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Original Research Article

Effects of Sodium Azide (NaN_3) on Seed Germination, Plantlets Growth and *In Vitro* Antimalarial Activities of *Phyllanthus odontadenius* Müll. Arg

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Abstract

Seeds of *Phyllanthus odontadenius* were obtained after oven drying at 45°C and they were immersed in sodium azide (SA) at concentrations ranging firstly between 0 to 10 mM; secondary between 0 to 20 mM; Seeds were germinated on media and plantlets were transferred *in situ*. 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. *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. IC_{50} values were ranged between 1.04±0.02 µg/ml (10 mM) to 12.77±5.83 µg / ml (0.26 mM) for the first assay. 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. SA lethal doses were 4.76 mM for LD_{30} and 10.99 mM for LD_{50}. *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. 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 justified the usefulness of SA in the increasing production of secondary metabolite against malaria in Nigeria.

Keywords

Antimalarial activity
Antiplasmodial activity
Clinical isolates
Phyllanthus odontadenius
Sodium azide

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 (Adjanohoum, 1982; Oksman-Caldentey and Barz, 2002). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (Igbinosa et al., 2009). In the Democratic Republic of Congo (DRC), among the species used in the treatment

against malaria, *Phyllanthus odontadenius* Müll.-Arg. (Family: Euphorbiaceae) is well positioned for different previous studies on this plant (Pauwels, 1993; Tona et al., 1999; Cimanga et al., 2004). *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 (Kerharo and Adam, 1974; Ishimari et al., 1999; Paranjape, 2001); 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 (WHO, 2010; Orhan et al., 2006; Guédé et al., 2010). 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 antimalarial 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 (Bhat and Surolia, 2001; Céu de Madureira et al., 2002).

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 (Al-Qurainy, 2009). It is known that various chemicals have positive or negative effects on living organisms. Chemical mutagens 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 (Al-Qurainy, 2009; Khan et al., 2009). These chemo mutagens 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 (Rines et al., 1985; Grant and Salomone, 1994). The mutagenicity created by NaN_3 is mediated through the production of an organic metabolite of azide compound, presumably azidoalanine (N_3-CH_2-CH ($NH)_2$-COOH). The production of this metabolite was found to be dependent on the enzyme O-acetyl serine sulfhydrylase (E.C.4.2.99.8.) (Khan et al., 2009). In order to understand that NaN_3 is mutagenic mechanism used for the improvement economic characters to many studies in rice, wheat, Barley and Sorghum (Maluszynski et al., 2009).

In this study, firstly the mutagenic effect of NaN_3 on growth and yield traits of *Phyllanthus odontadenius* was studied. Secondary, to monitor the effects of NaN_3 on the production of active secondary metabolites in *P. odontadenius* aerials parts in order to amplify those with *in vitro* antimalarial activity.

Materials and methods

Plant material–Mutagenesis–*In vitro* Germination

Plant material

The plant material used for harvesting fruits was identified by taxonomist Gallah U.S, Department of Biological Sciences (Faculty of Science) where a voucher number *ABU/BIO4578* was deposited for the plant. The seeds of *P. odontadenius* were used for the study.

Immersion of seeds in SA solutions

Seeds of *P. odontadenius* obtained from drying fruits harvested on IAR Samaru Zaria 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 94 various concentrations of NaN_3 for 2 h 30 min of time with agitation on shaker and the same time seeds were submerged in deionised water for the same period of time served as control. After NaN_3 treatment, seeds were washed properly with autoclaved distilled water 4-5 times to remove excess NaN_3.

In vitro seeds germination

Seeds were disinfected with 70% (v/v) ethanol for 1 min, sterilized with 0.125% (w/v) $HgCl_2$ for 100 3 min, and washed with sterile distilled water. They were then handled with gibberellic acid (GA3) 200; 101 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 (Zao et al., 2006**;** Maluszynski et al., 2009). The pH of the media was adjusted to 5.6 before autoclaving at 121°C for 15 min. Cultures were incubated at 25 ± 104 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 the Petri dish. The reduction of emergence (%) was also determined by the relationship from Maluszynski et al. (2009) as shown:

Emergence reduction (%) = 100 – (Avg. emergence in the dose×100)/Avg. emergence in the control).

In situ seedling transfer

Plantlets growths in vitro were transferred in polyethylene bags containing 300 g of soil for *in situ* growth (Chaves, 2006). Bags were then buried in 3/4 in the ground in randomized complete block (RCB) design with 3 replications (Maluszynski et al., 2009; Rohrmoser, 1986; Khan et al., 2005; Nouri et al., 2012). 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 six plants from each replicate were used to measure the plant growth.

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.

Phytochemical Analysis

Preparation of crude extracts

Ten gram of dried plant material was 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.

Phytochemical screening

The chemical screening was carried out on all crude extracts. Alkaloids 134 were detected with Dragendorff's and Mayer's reagents, Saponins were detected by fronting test. Presence of tannins was detected using Stiasny reagent and ferric chloride 2% (Harborne, 1998; Leconte et al., 1997; Hagerman, 1988; Peng and Jay-Allemand, 1991). Flavonoids were detected using Shinoda's reagent or aluminium chloride 5% (Mabry et al., 1970). Anthraquinones were detected using Borntrager's reagent. Anthocyanins were identified using HCl 2N, heating and add iso-amylic alcohol. Steroids and terpenoids were identified using Liebermann-Bouchard's reagent (Harborne, 1998).

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 $CHCl_3$/MetOH/NH_4OH (9:2:0.5) and EtOAc/Iso-PrOH/NH_3 (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% diphenyl

boric acid ethanolamine complex, methanol solution 1%). Steroids and terpenoids were detected using $CHCl_3$/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 colours. Tannins, mainly pro-anthocyanidins were detected using EtOAc/ HOAc/ HCOOH/H_2O (30:02:1.2.8) (upper phase) as mobile phase and 1% Vaniline and 5% H_2SO4 as reagents. After spraying, drying and heating the plate, pro-anthocyanidins were coloured in red while flavonoids appear in yellow colour. Anthraquinones were identified using $CHCl_3$/MeOH (7:3) as mobile phase and Borntrager's reagent (Harborne, 1998).

In vitro antimalarial activity

Antimalarial activity assays were performed at the National Institute for Pharmaceutical Research and Development (NIPRD) in Idu-Abuja, Nigeria. 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 St. Paul's Bali, Taraba State Nigeria. 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. One 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 (Lekana-Douki et al., 2011).

Plates were then maintained at 37°C in a humid atmosphere containing 5% CO_2. 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 Zeis Primo Star microscope (GmbH/Germany) (Ljungström et al., 2004).

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 IC_{50} of each sample was obtained using the dose-response curves.

Statistical analysis

Data were subjected to Analysis of Variance (ANOVA) using MSTAT-173 C Software Borzouei et al. (2010) and compared to the software statistical 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.

Results

In 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 ± 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 ± 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 1. The DL_{50} and DL_{30} calculated from these equations of linear regression.

Equation / Dilution	**y=-3.2x+85.27 (0-10mM)**	**y=-0.579x+133.2 (0-20mM)**	**y=7.448x+78.17 (0-10mM)**	**y=0.16x+3.438 (0-20mM)**
30	4.7 mM	107.43 mM	-1.10 mM	408.36 mM
50	10.99 mM	141.97 mM	-3.78 mM	285.66 mM

Table 2 shows that the control plants exhibit lower values for most cases compared to plants from seeds soaked in 20 mM NaN_3 solution. The lowest values were found in most plants whose seeds were soaked in 7.5 mM of NaN_3 solution. When control values were compared with those of 10 mM, the later has higher values than the control for all measured parameters. With the exception of germination 11.33 ± 2.67% for 5 mM and 6.67 ± 2.0% for 10 mM, the measured values of other parameters in plants whose seeds were soaked in 5 mM and 10 mM does not differ significantly (Table 2).

Table 2. Effect of sodium azide (NaN_3) on seed germination and plant growth of *Phyllanthus odontadenius.*

Parameter	Treatment concentrations of NaN_3 (mM)						CV	LSD
	0	2.5	7.5	12.5	15.0	20.0		
Rate (%) of seed germination	5.33^{cde} ± 0.9	7.33^{bc} ± 1.11	4.33^{e} ± 1.0	7.0b^{c} ± 15	5.67^{cd} ± 3.78	8.33 ± 0.92	15.6^{a}	2.208^{c}
Emergency reduction (%)	0	-37.52	18.76	-31.33	-6.38	-56.29	-	-
Size (cm) of Plants	11.83^{ab} ± 2.55	10.87^{ab} ± 2.9	8.18^{b} ± 2.18	15.22^{a} ± 082	11.73^{ab} ± 1.56	14.47^{a} ± 0.55	12.3^{a}	5.465^{a}
Collar diameter of plant (mm)	1.15^{cde} ± 0.21	1.05^{de} ± 0.27	0.85^{e} ± 0.25	1.82^{ab} ± 0.06	1.47^{abcd} ± 0.26	1.95^{a} ± 0.04	10.7^{c}	0.53^{c}
No. of branches	10.53^{cde} ± 1.33	9.6^{de} ± 1.85	7.43^{e} ± 1.68	14.37^{ab} ± 0.71	10.93^{bcde} ± 0.95	15.5a ± 1.21	8.86^{b}	3.64^{cd}
Biomass of aerial parts (g)	2.03^{a} ± 1.1	1.72^{a} ± 0.95	2.05^{a} ± 0.60	2.57^{a} ± 1.56	2.24^{a} ± 0.58	3.05^{a} ± 022	28.85^{a}	2.46^{ab}

* For the same parameter, value with identical superscript letters were not significantly different at $p<0.05$ by One-way ANOVA.

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 NaN_3. They are absent in the controls in Tables 3 and 4 and then in the extracts plants from seeds treated with NaN_3 at 2.5 mM; 12.5 mM and 15 mM concentrations (see Table 4). Saponins were absent in all the treatments, while flavonoids and tannins were found all the treatments of M1 and M2 plants (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 NaN_3 (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 NaN_3 (Table 3).

Table 3. Effect of sodium azide (NaN_3) on seed germination and plant growth of *Phyllanthus odontadenius,*

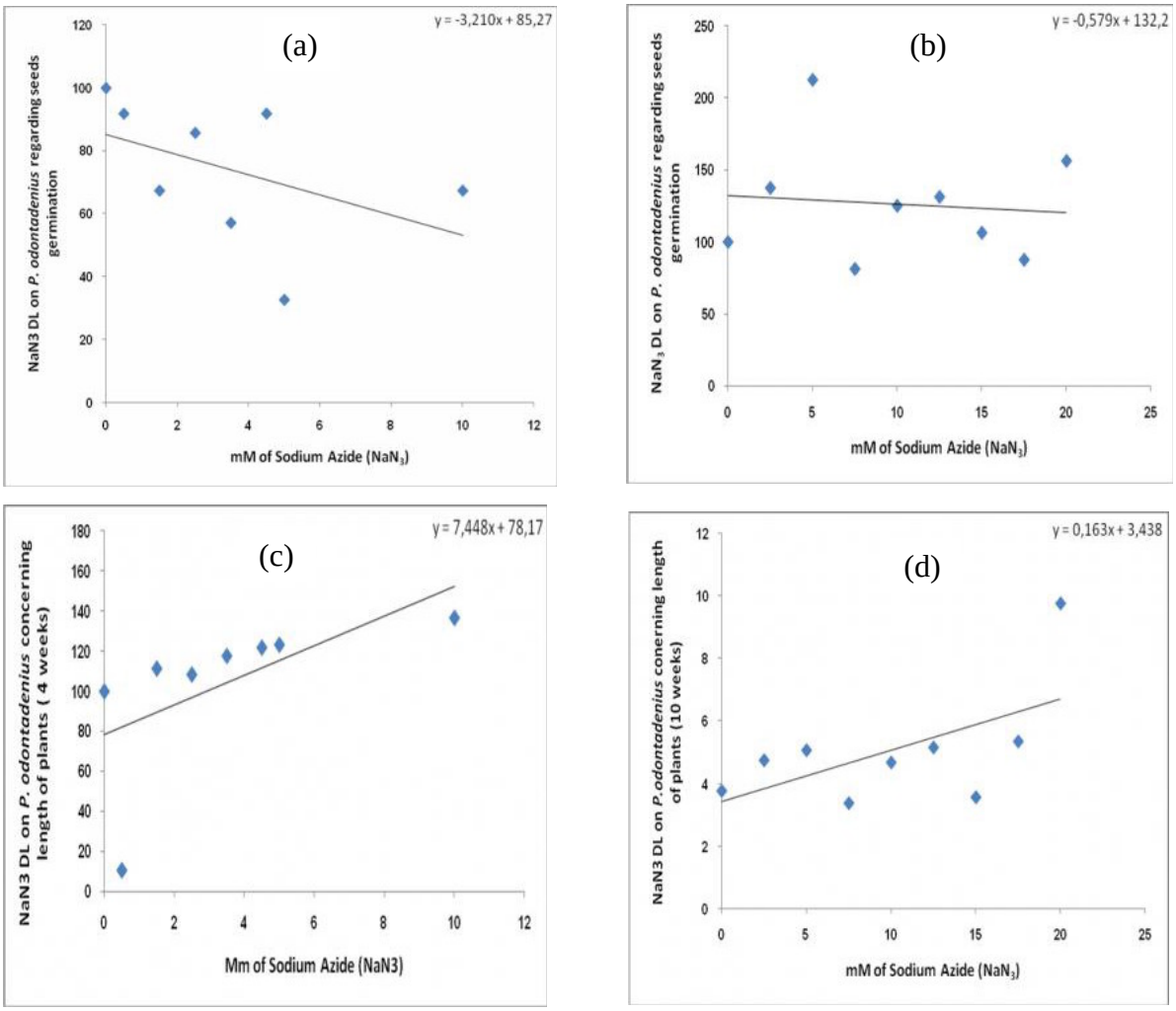
Parameters	Treatment concentrations of NaN_3 (mM)								CV	LSD
	0	0.5	1.5	2.5	3.5	4.5	5.0	10.0		
Rate (%) of seed germination	16.33^{a} ± 0.58	15.00^{a} ± 0.77	11.0^{a} ± 0.29	14.0^{a} ±0.5	9.33^{a} ± 0.36	15^{a} ± 0.44	5.33^{a} ± 0.32	11.0^{a} ± 1.32	23.11	12.44^{c}
Emergency restriction (%)	0	37.51	79.97	39.98	79.97	92.50	87.47	69.99	-	-
Size of Plants (cm)	16.02^{b} ± 2.56	18.21^{ab} ± 4.22	166.0^{b} ± 4.36	17.32^{b} ± 2.92	17.65^{b} ± 3.30	18.30^{ab} ± 3.51	18.50^{ab} ± 4	20.16^{c} ±3.89	21.39^{b}	14.66^{a}
Collar diameter of plant (mm)	1.5^{bc} ± 0.27	1.59^{bc} ± 0.17	1.60^{bc} ± 0.24	1.60^{c} ± 0.30	1.61^{bc} ± 0.30	1.72^{ab} ± 0.25	1.68^{ab} ± 0.24	1.83^{a} ± 0.22	15.87^{c}	0.067^{a}
No. of branches	12.0^{bcd} ± 1.96	12.05^{d} ± 2.96	12.40^{abcd} ± 2.0	11.85^{cd} ± 1.35	12.85^{a} ± 1.84	12.95^{abc} ± 1.74	13.6^{ab} ± 2.4	13.8^{a} ± 20	18.14^{cd}	5.313^{b}
Biomass of aerial parts (g)	1.63^{a} ±0.52	1.45^{ab} ± 0.46	1.75^{ab} ± 0.56	1.66^{ab} ± 0.59	1.79^{ab} ± 0.51	1.8^{ab} ± 0.50	1.8^{ab} ± 1.0	20.8^{a} ± 0.61	32.72^{c}	0.334^{c}

* For the same parameter, value with identical superscript letters were not significantly different at $p<0.05$ by One-way ANOVA.

Sodium azide chemosensibility of *P. odontadenius*

NaN_3 chemosensibility of *P. odontadenius* was determined by seeds germination or by length of plantlets. Results obtained were shown in Fig. 1 (a-d). In Fig. 1, it is apparent 4 linear regression equations as y = -3.210× + 85.27 for Fig. 1a; y = -0.579× + 132.2 for Fig. 1b; y = 7.448 + 78.17× for Fig. 1c and finally the equation: y = 0.163× + 3.438 for Fig. 1d. In Table 5, it appears that the parameter showed a stimulation effect of 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 NaN_3 0-10 mM, which showed values of DL_{30} and DL_{50} which only deviates DL_{50} but also not too relevant concentrations.

Fig. 1: Determination of chemosensibility of sodium azide (NaN_3) on *Phyllanthus 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 weeks (0–10 mM); (d) by length of plants which were dured 10 weeks (0–20 mM).



In vitro antimalarial activities of aqueous extracts from *P. odontadenius*

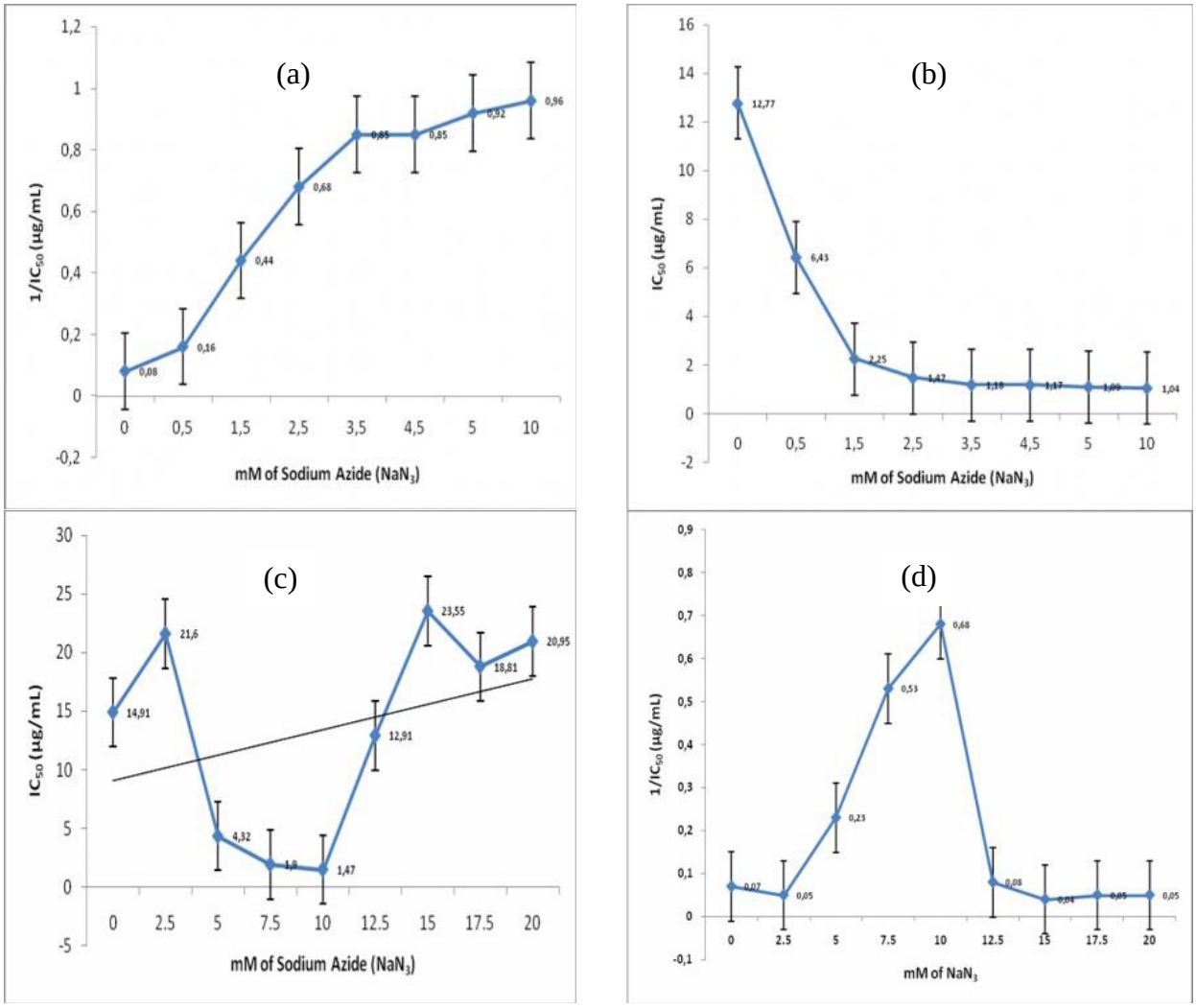
The Fig. 2 (a – d) showed that low values of IC_{50} (µg/mL) were those obtained with plants from seeds immerged in 5 and 10 mM solutions (1.09±0.13 and 1.04±0.02 µg/mL) (Fig. 2a). It's the same for plants obtained by immersion of seeds to 0 – 20 mM; the low values were those at 5 to 10 mM (4.32±0.38 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 shown in Fig. 2b and Fig. 2d than in all concentrations of NaN_3.

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

Constituents	Treatment concentrations of NaN_3 (mM)								Natural Plant
	0	0.5	1.5	2.5	3.5	4.5	5.0	10.0	N
Alkaloids	-	+	+	+	+	+	++	+++	-
Saponins	-	-	-	-	-	-	-	-	-
Anthocyanins	+	+	-	-	+	++	++	++	+
Tannins	+	++	++	+	+	+	+	+	+
Free Anthracene	-	+	+	+	+	-	-	++	-
Cb Anthracene	+	-	+	-	-	-	-	+	+
Steroids	-	+	+	-	-	+	+	+	-
Flavonoids	+	++	+	+	+	+	+	+	+

*N (natural harvested plant), ++ (highly present); + (moderately present); -(absent); Cb (combined).

Fig. 2: Effects of aqueous crude extracts of *Phyllanthus odontadenius* from plants obtained after seeds immerged in sodium azide solutions: (a) antiplasmodial activities with plants which seeds were treated with 0–10 mM; (b) $1/IC_{50}$ values from IC_{50} 0–10 mM; (c) IC_{50} (mg/mL) of plant extracts from 0–20 mM treated seeds; (d) $1/IC_{50}$ values from IC_{50} 0–10 mM.



Comparing Fig. 1a of DL and those of inversing IC_{50} (Fig. 2b and Fig. 2d), concentrations of 5 to 10 mM NaN_3 were promising for breeding *P.odontadenius* with sodium azide for treatment of malaria disease. The IC_{50} values were in accordance with the DL_{50}.

Discussion

Sodium azide is a powerful chemical mutagen, safer and more efficient, which increases the yield and quality of field crops against harmful pathogens (Khan et al., 2009). This mutagen is limited to only a few plant species (Maluszynski et al., 2009). Khan et al. (2009) reported in addition that NaN_3 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 NaN_3. In general, all these parameters decrease with increasing doses of irradiation or chemical mutagen. Dhakshanamoorthy et al. (2010) 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 NaN_3.

Table 5. Phytochemical screening of *Phyllanthus odontadenius* crude extracts from M2 plants.

Constituents	Treatment concentrations of NaN_3 (mM)								
	0	2.5	5.0	7.5	8.5	12.5	15.0	17.5	20.0
Alkaloids		-	+	+	+	-	-	+	+
Saponins	-	-	-	-	-	-	-	-	-
Anthraquinones	+	+	+	+	+	+	-	+	+
Tannins	+	+	+	+	+	+	+	+	+
Free Anthracene	-	-	-	-	-	-	-	-	-
Cb Anthracene	-	-	-	-	-	-	-	-	-
Steroids	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Polyphenols	+	+	+	+	+	+	+	+	+

* + (moderately present); - (absent); Cb (combined anthracene)

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 307 affected when they were dipped in solutions ranging from 0-10 mM as confirmed by Dhakshanamoorthy et al. (2010), 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 (Cain et al., 2010; Joshi et al., 2011).

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 (Bhosale and Hallale, 2011; Devi and Mullainathan, 2012). Gicquel (2012) 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 with the findings of Gicquel (2012). 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 (Hopkins, 2003). Indeed, Moghaddam et al. (2011) showed that the amount of increase in total flavonoids of *Centella asiatica* when stems of this plant suffered acute radiation from 0 to120 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 Gicquel (2012). Luyindula et al. (2004) 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 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 (Hopkins, 2003). Ravikumar et al. (2012) reported that *Phyllanthus emblica* L. contains alkaloids 346 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* antiplasmodial activities of extracts or drugs (Batista et al., 2009; Lekana-Douki et al., 2011), extracts from two tests behaved differently. In the first trial (Table 3), the control (0 mM) extracts with 12.77 ± 5.83 µg/ml value showed moderate *in vitro* antiplasmodial activity (IC50 between 10-50 µg/ml). Extracts of plants from treated seeds at 0.5 to 10 mM with their respective values of 6.43 ± 6.13 µg/ml (0.5 mM), 2.25 ± 0.16 µg/ml (1.5 mM), 1.47 ± 0.14 µg/ml (2.5 mM), 1.18 ± 0.068 µg/ml (3.5 mM), 1.17 ± 0.06 µg/ml (4.5 mM) 1.09 ± 0.13 µg/ml (5 mM) and 1.04 ± 0.02 µg/ml (10 mM) showed good *in vitro* antiplasmodial activities (IC50 <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* antiplasmodial activities (values between 10 to 50 µg/ml) with respective IC50 values of 14.91±3.85 µg/ml, 21.6±7.13 µg/ml; 12.91±6.06 µg/ml; 23.55±10.73 µg/ml; 18.81±3.37 µg/ml and 20.95±8.30 µg/ml (Batista et al., 2009). And extracts of plants from seeds treated with concentrations of 5 mM; 7.5 mM and 10 mM of SA exhibited good *in vitro* antiplasmodial activity (IC50 <10 µg/ml) with respective values of 4.32 ± 0.38 µg/ml ; 1.90 ± 1.75 µg/ml and 1.47 ± 1.07 µg/ml.

The *in vitro* antiplasmodial 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 NaN_3 could be explained by the presence of alkaloids synthesized in plants which disclosed in some concentrations of SA. Batista et al. (2009) report that among the agents contain antimalarial or antiplasmodial activities from plants exist further alkaloids, terpenes and related compounds, flavonoids, chromones, xanthones, and anthraquinones and various related compounds and others such as S- isogeranyl and isovaleric acid. These compounds were found in all the different aqueous extracts of plants grown from seeds treated with NaN_3.

Conclusions

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 NaN_3 on *P. odontadenius* plants from seeds soaked in different solutions of NaN_3 show that this has more mutagenic stimulatory effects than inhibitors effects opposite to *P. odontadenius*.

The *in vitro* antiplasmodial activity of aqueous extracts of plants from seeds soaked in different solutions of NaN_3 shows that concentrations between 5-10 mM with respective IC50 of 1.09 µg/mL (5 mM) and 1.04 µg/mL (10 mM) or 4.32 µg/mL (5 mM) 1.90 µg/mL (7.5 mM) and 1.47 µg/mL (10 mM) are those 384 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 NaN_3 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 NaN_3. 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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