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 *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 , antimalarial activity.

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 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.