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EFFECTS OF SODIUM AZIDE (NAN_3) ON SEED GERMINATION, PLANTLETS GROWTH AND *IN VITRO* ANTIMALARIAL ACTIVITIES OF *PHYLLANTHUS ODONTADENIUS* MULL ARG

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ABSTRACT

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 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

Phyllanthus odontadenius, Sodium Azide and Antimalarial activity.

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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[4-6]. *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[7-9], 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[10-12]. 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 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[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 chemo mutagens induce a broad variation of morphological and yield structure parameters in comparison to normal plants. Sodium azide (NaN3), 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 NaN3 is mediated through the production of an organic metabolite of azide compound, presumably azidoalanine (N3-CH2-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.)[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 study, i 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.

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 sodium azide for 2 h 30 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 sodium azide treatment, seeds were washed properly with autoclaved distilled water 4-5 times to remove excess sodium azide.

***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[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 ± 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.

% of germinated seeds = *n* x 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 – (Average emergence in the dose x 100)/average 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[22]. Bags were then buried in 3/4 in the ground in randomized complete block (RCB) design with 3 replications[19,23-25]. 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.

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

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.

Phytochemical screening

The chemical screening was carried out on all crude extracts. Alkaloids 134 were detected with Dragendorff's and Mayer's reagents[26]. Saponins were detected by fronting 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 aluminium chloride 5%[30]. 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[26]. The presence of the different chemical groups was confirmed by Thin Layer Chromatography (TLC+0) performed on silica gel plates GF254 (Merck, Germany). Alkaloids were detected using CHCl3/MetOH/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, methanol 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 colours. Tannins, mainly pro-anthocyanidins were detected using EtOAc/HOAc/HCOOH/H2O (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 colored in red while flavonoids appear in yellow colour. Anthraquinones were identified using CHCl3/MeOH (7:3) as mobile phase and Borntrager's reagent[26,31].

***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. 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% 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)[33-34]. Inhibition of parasitaemia (percent) was calculated as following:

Inhibition (%) = (A - B/A) x 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[35] 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 No.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 No.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 ± 2.67% for 5 mM and 6.67 ± 2.0% for 10 mM in Table No.2, the measured values of other parameters in plants whose seeds were soaked in 5 mM and 10 mM does not differ.

In view of Table No.4, it is apparent that the alkaloids have been found present in all extracts plants from treated seeds (Table No.4) 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 Table No.4 and then in the extracts plants from seeds treated with SA at 2.5 mM; 12.5 mM and 15 mM concentrations (Table No.4). Saponins are absent everywhere, while flavonoids are found everywhere such as tannins in the two Table No.4. Anthocyanins are present in the control extracts as in the treated extracts except at concentrations of 1.5

mM and 2.5 mM and 15 mM in Table No.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 No.4). They are present in the control as treated extracts in Table No.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 No.4).

Sodium Azide Chemosensibility of *P. odontadenius*

The Sodium Azide (NaN3) chemosensibility of *P. odontadenius* was determined by seeds germination or by length of plantlets. Results obtained were showed in Figure No.1 (a-d).

In Figure No.1, it is apparent 4 linear regression equations as y = -3.210 x + 85.27 for Figure No.1a; y = -0.579 x + 132.2 for Figure No.1b; y = 7.448 + 78.17 x for Figure No.1c and finally the equation: y =0.163 x + 3.438 for Figure No.1d.

In Table No.3, it appears that the parameter showed a stimulation effect of sodium azide (NaN3) gives values of DL30 and DL50 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.

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

The Figure No.2 (a-d) showed that low values of IC50 (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) (Figure No.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±1.07µg/mL for 10 mM). These concentrations (5 – 10 mM) have high antiplasmodial activities which were showed in Fig. 2b and Figure No.2d. Than all concentrations of sodium azide. Comparing Figure No.1a of DL and those of inversing IC50 (Figure No.2b and Figure No.2d), concentrations of 5 to 10 mM were promising for plant breeding with sodium azide for treatment of malaria disease. IC50 were according with DL_{50}.

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[16, 19, 36]. 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 307 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[40-42]. 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 NaN3 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 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[42,46-50] 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 No.4) and in concentrations of 5 mM; 7.5 mM; 10 mM; 17.5 mM and 20 mM. 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 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[52,32], extracts from two tests behaved differently. In the first trial (Table No.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[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* 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 SA (NaN3) 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 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.

Table No.1: Effect of sodium azide (NaN_3) on seeds germination and plant growth of *Phyllanthus odontadenius*

S.No	mM of NaN_3 Parameters	0	0.5	1.5	2.5	3.5	4.5	5	10	C.V	LSd
1	Rate (%) of seed germination	16.33±0.58a	15.00±0.77a	11.0±0.29a	14±0.5a	9.33±0.36a	15±0.44a	5.33±0.32a	11±1.32a	2	12.44c
2	Emergency reduction (%)	0	37.51	79.97	39.98	79.97	92.50	87.47	69.99	-	-
3	Size (CM) of Plants	16.02±2.56b	18.21±4.22ab	166±4.36b	17.32±2.92b	17.65±3.30b	18.30±3.51ab	18.50±4ab	20.16±3.89	21.39	14.66
4	Collar diameter of plant (MM)	1.5±0.27bc	1.59±0.17bc	1.60±0.24bc	1.60±0.30c	1.61±0.30bc	1.72±0.25ab	1.68±.24ab	1.83±0.22a	15.87	0.067
5	No of branches	12±1.96bcb	12.05±2.96d	12.40±2.0abcd	11.85±1.35cd	12.85±1.84a	12.95±1.74abc	13.6±2.4ab	13.8±20a	18.14	5.313
6	Biomass of aerial	1.63±0.526	1.45±0.46ab	1.75±0.56ab	1.66±0.59ab	1.79±0.51ab	1.81±0.50ab	1.81±1.0ab	20.8±0.61a	32.72	0.334

Table No.2: Effect of sodium azide (NaN_3) on seeds germination and plant growth of *Phyllanthus odontadenius*

S.No	mM of NaN_3 Parameters	0	2.5	7.5	12.5	15	20	Cv(%)	Lsd
1	Rate (%) of seed germination	5.33+0.9cde	7.33±1.11bc	4.33±10e	7.0±15bc	5.67±3.78cd	8.33±092	15.60	2.208
2	Emergency reduction (%)	0	-37.52	18.76	-31.33	-6.38	-56.29	-	-
3	Size (cm) of Plants	11.83±2.55ab	10.87±2.9ab	8.18±2.18b	15.22±082a	11.73±1.56ab	14.47±0.55a	12.36	5.465
4	Collar diameter of plant (mm)	1.15±0.21cde	1.05±0.27de	0.85±0.25e	1.82±0.06ab	1.47±0.26abcd	1.95±0.04a	10.7	0.5328
5	No of branches	10.53±1.33cde	9.6±1.85de	7.43±1.68e	14.37±0.71ab	10.93±0.95bcde	15.5±1.21a	8.86	3.642
6	Biomass of aerial p	2.03±1.1a	1.72±0.95a	2.05±0.60a	2.57±1.56a	2.24±0.58a	3.05±022a	28.85	2.464

* For the same parameter, value with identical letters were not significantly different at 5% ($p \leq 0.05$); DMRT.

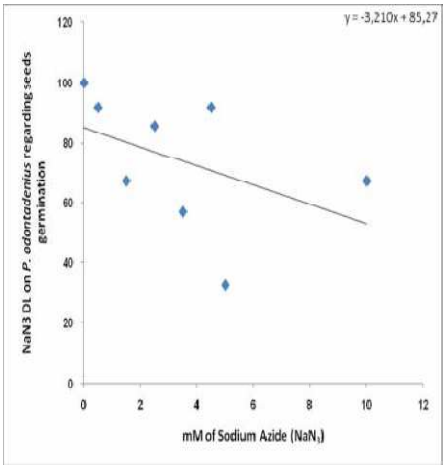
Table No.3: The DL_{50} and DL_{30} calculated from these equations of linear regression

S.No	Equation	y=-3.2x+85.27	y=-0.579x+133.2	y=7.448x+78.17	y=0.16x+3.438
	DL	(0-10mM)	(0-20mM)	(0-10mM)	(0-20mM)
1	30	4.7mM	107.43mM	-1.10mM	408.36mM
2	30	10.99mM	141.97mM	-3.78mM	285.66mM

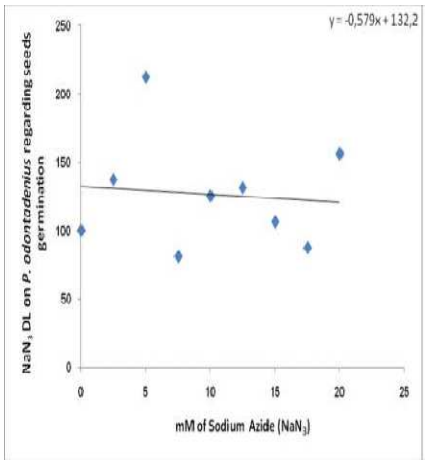
Table No.4: Phytochemical screening of *p. odontadenius* crude extracts from MI plant

S.No	Constituents Treatment									Natural Plant
	Test	0	0.5	1.5	2.5	3.5	4.5	5	10	N
1	Alkaloids	-	+	+	+	+	+	++	+++	-
2	Saponins	-	-	-	-	-	-	-	-	-
3	Anthocyanins	+	+	-	-	+	++	++	++	+
4	Tannins	+	++	++	+	+	+	+	+	+
5	Free Anthracene	-	+	+	+	+	-	-	++	-
6	CB Anthracene	+	-	+	-	-	-	-	+	+
7	Steroids	-	+	+	-	-	+	+	+	-
8	Flavonoids	+	++	+	+	+	+	+	+	+

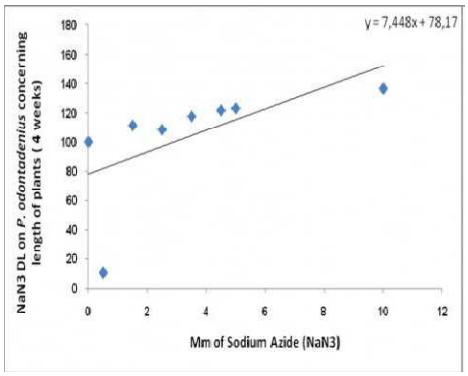
P (natural harvested plant), + present, - absent, cb combined



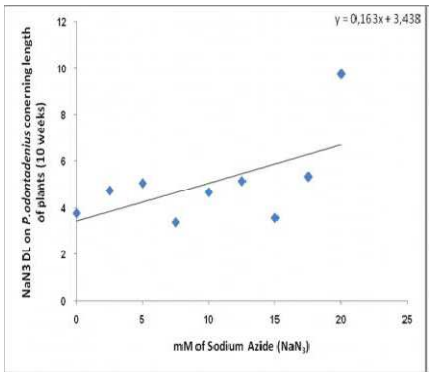
a.



b.



c.



d.

Figure No.1: Determination of chemosensibility of Sodium Azide (NaN3) on *P. odontadenius*; a: by seeds germination using 0 – 10 mM of NaN3; b: by seeds germination using 0 – 20 Mm of NaN3; 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).

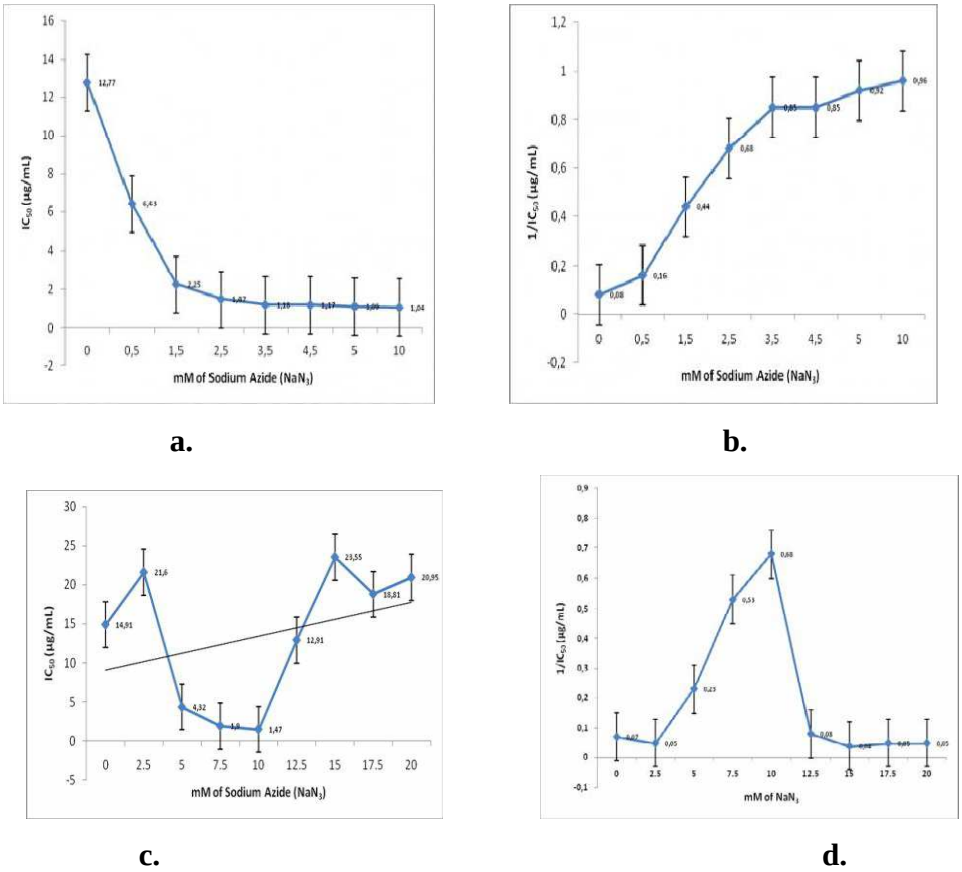


Figure No.2: Effects of aqueous crude extracts of *P. odontadenius* from plants obtained after seeds immerged in Sodium Azide solutions (a: antiplasmodial activities with plants which seeds were treated with 0 – 10 mM; b: 1/IC_{50} values from IC_{50} 0 – 10 mM. C: IC_{50} (mg/mL) of plant extracts from 0 – 20 mM treated seeds; d: 1/IC_{50} values from IC_{50} 0 – 10 mM).

CONCLUSION

This study showed that this chemical has effects on the parameters investigated and also the plant demonstrated a high level of antimalarial activities in vitro. The *in vitro* antiplasmodial 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 IC50 of 1.09 ± 0.13 µg/mL (5 mM) and 1.04± 0.02 µg/mL (10 mM) or 4.32 ± 0.38 µg/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*. These studies are essential in the selection of plants with *P. odontadenius* antiplasmodial activity enhanced by sodium azide.

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