | TC                | 103.82 ± 4.16  | 104.33 ± 3.30  | 106.30 ± 3.13  | 101.78 ± 4.28  |
|        | HDL               | 59.52 ± 1.48  | 54.60 ± 2.12  | 54.10 ± 1.99  | 54.60 ± 4.33  |
|        | TG                | 62.87 ± 4.59  | 91.18 ± 4.20  | 92.83 ± 3.67  | 72.24 ± 3.31  |
|        | LDL               | 31.72 ± 2.43  | 31.49 ± 1.45  | 33.63 ± 1.30  | 32.73 ± 4.65  |
| Males  | TC                | 80.10 ± 2.31  | 78.50 ± 3.10  | 78.00 ± 3.20  | 78.80 ± 3.30  |
|        | HDL               | 50.95 ± 0.41  | 52.08 ± 1.67  | 53.22 ± 1.86  | 52.46 ± 1.09  |
|        | TG                | 52.00 ± 5.80  | 50.71 ± 3.83  | 55.51 ± 6.28  | 50.26 ± 2.90  |
|        | LDL               | 19.46 ± 1.14  | 20.62 ± 3.73  | 20.41 ± 1.90  | 21.71 ± 2.45  |

The table values are presented as mean ± standard deviation of 4 repetitions. In the same line and by sex, the values bearing the different letters are significantly different according to Waller Duncan’s multiple comparison test (p < 0.05). Indicators: TG: triglyceride; TC: total cholesterol; HDL: high-density lipoproteins; LDL: Low-density lipoproteins.
treated with extract at dose 1000 mg/kg compared with control groups. In male rats, granulocytes were significantly higher in treated animals who received highest dose of extract; the lymphocyte level indicated significant reduction in same group of rats compared with the control group. The significant differences did not show in the rest of hematological parameters measured compared with the control group.

3.4.6. Histopathological Examination. Histopathological examinations were performed on the liver and kidneys to verify whether these organs or tissues had been damaged. No remarkable pathological change was shown on all organs after the microscopic observation compared with the control group. The effect of RVM on liver and kidneys histology in female and male rats during subchronic toxicity study is presented in (Figures 4(a) and 4(b)) and (Figures 5(a) and 5(b)).

4. Discussion

Several mineral elements and metabolic products of plant cells are capable to influence the metabolism. These minerals are very important as they have several biological functions, and their deficiency generally leads to nutritional disorders [39]. In this study, zinc (Zn) and sodium (Na) were detected. Zinc plays a vital role in human growth and development. High zinc content was observed in RVM (6.52 mg/100 g DM). The recommended daily dose is between 0.3 and 1 mg/kg in adults [40]. This result is not the same as that of Doungue [41] who obtained 0.88 mg/100 g of DM in Raphia (Raphia hookeri). This variation of values might be due to the difference in Raphia species used.

Medicinal plants are good cytotoxic agents if their IC$_{50}$ value is below 20 µg/mL; phytochemicals are significantly cytotoxic if their IC$_{50}$ < 10 µM and moderately cytotoxic if 10 µM < IC$_{50}$ < 50 µM [42]. Also, according to Suffness and Pezzuto, if the IC$_{50}$ values of plant extracts are lower than or around 30 µg/mL, they deserve to be purified in order to find active components [43]. Hence, plant extract with IC$_{50}$ values lower than 20 µg/mL and 30 µg/mL as obtained in this assay against 15/18 and 18/18 cancer cell lines, respectively. Compound 4 showed IC$_{50}$ equal to 10.42 against MaMel-80aBRAF-V600E homozygous mutant; compounds 4 and 1 showed 10 µM < IC$_{50}$ < 50 µM against 8/9 and 3/9 cell lines, respectively. Regarding criterion of anticancer activities, the plant extracts (RVM), compound 4 (Raphvinin), could therefore be considered as potential cytotoxic drug towards sensitive and resistant phenotypes. Those activities are due to different chemical compounds present in the plant extract. This result is in agreement with those of some authors [18, 26], which have shown that the Raphia vinifera compounds have cytotoxicity activity against cancer cell lines. Previous research showed that saponins from Raphia vinifera (Progenin III) induced necroptosis, autophagy, and apoptosis in leukemia cells [26]. Zhao et al. have shown that steroidal saponins

![Figure 4: (a). Effect of RVM on liver histopathology in female rats during subchronic toxicity study: (L0): control group; (L1): 250 mg/kg; (L2): 500 mg/kg; and (L3): 1000 mg/kg. Indicators: (Cb): bile duct; (VPH): hepatic portal vein; (H): hepatocytes; (S): sinusoid. (b) Effect of RVM on liver histopathology in male rats during subchronic toxicity study: (L0): control group; (L1): 250 mg/kg; (L2): 500 mg/kg; and (L3): 1000 mg/kg. Indicators: (VPH): hepatic portal vein; (H): hepatocytes; and (S): sinusoid. The liver photomicrographs presented in the document represent the general appearance observed in at least three of four animals in each group.](image)
previously exhibited cytotoxic effects by blocking the S phase of interphase [14].

The undesirable effects observed in animals after substance intake predict the toxic effects in humans after its administration [44]. The dosage of markers that ensures the correct functioning of the organism in rats can provide information on the toxicological effect of a substance. For acute toxicity, the single dose (5000mg/kg b.w.) of extract administered in rats did not cause animal deaths. Therefore, LD50 was estimated to be greater than 5000mg/kg since no acute toxicity was detected. The extract has low toxicity when their LD50 is between 2000mg/kg and 5000mg/kg after oral administration [35]. Some authors had obtained LD50 higher than 4000mg/kg b.w. after administration of the root extract of *Raphia spp.* (*Raphia hookeri*) in same experience [45]. The high safety margin presented by this genus proof its safety for consumers [45].

For the subchronic toxicity study, the rats received 3 doses (250, 500, and 1000 mg/kg) of extract. The results showed that the amount of food intake had a direct effect on animal growth. The significant reductions of food consumption and animal growth were observed in male rats treated at highest dose (1000mg/kg) from the 16th day of treatment with respect to the control group. The weight loss of animals during this work can be explained by the presence of tannins and saponins (antinutritional substances) in this plant extract. These substances that have the ability to reduce absorption of nutrients in the body [46] would be responsible for the reduction of food consumption, and thus, the reduction in body weight of rats treated at the highest dose of extract. This is agreed with that of Felix and Mello [47] who have reported that tannins showed inhibitory activities on digestive enzymes and decrease the protein quality of foods.

The levels of transaminases and ALP are generally used as biomarkers associated with liver damage [48, 49]. The decrease in serum transaminase, total proteins, and alkaline phosphatase levels at all doses observed in the female rats with respect to control groups could reflect the hepatoprotective activity of secondary metabolite contents in *RVM*. This result is in agreement with those obtained by Kang and Russell [9, 12] who have shown that the young leaves of *Raphia vinifera* are used against liver problems. Also, many types of research showed that many flavonoids and saponins present in root, leaf, and epicarp of *Raphia ssp* (*R. hookeri*) have hepatoprotective, antioxidative, anti-inflammatory, and anticancer activities [50–52]. This hepatoprotective activity is proven by histopathological analysis of the liver that revealed no damage in both sexes in rats treated at all doses.

The significant increase of triglyceride levels (TG) was remarked in male rats treated at all doses of extract with respect to their control groups. These could be due to the fact that the extract contains oil, which is rich in triglyceride. This is in accordance with the idea of Igwenyi et al. [15] who have
extracted oil from the dried pulp of *Raphia vinifera*. Noubangue et al. [39] have extracted oil from the dried pulp of *Raphia spp* using the maceration method.

Kidney is an organ, which excrete waste product of metabolism outside organism. However, prolonged exposure of the kidney to toxic substances may be altered the renal tubules [53]. The significant reduction of urea and creatinine levels in rats treated with lower doses (250 mg/kg and 500 mg/kg) of extract compared with the control group would be due to the fact that the extract contains secondary metabolism responsible for nephroprotective effect. Some studies have shown that the phenolic compound contents in the extract have nephroprotective activities [52]. However, the apical bud is taken to treat gonorrhea and other genitourinary infections [8]. These results are reinforced by the kidney sections of rats, which present no alteration. The possible kidney malfunction is suspected when the serum levels of creatinine and urea are abnormally high [54]. The increase in urinary creatinine levels in rat treated at dose 1000 mg/kg is due to antinutrient (oxalate) contained in the extract. In fact, the oxalic acid is nocive to the kidney and heart [55] and the symptoms of mild oxalate poisoning include kidney diseases [56].

Hematological components are useful for assessing food’s toxicity [57]. The significant increase of lymphocyte levels in female rats treated at highest dose (1000 mg/kg) of extract with respect to their control group was observed since extract contains lactic acid responsible for immunoprotection effect. The investigations performed by some authors showed that raffia wine of *Raphia vinifera* contains lactic acid bacteria [5] that stimulate the immune system of rats [6]. These results agree with those of some authors [58] who have shown that lymphocyte and monocyte levels increase at dose 1000 mg/kg in rat treated with ethanol extracts. The significant increase in blood platelets in both sexes treated at highest dose would indicate thrombocytosis. Moreover, the investigation carried out showed that extracts of *Raphia vinifera* fruit showed an increase in platelet indices counts [58]. The significant reduction of hemoglobin and hematocrit level in female rats treated at highest dose of extract could be because these rats had anemia. Several types of research demonstrate that, when the hemoglobin level is decrease, the patient has anemia [59]. However, investigations carried out by Ogidi et al. [58] showed that methanol and ethanol extracts of RVM showed an increase in red blood cell with respect to control groups. These contradictory results could be explained by the difference in the qualitative or quantitative composition of those two extracts of *Raphia*.

5. Conclusion

The aim of this investigation was to evaluate the antiproliferative potential of *Raphia vinifera* extract and its constituents on cancer cells, as well as the toxicity of the crude extract. It also showed that this extract is relatively nontoxic. However, caution should be taken when consuming the extract of the fruit mesocarp of *Raphia vinifera* during 28 days of treatment at highest dose, as it may induce some liver and kidney injuries. In general, *Raphia vinifera* is a safe medicinal plant that deserves further investigation to afford an anticancer phytomedicine.

Data Availability

All data obtained or generated during this work are incorporated in this published article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

GSN and INB carried out the study; GFC, VK, and ATM designed the experiments; GSN wrote the manuscript; VK supervised the work; and all authors read and approved the final manuscript.

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Supplementary Materials

S1: 1H NMR (400MHz DMSO) Spectrum of Compound 1; S2: 13C NMR (100MHz DMSO) Spectrum of Compound 1; S3: 1H NMR (600 MHz, CD3OD) Spectrum of Compound 2; S4: 13C NMR (150 MHz, CD3OD) Spectrum of Compound 1; S5: 1H NMR (400 MHz CD3OD) Spectrum of Compound 3; S6: 13C NMR (100 MHz CD3OD) Spectrum of Compound 3; S7: 1H NMR (400 MHz CD3OD) Spectrum of Compound 4; S8: 13C NMR (100 MHz CD3OD) Spectrum of Compound 4; S9: 1H NMR (400 MHz CDCl3) Spectrum of Compound 5; S10: 13C NMR (100 MHz CDCl3) Spectrum of Compound 5; S11: 1H NMR (300 MHz DMSO) Spectrum of Compound 6; S12: 13C NMR (75 MHz DMSO) Spectrum of Compound 6; S13: 1H NMR (400 MHz DMSO) Spectrum of Compound 7; S14: 13C NMR (100 MHz DMSO) Spectrum of Compound 7; S15: 1H NMR (400 MHz CD3OD) Spectrum of Compound 8; S16: 13C NMR (100 MHz CD3OD) Spectrum of Compound 8; S17: 1H NMR (400 MHz, CDCl3) Spectrum of Compound 9; S18: 13C NMR (100 MHz, CDCl3) Spectrum of Compound 9.

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