

Concentrations of Fat-soluble Vitamins of *Trema guineensis* Aqueous and Ethanolic Extracts and Rats Supplemented

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Abstract This study was undertaken to determine vitamins A, D and E composition of aqueous and ethanolic extracts of *Trema guineensis* and effect of supplementation of these extracts on serum concentrations of these vitamins. Aqueous and ethanolic extracts of *Trema guineensis* were obtained by decoction and maceration of leaves powder, respectively. Then, they were administered to animals at doses of 100 and 200 mg/kg body weight against a control group treated with distilled water for two weeks. Composition of vitamins in extracts and serum concentrations of vitamins A, D and E were performed by HPLC. Results showed that *Trema guineensis* extracts contained high concentrations of vitamins A, D and E. Thus, vitamins A and D serum concentrations were weakly influenced while that of vitamin E increased strongly during administration of *Trema guineensis* extracts. This study therefore showed that extracts of *Trema guineensis* had vitamins A, D and E and a beneficial effect on serum concentrations of these vitamins.

Keywords *Trema guineensis*, Vitamins A, D and E, Côte d'Ivoire

calcium and phosphorus homeostasis and good skeletal formation [5]. Vitamin E is basically known as antioxidant. It protects cell membranes against oxidation caused by free radicals [1; 6]. Several work indicate that the serum disturbances of vitamins A, D and E are often associated with hepatic, renal and hematologic pathologies [7; 8; 9; 10]. These vitamins could be found in plants whose biological activities have been studied. *Trema guineensis* is a plant used for feeding and various pathologies treatment [11; 12; 13]. Several studies showed that this plant has pharmacological properties such as nephroprotective [14], hepatoprotective [15] and antianemic activity [16] and chemical compounds that can justify its activities have been identified [17]. However, little information is available regarding the composition of vitamins A, D and E in *Trema guineensis* leaves and effect of supplementation of this herbal extract on serum concentrations of these vitamins. Study objective was to find vitamins A, D and E in aqueous and ethanolic extracts of *Trema guineensis* and to determine effect of these extracts on serum concentrations of these vitamins.

1. Introduction

Vitamins are organic compounds essential to life. They are generally obtained in very small quantities by food and provide various biological functions [1]. Vitamins A, D and E constitute the class of fat-soluble vitamins [2]. Vitamin A refers to a group of compounds essential for vision, growth, cellular differentiation and proliferation, reproduction and immune system integrity [3]. It also has antioxidant properties [4]. Vitamin D plays a central role in

2. Materials and Methods

2.1. Collection and Extracts Preparation

Trema guineensis leaves were collected from Abobo (Abidjan district) in January 2017. This plant was identified and authenticated by Botanical Department of Felix Houphouët Boigny University, Côte d'Ivoire. These leaves were dried at ambient temperature safe from light during two weeks then pulverized using an electric crusher (IKA-type MAG®). The powder obtained, was useful for the various extractions.

2.2. Aqueous Extraction

Hundred grams of plant powder was boiled in one Liter of distilled water for 10 minutes. The decoction was filtered twice on hydrophilic cotton and then once on filter paper Whatman N° 3. The filtrate was dried in an oven at 40°C. [18; 19].

2.3. Ethanol Extraction

Hundred grams powder of *Trema guineensis* leaves was macerated in one liter of the mixture ethanol-water 70% for 24 hours. The macerate was filtered twice on hydrophilic cotton and then once on filter paper Whatman N° 3. The filtrate was concentrated at a temperature of 40°C using a rotary evaporator Büchi-type and then dried in an oven at 40°C. [20].

2.4. Animals

Healthy adult Wistar albino rats of mean weight 134.7 ± 0.18 g were used for the study. These animals came from animal house of Pharmacology Laboratory of Training and Research Unit of Pharmaceutical and Biologic Sciences, Felix Houphouet Boigny University, Côte d'Ivoire where they were kept under favorable conditions of breeding and ethics. These rats were fed a standard complete food in the form of FACI (Fabrication d'Aliments de Cote d'Ivoire) pellets and drank tap water served *ad libitum* and renewed daily.

2.5. Experimental Treatment

Extracts administration was carried out according to method described by Adekunle *et al.* [21].

Thirty healthy rats divided into five groups of six animals were used for this study. These animals received daily gavage of distilled water or plant extracts with different doses for two weeks.

- Group control received distilled water for 14 days;
- Groups A 100 and A 200 were treated with aqueous extract *Trema guineensis* at respective doses of 100 mg / kg and 200 mg / kg for 14 days;
- Groups E 100 and E 200 received ethanolic extract of *Trema guineensis* with respectively doses of 100 mg/kg and 200 mg/kg for 14 days.

Each animal was anesthetized with ether and blood was collected from caudal vein on days 0, 7 and 14 (D0, D7 and D14). Blood was centrifuged at 3000 rpm for 10 minutes (centrifuge B4i) to obtain serum, put in an eppendorf tube and protected by aluminum foil then stored at -20°C until fat-soluble vitamins A, D and E analysis.

2.6. Vitamins Analysis

Vitamins A (retinol), D (calciferol) and E (α -tocopherol)

concentrations were determined after extraction by high performance liquid chromatography (HPLC) of Waters® type in isocratic mode coupled with a fluorometric detector [22].

Fat-soluble vitamins were extracted from 0.5 g of extract or 0.5 mL of serum. To this quantity, 10 mL of a 10% KOH solution are added in a methanol-water mixture (1:1; v/v). Then 0.025 g of ascorbic acid was added to avoid oxidation process during saponification. Mixture is then refluxed in a water bath at 70°C. for 30 minutes. After cooling mixture, 3 x 5 mL of hexane was added. Hexanic phases were combined, dried over anhydrous sodium sulfate and evaporated to dryness. The residue obtained was taken up in methanol and 20 μ l of the methanol solution were injected into the chromatographic system for the quantitative analysis of the vitamins. Standard ranges were obtained from standard vitamin initial concentrations (vitamin A concentration: 0.9 mg/mL, vitamin D concentration: 0.091 mg/mL, vitamin E concentration: 2.8 mg/mL). Fluorometric detection of vitamins A, D and E was done at respective wavelengths of 455 nm, 245 nm and 295 nm. Vitamins concentrations in sample are determined from peaks area of standard molecule and sample by following formula:

$$CV_s = \frac{SV_s \times CV_{sr}}{SV_{sr}}$$

CVs: concentration of vitamin in sample; SVs: peak area of the vitamin in sample; CVsr: concentration determined from standard range for each vitamin; SVsr: peak area corresponding to concentration vitamin selected from standard range.

2.7. Statistical Analysis

Values are expressed as mean \pm standard deviation (SD). Statistical analysis was carried out using the software GraphPad Prism 7. The analysis of the variances was performed using ANOVA followed by Tukey tests for comparison of vitamin concentrations in extracts and Dunnett for comparison of vitamins serum concentrations between different groups. The value of $p < 0.05$ was considered significant.

3. Results

Concentrations of fat-soluble vitamins A, D and E in the *Trema guineensis* extracts have been shown in Table 1. Results revealed that vitamins A, D and E were present in aqueous and ethanolic extracts of *Trema guineensis* leaves but in variable amounts. The contents of vitamins A (1.798 ± 0.30 mg/g), D (354.34 ± 27.22 μ g/g) and E (3 ± 0.28 mg/g) of the extract ethanolic were high in comparison to those of vitamins A (1.342 ± 0.58 mg/g), D (317.20 ± 42.70 μ g/g) and E (2.08 ± 0.66 mg/g) in the aqueous extract.

However, this difference was not significant. Fat-soluble vitamins in serum of rats supplemented with *Trema guineensis* extracts at doses of 100 and 200 mg/kg body weight and control group showed in Table 2. At day 0 (D0) there was no significant difference in vitamins concentrations between all groups. Administration of aqueous and ethanolic extracts of *Trema guineensis* to doses of 100 and 200 mg/kg body weight resulted in a non-significant increase in serum levels of vitamins A and D on day 7 (D7) in comparison to control group. However, at day 14 (D14), this increase was significant ($p < 0.05$) in rats treated with aqueous and ethanolic extracts at a dose of 200 mg/kg body weight in comparison to control group rats. During treatment, the additional intake of *Trema guineensis* extracts to rats increased significantly ($p < 0.01$ and $p < 0.001$) vitamin E serum levels in comparison to control group.

4. Discussion

Vitamin A provides a variety of functions throughout the body. These include: vision, gene transcription, immune function, embryonic development and reproduction, bone metabolism, hematopoiesis, skin and cell structure maintenance as well as antioxidant activity [23; 24]. Vitamin D contributes to phosphocalcic balance maintenance. It is essential for bone health and plays a role in gene expression, muscle and immune function [25; 26; 27; 28]. Vitamin D induces several molecules expression such as glutathione and superoxide dismutase involved in antioxidant defense. It also has a direct antioxidant effect on cell membranes [29; 30]. Vitamin E, exclusively provided by diet, is a recognized antioxidant [31]. It also protects the membrane of red blood cells and limits the premature lysis of the erythrocytes [4]. Other known functions of vitamin E include enzymatic activities, gene expression, neurological function and cell signaling [31; 32; 33].

Compounds analysis showed that *Trema guineensis* leaves extracts contained vitamins A, D and E with high amounts in ethanolic extract as aqueous extract. Other authors showed the presence of fat-soluble vitamins in various parts of plant [31; 34]. A level (1.798 ± 0.30 mg/g and 1.342 ± 0.58 mg/g) of ethanolic and aqueous extracts respectively were higher than that (0.30 ± 0.15 mg/g) in *Citrullus vulgaris* Schrad extract reported by Hannah and Krishnakumari [35] and (0.045 ± 0.003 mg/g) in *Vitex chrysoarpa* leaves obtained by Ilodibia *et al.* [36]. Vitamin D amounts (317.20 ± 42.70 µg/g and 354.34 ± 27.22 µg/g) in extracts of *Trema guineensis* was very high compared to that 0.0835 µg/100g obtained in *Canarium schweinfurthi* pulp by Monanu *et al.* [31] works. Assi *et al.* [37] and Ajayi and Malachi [23] studies found vitamin E

concentrations of 70.83 ± 9.73 mg/100g in *Irvingia gabonensis* almond consumed in Côte d'Ivoire and 49.43 ± 0.58 mg/100g in vegetable oil "Executive chef soybean" consumed in Nigeria. However, these values remained below vitamin E concentrations (208 ± 66 mg/100 g and 300 ± 28 mg/100 g) obtained from *Trema guineensis* leaves extracts. High content of fat-soluble vitamins observed in the aqueous extract is attributable to the rupture of the cell structure leaves by heat allowing the release of the micronutrients [31].

The aqueous and ethanolic extracts of *Trema guineensis* leaves administration to rats resulted in a small increase in serum vitamin A concentration after fourteen days of treatment. This result was in agreement with those of Raila *et al.* [38] who obtained a small increase in the serum concentration of vitamin A after vitamin A supplementation to animals. Also, Beketova *et al.* [39] reported a vitamin A serum concentration increase in rats following a supplementation of the Sunflower and Lard oil mixture. However, Yildirim *et al.* [40] reported no change in vitamin A serum concentration after the supplementation of Cocoa butter and Sunflower oil to rats. Treatment of rats by *Trema guineensis* extracts resulted in a large increase in serum concentrations of vitamins D and E. These results are supported by those of Jang *et al.* [41] who showed that supplementation of vitamins D and E increased serum levels of these vitamins in pigs. Adekunle *et al.* [21] also observed a vitamin E serum increase in rats supplemented with *Bryophyllum pinnatum*. However, Yildirim *et al.* [40] showed no vitamin E concentration increase in serum of rats following supplementation of Cocoa butter and Sunflower oil.

Moreover, heat treatment as boiling also improves the availability of the vitamins A, D and E by destroying some anti-nutriments such as polyphenols and the tannins which interfered with the absorption of these compounds [42; 43; 44; 45]. In addition, Kouakou *et al.* [17] showed that the ethanolic extract of *Trema guineensis* was rich in phenolic compounds. Consequently, they would reduce bioavailability of vitamins A, D and E.

Increased serum levels of vitamins A, D and E after supplementation may prevent these vitamins disturbances in body. Also, it would justify some biological properties of *Trema guineensis* such as nephroprotective [14], hepatoprotective [15] and antianemic activity [16].

Table 1. Fat-soluble vitamins content of *Trema guineensis* extracts leaves

	Vitamin A (mg/g)	Vitamin D (µg/g)	Vitamin E (mg/g)
Aqueous extract	1.342±0.58	317.20±42.70	2.08±0.66
Ethanolic extract	1.798±0.30	317.20±42.70	3±0.28

Values are expressed as mean ± standard deviation with n = 3. $P > 0.05$: no significant difference between extracts.

Table 2. Serum concentrations of vitamins A, D and E after administration of the extracts aqueous and ethanolic of *Trema guineensis*

		Control	A 100	A 200	E 100	E 200
Vitamin A (mg/mL)	D0	0.85±0.16	0.86±0.14	0.84±0.06	0.86±0.29	0.84±0.03
	D7	0.88±0.14	1.09±0.13	1.17±0.05	1.03±0.17	1.13±0.02
	D14	0.91±0.17	1.19±0.15	1.31±0.05*	1.16±0.14	1.28±0.35*
Vitamin D (µg/mL)	D0	8.36±0.89	8.11±1.71	8.33±0.32	8.26±1.99	8.84±0.23
	D7	8.94±0.24	9.18±0.69	9.49±0.57	9.13±0.71	9.36±0.82
	D14	8.98±0.97	10.85±1.22	11.80±2.60*	10.63±0.33	11.65±1.61*
Vitamin E (µg/mL)	D0	66.07±100.2	63.35±18.21	60.18±2.11	65.03±2.18	57.04±8.71
	D7	69.03±8.97	82.94±3.11**	89.76±1.70***	81.43±8.71**	88.16±1.52***
	D14	72.18±31.12	117.15±7.04***	137.02±13.08***	115.43±4.11***	133.06±1.61***

Values are expressed as mean ± standard deviation with n = 6. * p<0.05; ** p<0.01; *** p<0.001. D0: day 0; D7: Day 7; D14: Day 14 ; Control: rats received distilled water; A 100 and A 200: groups treated with aqueous extract of *Trema guineensis* at doses of 100 and 200 mg/kg of body weight; E 100 and E 200: groups treated with *Trema guineensis* ethanolic extract at doses of 100 and 200 mg/kg of body weight

5. Conclusions

Vitamins A, D and E composition of aqueous and ethanolic extracts of *Trema guineensis* leaves and effect of these extracts on serum concentrations of these vitamins were determined in this study. *Trema guineensis* leaves extracts contain a high concentration of fat-soluble vitamins A, D and E. Thus, *Trema guineensis* extracts supplementation positively influences serum levels of vitamins A, D and E. The presence of vitamins in leaves of *Trema guineensis* make this plant beneficial for health and justify its consumption and therapeutic use.

Ethical Approval

The experimental procedures were conducted after the approval of the Ethical Guidelines of University (Côte d'Ivoire) Committee on Animal Resources. All these procedures used, were in strict accordance with the guidelines for Care and Use of Laboratory Animals and the statements of the European Union regarding the handling of experimental animals (86/609/EEC).

Competing Interests

Authors have declared that no competing interests exist.

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