

Original Research Article

Effects of Sodium Azide (NaN₃) on Seeds germination, Plantlets Growth and *In vitro* Antimalarial Activities of *Phyllanthus odontadenius* Müll Arg.

ABSTRACT

Aims: To increase the production of secondary metabolites of *P. odontadenius* using sodium azide (NaN₃) in order to amplify those with *in vitro* antimalarial activity.

Study Design: Laboratory experiment tests; Identification of plant material, Immersion of seeds in SA concentrations, *In vitro* culture of seeds, *In situ* culturing plantlets, Extraction of *Phyllanthus odontadenius* aerial parts, Phytochemical screening, *in vitro* antiplasmodial tests to determine the inhibition of concentration killing 50% of parasite population (IC₅₀).

Place and Duration of Study: Department of Biotechnology and Molecular Biology, Department of Biochemistry: General Atomic Energy Commission, Regional Center of Nuclear Studies, P.O. Box. 868 Kinshasa XI, in Democratic Republic of the Congo (RDC). National Institute of Biomedical Research (NIBR) at Kinshasa/Gombe (RDC). The experiments were conducted during August 2011 to December 2011 firstly and Mai 2012 to September 2012 for the secondarily.

Methodology: Seeds of *P. odontadenius* were obtained after oven drying at 45°C and they were immersed in SA at concentrations ranging firstly between 0 to 10 mM ; secondary between 0 to 20 mM. Seeds were germinated on Murashige and Skoog medium and plantlets were transferred *in situ*. In addition some parameters such as height, collar diameter, number of branches and biomass from first generation were analyzed. Phytochemical screening was released. The *in vitro* antiplasmodial activities assays on clinical isolates of *P. falciparum* was determined.

Results: Results showed that SA had POSITIVE effects on growth parameters of *P. odontadenius* in the M1 generations with greater effects observed with treatment exceeding 10 mM. For the *in vitro* antimalarial activities from to extracts obtained with aerial materials part from directly immersed seeds (M1), the effects observed with extracts plant from seeds dipped in SA were higher than those from untreated seeds. IC₅₀ values were ranged between 1.04±0.02 µg/ml (10 mM) to 12.77±5.83 µg / ml (0 mM) for the first assay. And for the second test, the *in vitro* antiplasmodial activities varied between 1.47±1.07 µg/ml (10 mM) to 21.60±7.13 µg/ml (2.5 mM) for. The best activities were observed with SA solutions exclusive of 5 mM to 10 mM. The SA lethal doses were 4.76 mM for LD₃₀ and 10.99 mM for LD₅₀.

Conclusion: Treatment of *P. odontadenius* seeds with SA induced stimulation of parameters which increase linearly with increasing concentrations of SA. Some secondary metabolites were synthesized for example alkaloids compounds compared to the untreated seeds of *P. odontadenius* with a more important synthesis in phenolic compounds. The *in vitro* antiplasmodial activity on the clinical isolates *P. falciparum* showed low antimalarial activities from M1 controls (0 Gy) than that of extracts from treated plants. The high inhibitory effects (1.04±0.02 µg/mL or 1.47±1.07 µg/mL for 10 mM) of crude extracts plants from treated seeds have justified the usefulness of SA in the increasing production of secondary metabolite against malaria in the Word particularly in DRC.

Keywords: *Phyllanthus odontadenius*; Sodium Azide (SA); secondary metabolites; malaria; antimalarial activity.

1. INTRODUCTION

Plants have been used in traditional medicine since a long time. About 13,000 plant species have been used as drugs throughout the world, and approximately 25% of the current materials medical are derived from plants in form of teas, extracts, or pure substances [1]; [2]. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world [3]. In the Democratic Republic of Congo (DRC), among the species used in the treatment against malaria, *Phyllanthus odontadenius* is well positioned for different previous studies on this plant [4]; [5]; [6]. *P. odontadenius* is one of the most important medicinal plants used in different regions in the world for the treatment of various diseases such as jaundice, asthma, hepatitis, flu, dropsy, diabetes, fever causing by malaria [7]; [8]; [9] but its availability is drastically decreasing because of numerous harvests.

Malaria is the most important parasitic disease in tropical areas. The estimated clinical cases for WHO were 216 million in 2010, approximately 40% of world's population were at risk of malaria. Nearly 655,000 died from to malaria disease, mainly children under 5, pregnant women and elderly [10]; [11]; [12]. A major obstacle to malaria control is the emergency and spread of antimalarial

resistance drugs, and urgent efforts are necessary to identify new classes of antimalarial drugs. In the last decades resistance to several antimalarials drugs became widely disseminated, while the cost of effective treatment is prohibitive for the large majority of the populations in these areas. It continues to cause morbidity and mortality on a large scale in tropical countries. There is an urgent need for new chemotherapeutic compounds, which are easy to administer and store, and which are of low cost [13]; [14].

Mutations are the tools used by the geneticists to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops [15]. It is known that various chemicals have positive or negative effects on living organisms. Chemical mutagen generally produce induced mutations which lead to base pair substitution especially GC → AT (guanine:cytosine to adenine:thymine) resulting in amino acid changes, which change the function of proteins but do not abolish their functions as deletions or frame shift mutations mostly [15]; [16]. These chemomutagens induce a broad variation of morphological and yield structure parameters in comparison to normal plants.

Sodium azide (NaN_3), which has been demonstrated to have these effects, is a mutagen and it has proved to be one of the most powerful mutagens in crop plants. It is a common bactericide, pesticide and industrial nitrogen gas generator if known to be highly mutagenic in several organisms, including plants and animals [17]; [18]. The mutagenicity created by NaN_3 is mediated through the production of an organic metabolite of azide compound, presumably azidoalanine ($\text{N}_3\text{-CH}_2\text{-CH(NH)}_2\text{-COOH}$). The production of this metabolite was found to be dependent on the enzyme O-acetylserine sulfhydrylase (E.C.4.2.99.8.) [16]. In order to understand that sodium azide is mutagenic mechanism used for the improvement economic characters to many studies in rice, wheat, Barley and Sorghum [19].

In this report, we studied firstly the mutagenic effects of sodium azide on growth and yield traits of *Phyllanthus odontadenius*. Secondary, to monitor the effects of sodium azide on the production of active secondary metabolites in *P. odontadenius* aerials parts in order to amplify those with *in vitro* antimalarial activity.

2. MATERIALS AND METHODS

2.1 Plant material – Mutagenesis – *In vitro* Germination

2.1.1 Plant material

The plant material used for harvesting fruits was identified by the senior Assistant research Anthony KIKUFI, Laboratory of Systematic Botany and Plant Ecology, Department of Biology (Faculty of Science). The seeds of *P. odontadenius* were used such as study material.

2.1.2 Immersion of seeds in SA solutions

Seeds of *P. odontadenius* obtained from drying fruits harvested on the Kinshasa university site were placed firstly in the Eppendorf tubes (1.5 mL). Stock solution of sodium azide (Merck) was prepared in 1 M phosphate buffer, pH 3, filter sterilized and stored frozen it, at -20°C . Stock solution was diluted in water as well as in phosphate buffer of pH 3 to give various concentrations (0.5 mM, 1.5 mM, 2.5 mM, 3.5 mM, 4.5 mM, 5 mM and 10 mM) and (2.5 mM, 5 mM, 7.5 mM, 10 mM, 12.5 mM, 15 mM, 17.5 mM and 20 mM) to treat the seeds. The seeds were counted per 100 and then imbibed in

sterilized water for 1 h with agitation on shaker. 100 seeds were kept under various concentrations of sodium azide for 2 h 30 of time with agitation on shaker and the same time seeds were submerged in deionized water for the same period of time served as control. After sodium azide treatment, seeds were washed properly with autoclaved distilled water 4-5 times to remove excess sodium azide.

2.1.3 *In vitro* seeds germination

Seeds were disinfected with 70% (v/v) ethanol for 1 min, sterilized with 0.125% (w/v) HgCl₂ for 3 min, and washed with sterile distilled water. They were then handled with gibberellic acid (GA₃) 200 mg/L for 4 h and finally drained before being cultivated on modified Murashige and Skoog (MS) basal media without sucrose or growth regulators and supplemented with 0.8% agar [20]; [21]. The pH of the media was adjusted to 5.6 before autoclaving at 121°C for 15 min. Cultures were incubated at 25 ± 1°C under fluorescent light with 16 h photoperiod. Percentage of germinated seeds or the germination rate for each dose was determined by the equation before.

$$\% \text{ of germinated seeds} = n \times 100 / N.$$

Where *n*: number of germinated seeds and *N*: the number of seeds in de Petri dish.

The reduction of emergence (%) was also determined by the relationship from Maluszynski et al. (2009) below:

Emergence reduction (%) = 100 – (Average emergence in the dose x 100)/average emergence in the control).

2.1.4 *In situ* seedling transfer

Plantlets growths *in vitro* were transferred in polyethylene bags containing 300 g of soil for *in situ* growth [22]. Bags were then buried in 3/4 in the ground in randomized complete block (RCB) design with 3 replications [19]; [23]; [24]; [25]. The plantlets placed *in situ* were watered three times a week, the odd days, with the same amount of water (20 L per plot 5 dm/6 dm). and 6 plants from each replicate were used to measure the plant growth.

2.1.5 Seedling growth

Parameters such as collar diameter shoot length, number of branches for the selected M1 plants were measured after four months of culturing. The length of plants was performed using a lathe measuring 50 cm. The collar diameter was measured using Slot-foot Digital CALIPER 150 mm (6") and the number of branches was measured manually. Fresh Biomass and dried biomass for aerial parts after plants harvest were measured using a balance DENVER APX-100.

2.2 Phytochemical Analysis

2.2.1 Preparation of crude extracts

10 g of dried plant material were macerated separately with ethanol and dichloromethane (300 ml each) for 24 h. Each mixture was filtered and dried at 45°C for 72 h. The aqueous extract was prepared by mixing 10 g of dried plant material with 300 ml distilled water. The mixture was boiled at 100°C for 15 min, cooled, filtered and dried at 45°C for 72 h.

2.2.2. Phytochemical screening

The chemical screening was carried out on all crude extracts. Alkaloids were detected with Dragendorff's and Mayer's reagents [26]. Saponins were detected by frothing test [26]; [27]. Presence of tannins was detected using Stiasny reagent and ferric chloride 2% [26]; [28]; [29]. Flavonoids were detected using Shinoda's reagent or aluminum chloride 5% [30]. Anthraquinones were detected using Böttranger's reagent. Anthocyanins were identified using HCl 2N, heating and add iso-amylic alcohol. Steroids and terpenoids were identified using Liebermann-Bouchard's reagent [26].

The presence of the different chemical groups was confirmed by Thin Layer Chromatography (TLC) performed on silica gel plates GF254 (Merck, Germany). Alkaloids were detected using CHCl₃/MeOH/NH₄OH (9:2:0.5) and EtOAc/Iso-PrOH/NH₃ (85:15:5) as mobile phases and Dragendorff's as reagent. Flavonoids were detected using *n*-BuOH/water/acetic acid (4:1:5) (Top layer) as mobile phase with NEU's reagent (1% diphenylboric acid ethanolamine complex, methanolic solution 1%). Steroids and terpenoids were detected using CHCl₃/MeOH (9:1) and *n*-Hexane/MeOH: (9:1) on mobile phases and Liebermann-Bouchard's reagent. After drying, the plate was heated at 110°C for 10 min intensify the spot colors. Tannins, mainly proanthocyanidins were detected using EtOAc/HOAc/HCOOH/H₂O (30:02:1.2.8) (upper phase) as mobile phase and 1% Vaniline and 5% H₂SO₄ as reagents. After spraying, drying and heating the plate, proanthocyanidins were colored in red while flavonoids appear in yellow color. Anthraquinones were identified using CHCl₃/MeOH (7:3) as mobile phase and Böttranger's reagent [26], [31].

2.3. *In vitro* Antimalarial Activity

Antimalarial activity assays were performed at the National Institute of Biomedical Research (NIBR) in Kinshasa/Gombe, DR Congo.

The stock solutions were 20 mg/ml extracts. These solutions were prepared in 1% DMSO and diluted in two fold to have test concentration. Clinical isolates of *P. falciparum* were obtained from symptomatic malaria children (0-5 years) with high parasitaemia and who did not receive antimalarial treatment in the three weeks preceding the diagnosis at the Maternity Hospital of the Sisters of Kindele, Mont-Ngafula, Kinshasa. Venous blood samples (4 ml) were collected in tubes containing 1% heparin, and centrifuged for 5 min at 3000 rpm to separate the plasma and the erythrocytes. 1 ml of erythrocytes was mixed with 9 ml of RPMI 1640 containing 25 mM HEPES, 25 mM sodium bicarbonate and 10% of pooled human serum. After homogenization, 50 µl of the suspension were distributed in each well of a spot plate containing decreasing concentrations of extracts [32]. Plates were then maintained at 37°C in a humid atmosphere containing 5% CO₂. Quinine was used as control. After 48 h of incubation, thin smears were made and stained with GIEMSA 5% and parasitaemia were determined with a Zeiss Primo Star microscope (GmbH/Germany) [33]; [34]. Inhibition of parasitaemia (percent) was calculated as following:

$$\text{Inhibition (\%)} = (A - B/A) \times 100,$$

Where A is the parasitaemia in the negative control and B, the parasitaemia in the treated plates bucket. The IC₅₀ of each sample was obtained using the dose-response curves.

2.4. Statistical analysis

Data were subjected to Analysis of Variance (Anova) using MSTAT-C Software [35] and compared to the software Statistica software with General Linear and LSD test (Least Significant Difference) in order to identify differences between treatments. Means of different treatments were separated with LSD at 5% level of probability.

1. RESULTS

3.1. Effects of sodium azide (NaN₃) on the growth of *P. odontadenius*

Results obtained in this work were mentioned in Tables 1 to 5 and in figures 1 (a - d) and 2 (a - d).

Table 1.: Effects of Sodium Azide (NaN₃) on Seeds germination and plant growth of *Phyllanthus odontadenius*

mM of NaN ₃ Parameters	0	0.5	1.5	2.5	3.5	4.5	5	10	CV (%)	Lsd
Rate (%) of seeds germination	16.33±0.58a	15.00±0.77a	11.0±0.29 a	14±0.5 a	9.33±0.36 a	15±0.44 a	5.33±0.32 a	11±1.32 a	29.10	12.446
Emergence reduction (%)	0	37.51	79.97	39.98	79.97	92.50	87.47	69.99	—	—
Size (cm) of plants	16.02±2.56 b	18.21±4.22 ab	16.6±4.36 b	17.32±2.92 b	17.65±3.30 b	18.30±3.51 ab	18.50±3.21 ab	20.16±3.89 a	21.39	14.66
Collar diameter (mm) of plants	1.56±0.27 bc	1.59±0.17 bc	1.60±0.24 bc	1.60±0.30 c	1.61±0.30 bc	1.72±0.25 ab	1.68±0.24 ab	1.87±0.22 a	15.87	0.067
Number of branches	12±1.96 bcd	12.05±2.96 d	12.40±2.0 abcd	11.85±1.35 cd	12.85±1.84 a	12.95±1.74 abc	13.6±2.43 ab	13.8±2.20 a	18.14	5.313
Biomass of aerial part (g)/plant	1.63±0.52 b	1.45±0.46 ab	1.75±0.56 ab	1.66±0.59 ab	1.79±0.51 ab	1.81±0.63 ab	1.81±0.50 ab	2.08±0.61 a	32.72	0.334

For the same parameter, values with identical letters were not significantly different at 5% ($p < 0.05$)

Table 2.: Effects of Sodium Azide (NaN₃) on Seeds germination and plant growth of *Phyllanthus odontadenius*

mM of NaN ₃ Parameters	0	2.5	5	7.5	10	12.5	15	17.5	20	CV (%)	Lsd
Rate (%) of seeds germination	5.33±0.89 cde	7.33±1.11 bc	11.33±2.67 a	4.33±1.0 e	6.67±2.0 bc	7.0±1.5 bc	5.67±3.78 cd	4.67±0.83 de	8.33±0.92 b	15.60	2.208
Emergence reduction (%)	0	-37.52	-112.57	18.76	-25.14	-31.33	-6.38	12.38	-56.29	-	-
Size (cm) of plants	11.83±2.55 ab	10.87±2.9 ab	14.31±2.54 a	8.18±2.18 b	12.6±2.35 ab	15.22±0.82 a	11.73±1.56 ab	14.05±2.26 a	14.47±0.55 a	12.36	5.465
Collar diameter (mm) of plants	1.15±0.21 cde	1.05±0.27 de	1.60±0.31abc	0.85±0.25 e	1.38±0.15 bcde	1.82±0.06 ab	1.47±0.26 abcd	1.61±0.34 abc	1.95±0.04 a	10.7	0.5328
Number of branches	10.53±1.33 cde	9.6±1.85 de	13.33±1.47 abc	7.43±1.68 e	11.3±2.31 bcd	14.37±0.71 ab	10.93±0.95 bcde	12.3±2.78 abcd	15.5±1.21 a	8.86	3.642
Biomass (g)/plant	2.03±1.1a	1.72±0.95 a	2.79±1.40 a	2.05±0.60 a	2.81±1.30 a	2.57±1.56 a	2.24±0.58 a	2.62±0.81 a	3.05±0.22 a	28.85	2.464

For the same parameter, values with identical letters were not significantly different at 5% ($p < 0.05$)

In view of Table 1, it is clear that with the exception of the rate of *P. odontadenius* seeds germination the witness showed high value is $16.33 \pm 0.58\%$, all other parameters showed weak values comparing to the control. The high values are 10 mM. However the values of the witness not differ significantly from that at 10 mM ($11.0 \pm 1.32\%$). With the exception of germination where the

witness does not differ significantly from 10 mM, the values of other parameters (size, collar diameter, number of branches and the fresh biomass of plant) with higher values than the control significantly different to those of the latter to a confidence level of 5%.

Table 2 shows that the control plants exhibit lower values for most cases compared to plants from seeds soaked in 20 mM sodium azide solution. The lowest values are found in most plants whose seeds were soaked in 7.5 mM of sodium azide solution. When control values are compared with those of 10 mM, the latter has higher values than the control for all measured parameters. With the exception of germination $11.33 \pm 2.67\%$ for 5 mM and $6.67 \pm 2.0\%$ for 10 mM in Table 2, the measured values of other parameters in plants whose seeds were soaked in 5 mM and 10 mM does not differ.

Tableau 3 : Phytochemical screening of *P. odontadenius* crude extracts from M1 plants

Chemical Groups analyzed	Treatments								Natural plant
	0	0,5	1,5	2,5	3,5	4,5	5	10	N
Alkaloids	—	+	+	+	+	+	+	+	—
Saponins	—	—	—	—	—	—	—	—	—
Anthocyanins	+	+	—	—	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Free quinones	—	+	+	+	+	—	—	+	—
Anthraquinones	+	—	+	—	—	—	—	+	+
Steroids and terpenoids	—	+	+	—	—	+	+	+	—
Flavonoids	+	+	+	+	+	+	+	+	+

Legend: N: Harvested natural plant ; —: Absence; +: Presence.

Table 4: Phytochemical screening of *P. odontadenius* crude extracts from M1 plants

Chemical Groups analyzed	Concentrations (mM) of Sodium of Azide (NaN ₃)								
	0	2,5	5	7,5	10	12,5	15	17,5	20
Alkaloids	—	—	+	+	+	—	—	+	+
Saponins	—	—	—	—	—	—	—	—	—
Anthocyanes	+	+	+	+	+	+	—	+	+
Tannins	+	+	+	+	+	+	+	+	+
Free quinones	—	—	—	—	—	—	—	—	—
Anthraquinones	—	—	—	—	—	—	—	—	—
Terpoids and steroids	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Polyphenols	+	+	+	+	+	+	+	+	+

Legend : + : présence ; — : absence ; +/- : sous forme trace.

In view of Tables 3 and 4, it is apparent that the alkaloids have been found present in all extracts plants from treated seeds (Table 3) and they were only present in extracts plants from seeds

treated at 5 mM; 7.5 mM; 10 mM; 17.5 mM and 20 mM concentrations of SA. They are absent in the controls in Tables 3 and 4 and then in the extracts plants from seeds treated with SA at 2.5 mM; 12.5 mM and 15 mM concentrations (see Table 4). Saponins are absent everywhere, while flavonoids are found everywhere such as tannins in the two tables (3 and 4). Anthocyanins are present in the control extracts as in the treated extracts except at concentrations of 1.5 mM and 2.5 mM in Table 3 and 15 mM in Table 4. Steroids and terpenoids are absent in the control extracts as extracts plant from seeds treated at 2.5 mM and 3.5 mM concentrations of SA (Table 3). They are present in the control as treated extracts in Table 4 where the anthraquinones and free quinones are absent. However, the free quinones are absent in controls and in 4.5 mM and 5 mM. Anthraquinones are present in the control and in 1.5 and 10 mM concentrations of SA (Table 3).

3.2 Sodium Azide Chemosensitivity of *P. odontadenius*

The Sodium Azide (NaN_3) chemosensitivity of *P. odontadenius* was determined by seeds germination or by length of plantlets. Results obtained were showed in figures 1 (a-d).

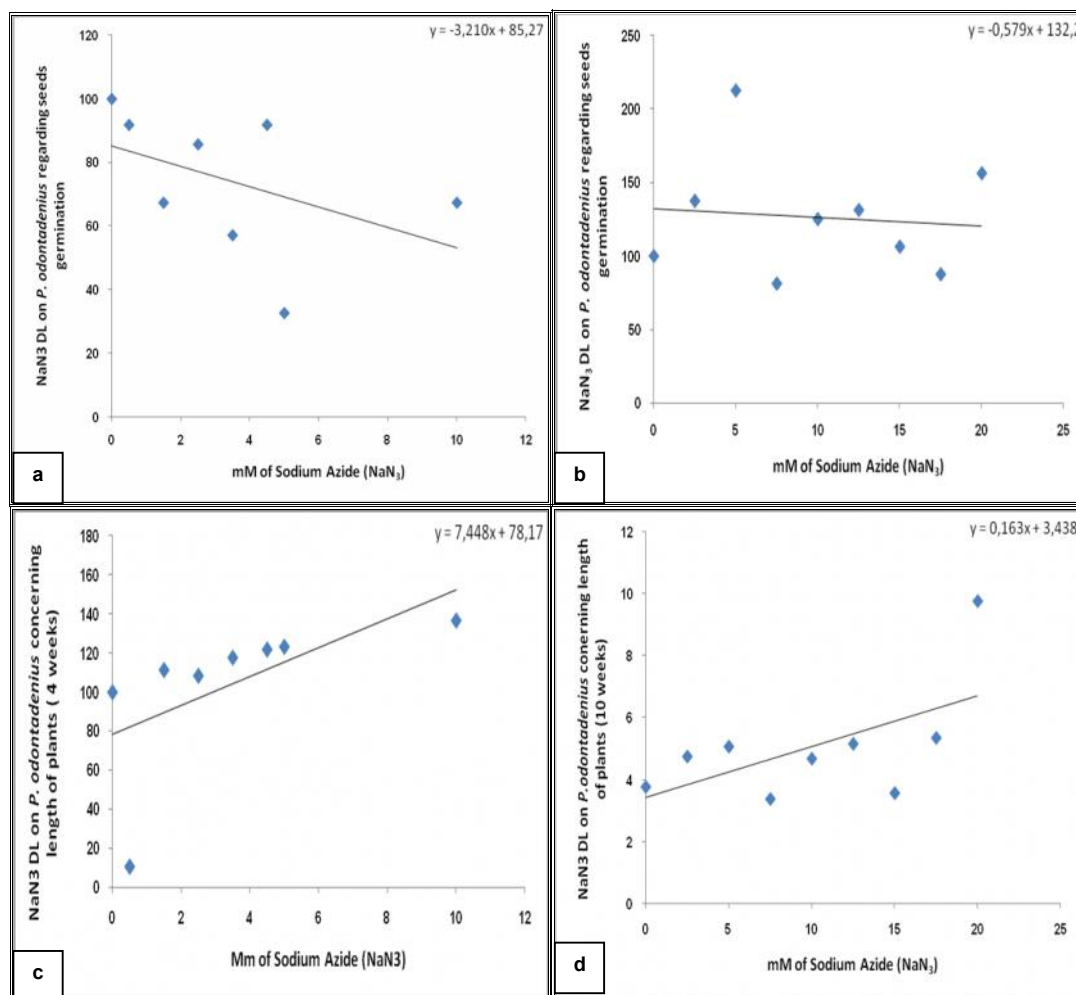


Fig. 1 : Determination of chemosensitivity of Sodium Azide (NaN_3) on *P. odontadenius*; a : by seeds germination using 0 – 10 mM of NaN_3 ; b: by seeds germination using 0 – 20 Mm of NaN_3 ; c: by length of plants which were dured 4 weeds (0 – 10 mM); d: by length of plants which were dured 10 weeds (0 – 20 mM).

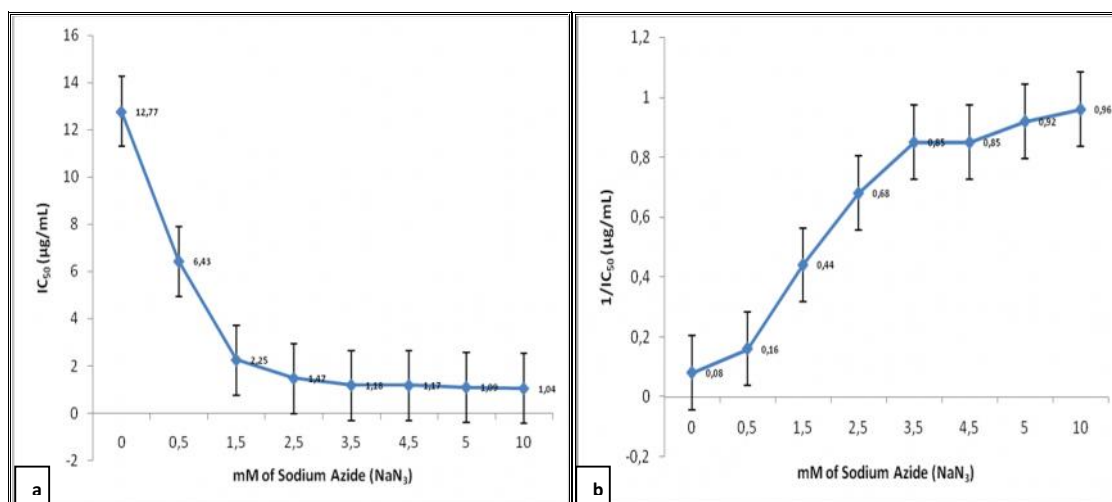
In view of Figure 1, it is apparent 4 linear regression equations as $y = -3.210x + 85.27$ for Fig.1a; $y = -0.579x + 132.2$ for Fig.1b; $y = 7.448x + 78.17$ for Fig.1c and finally the equation: $y = 0.163x + 3.438$ for Fig.1d.

The DL50 and DL30 calculated from these equations of linear regression are shown in Table 5.

Equation DL	$y = -3.210x + 85.27$ (0 – 10 mM)	$y = -0.579x + 132.2$ (0 – 20 mM)	$y = 7.448x + 78.17$ (0 – 10 mM)	$y = 0.163x + 3.438$ (0 – 20 mM)
30	4,76 Mm	107,43 mM	-1,10 mM	408,36 mM
50	10,99 mM	141,97 mM	-3,78 mM	285,66 mM

In view of Table 5, it appears that the parameter showed a stimulation effect of sodium azide (NaN_3) gives values of DL_{30} and DL_{50} that far exceed the concentrations used for testing. Only the germination of soaked seeds at concentrations varying from sodium azide 0 - 10 mM, which showed values of DL_{30} and DL_{50} which only deviates DL_{50} but also not too relevant concentrations.

3.3 *In vitro* Antimalarial Activities of Aqueous Extracts from *P. odontadenius*



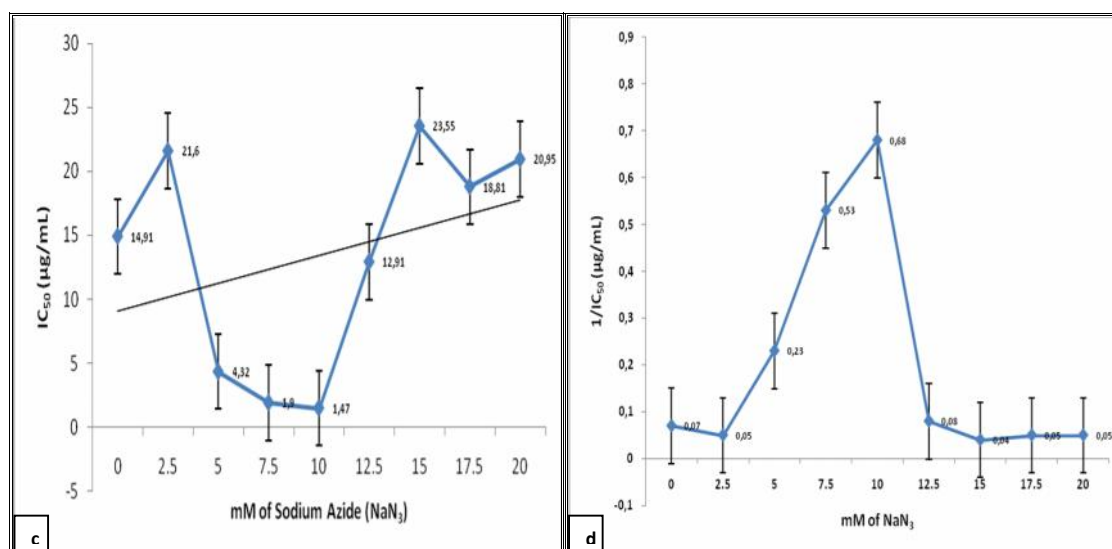


Fig. 2 : Effects of aqueous crude extracts of *P. odontadenius* from plants obtained after seeds immersed in Sodium Azide solutions (a: antiplasmodial activities with plants which seeds were treated with 0 – 10 mM; b: 1/IC₅₀ values from IC₅₀ 0 – 10 mM; c: IC₅₀ (mg/mL) of plant extracts from 0 – 20 mM treated seeds ; d: 1/IC₅₀ values from IC₅₀ 0 – 10 mM)

The figures 2 (a – d) showed that low values of IC₅₀ (µg/mL) were those obtained with plants from seeds immersed in 5 and 10 mM solutions (1.09±0.13 and 1.04±0.02 µg/mL)(Fig.2a). It's the same for plants obtained by immersion of seeds to 0 – 20 mM ; the low values were those at 5 to 10 mM (4.32±0.38 µg/mL for 5 mM ; 1.90±1.75 µg/mL for 7.5 mM ; 1.47±1.07 µg/mL for 10 mM). These concentrations (5 – 10 mM) have high antiplasmodial activities which were showed in Fig. 2b and Fig.2d. than all concentrations of sodium azide.

Comparing Fig.1a of DL and those of inversing IC₅₀ (Fig.2b and Fig.2d), concentrations of 5 to 10 mM were promising for plant breeding with sodium azide for treatment of malaria disease. IC₅₀ were according with DL₅₀.

4. Discussion

Sodium azide (NaN₃) is powerful chemical mutagen, safer and more efficient, which increases the yield and quality of field crop against harmful pathogens [16]. This mutagen is limited to only a few plant species [36]; [19]. [16] reported in addition that sodium azide (NaN₃) affects the rate of seed germination, shoot and root length, and seed germination delay. They reported further that chemical mutagens induce a large variation to the parameters of morphological structure and performance compared to plants whose seeds were not treated with NaN₃. In general, all these parameters decrease with increasing doses of irradiation or chemical mutagen. [37] confirm a reduced germination rate and height with the seeds of *Jatropha curcas* L. where mutagens (EMS and gamma rays) are used to obtain the interesting traits.

The results obtained in this study show that *P. odontadenius* is likely to be improved by sodium azide (NaN₃). It has been found that, contrary to the effects of chemical mutagens reported by the authors above; including reduced height, collar diameter, number of branches and fresh biomass;

SA stimulated most of these parameters. If seed germination was affected when they were dipped in solutions ranging from 0-10 mM as confirmed [37], the other parameters indicate that the parameter values have been stimulated at high concentrations and are low concentrations (0 - 2.5 mM or 0 - 7.5 mM) most surprisingly affected plant growth.

Height, collar diameter and number of branches per plant would be subject to the expression of related genes and disruption at the molecular level that affects the gene or groups of genes automatically lead to the same effects on these three parameters. These genes could be those controlling the synthesis of growth regulators such as auxins and cytokinins [38]; [39].

Results on length, collar diameter and number of branches of *P. odontadenius* plant confirm also the hypothesis by the fact that at the same concentration, when the length showed a high or low value, automatically the other two parameters, collar diameter and number of branches have also the same effects. Otherwise the observed value of a parameter not showed significant difference at the 5% confidence values between low or high values of other parameters.

The decrease in biomass could be attributed to disturbances in the synthesis of chlorophyll, reliable molecular index for the assessment of genetic effects and gas exchange at the plant [40]; [41]. [42] showed that the fresh and dry biomass of *Arabidopsis thaliana* seedlings subjected to low doses of gamma irradiation were not changed significantly.

The results obtained on the fresh biomass of *P. odontadenius* plants from seeds treated with NaN_3 corroborate [42]. Results on fresh biomass show that they have little varied and showed no significant difference in confidence level of 5%.

It is known that besides conventional primary metabolites (carbohydrates, proteins, lipids and nucleic acids), plants often accumulate some compounds so-called "secondary metabolites". These molecules are not directly involved in plant development but rather involved in relations with biotic or abiotic stress. On the one hand, they increase the efficiency of breeding and secondly, they represent an important source of molecules used by humans in areas as diverse as pharmacology or food. Secondary metabolites are present in all plant species but differ from one species to another and play an essential role in plant metabolism and development [43]; [44].

Indeed, [45] showed that the amount of increase in total flavonoids of *Centella asiatica* when stems of this plant suffered acute radiation from 0 to 120 Gy for 5 days compared with the control. Many other authors have worked to highlight the role that play secondary metabolites in protecting plants against stress due to ionizing radiation [42]; [46]; [47]; [48]; [49].

[50] had reported that to *Phyllanthus* they have described as *Phyllanthus niruri* plant contained alkaloids, polyphenols, flavonoids, tannins, terpenes and / steroids but not contained anthraquinones and saponins. The chemical analysis of large groups of *P. odontadenius* plants from seeds treated with sodium azide (NaN_3) reveals the presence of alkaloids in concentrations of 0.5 mM; 1.5 mM; 2.5 mM; 3.5 mM; 4.5 mM; 5 mM and 10 mM (Table 3) and in concentrations of 5 mM; 7.5 mM; 10 mM; 17.5 mM and 20 mM (Table 4). This presence could be explained by a possible stimulation of the biosynthesis of the alkaloids which were synthesized in most cases from to a small number of amino-acids such as tyrosine, tryptophan, ornithine, arginine and lysine [43].

[51] reported that *Phyllanthus emblica* L. contains alkaloids and saponins that are not found in *P. odontadenius*. However, flavonoids, phenols, triterpenoids and tannins are present both in *P. odontadenius* and in *P. emblica*. Quinones are absent in the two species of *Phyllanthus*.

Considering the results obtained in this work, it appears that according to the criteria of the WHO classification of *in vitro* antiparasmodial activities of extracts or drugs [52] ; [32] , extracts from two tests behaved differently. In the first trial (Table 3), the control (0 mM) extracts with 12.77 ± 5.83 $\mu\text{g/ml}$ value showed moderate *in vitro* antiparasmodial activity (IC_{50} between 10-50 $\mu\text{g/ml}$). Extracts of plants from treated seeds at 0.5 to 10 mM with their respective values of 6.43 ± 6.13 $\mu\text{g/ml}$ (0.5 mM), 2.25 ± 0.16 $\mu\text{g/ml}$ (1.5 mM), 1.47 ± 0.14 $\mu\text{g/ml}$ (2.5 mM), 1.18 ± 0.068 $\mu\text{g/ml}$ (3.5 mM), 1.17 ± 0.06 $\mu\text{g/ml}$ (4.5 mM) 1.09 ± 0.13 $\mu\text{g/ml}$ (5 mM) and 1.04 ± 0.02 $\mu\text{g/ml}$ (10 mM) showed good *in vitro* antiparasmodial activities ($\text{IC}_{50} < 10$ mcg / ml). In the second experiment; extracts from control plants (0 mM), 2.5 mM, 12.5 mM, 15 mM, 17.5 mM and 20 mM exhibited moderate *in vitro* antiparasmodial activities (values between 10 to 50 $\mu\text{g/ml}$) with respective IC_{50} values of 14.91 ± 3.85 $\mu\text{g/ml}$, 21.6 ± 7.13 $\mu\text{g/ml}$; 12.91 ± 6.06 $\mu\text{g/ml}$; 23.55 ± 10.73 $\mu\text{g/ml}$; 18.81 ± 3.37 $\mu\text{g/ml}$ and 20.95 ± 8.30 $\mu\text{g/ml}$ [52]. And extracts of plants from seeds treated with concentrations of 5 mM; 7.5 mM and 10 mM of SA exhibited good *in vitro* antiparasmodial activity ($\text{IC}_{50} < 10$ $\mu\text{g/ml}$) with respective values of 4.32 ± 0.38 $\mu\text{g/ml}$; 1.90 ± 1.75 $\mu\text{g/ml}$ and 1.47 ± 1.07 $\mu\text{g/ml}$.

The *in vitro* antiparasmodial activity of plants aqueous extracts from seed treated firstly from 0.5 mM to 10 mM and secondly from 5 mM to 10 mM of SA (NaN_3) could be explained by the presence of alkaloids synthesized in plants which disclosed in some concentrations of SA. [52] report that among the agents contain antimalarial or antiparasmodial activities from plants exist further alkaloids, terpenes and related compounds, flavonoids, chromones, xanthenes, and anthraquinones and various related compounds and others such as S- isogeranyle and isovaleric acid. These compounds were found in all the different aqueous extracts of plants grown from seeds treated with NaN_3 .

5. Conclusion

The main objective of this study was to improve the plant *P. odontadenius* by chemical mutagenesis in order to a possible fight against parasite of malaria. The effects of sodium azide (NaN_3) on *P. odontadenius* plants from seeds soaked in different solutions of sodium azide (NaN_3) show that this has more mutagenic stimulatory effects than inhibitors effects opposite to *P. odontadenius*.

This study showed that the length of plants ranged from 16.02 ± 2.56 cm (0 mM) to 20.16 ± 3.89 cm (10 mM); collar diameter from 1.56 ± 0.27 mm (0 mM) to 1.87 ± 0.22 mm (10 mM); the number of branches from 11.85 ± 1.35 (2.5 mM) to 13.80 ± 2.20 (10 mM) and fresh biomass from 1.45 ± 0.46 mg (0.5 mM) to 2.08 ± 0.16 mg (10 mM).

The *in vitro* antiparasmodial activity of aqueous extracts of plants from seeds soaked in different solutions of sodium azide (NaN_3) shows that concentrations between 5-10 mM with respective IC_{50} of 1.09 ± 0.13 $\mu\text{g/mL}$ (5 mM) and 1.04 ± 0.02 $\mu\text{g/mL}$ (10 mM) or 4.32 ± 0.38 $\mu\text{g/mL}$ (5 mM) 1.90 ± 1.75

µg/mL (7.5 mM) and 1.47 ± 1.07 µg/mL (10 mM) are those that deserve to be used in the improvement program of *P. odontadenius* secondary metabolites against *Plasmodium falciparum*.

Studies of the next generation or M2 are necessary to verify the heritable effects for to choose possible concentrations of plant breeding. The analysis of active principles involved in the death of trophozoites would be also necessary to justify the molecular effects of sodium azide in *P. odontadenius*.

However, it is necessary to know whether the plant extracts from these doses showed high antiplasmodial activity and also which one would present less toxicity on human cells. These studies are essential in the selection of plants with *P. odontadenius* antiplasmodial activity enhanced by sodium azide. Furthermore, the study of the fractions or molecules involved in improving the *in vitro* antiplasmodial activity by sodium azide is indispensable.

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