

EFFECTS OF GAMMA IRRADIATION ON SEEDS GERMINATION, PLANTLETS GROWTH AND ANTIMALARIAL ACTIVITIES OF PHYLLANTHUS ODONTADENIUS Müll Arg.

Abstract

The aim of this work was to increase the production of secondary metabolites of *P. odontadenius* using gamma radiation in order to amplify those with *in vitro* antimalarial activity. Thus against *P. falciparum* isolate and chloroquino-resistant strain *P. falciparum*. Seeds of *P. odontadenius* were irradiated by gamma-irradiation (^{137}Cs) at dose ranging between 0 to 300 Grays (Gy).

In addition some parameters such as height, collar diameter, number of branches and biomass from first and second generations were analyzed.

Results showed that gamma irradiation had negative effects on growth parameters of *P. odontadenius* in the M1 and M2 generations with greater effects observed with treatment exceeding 100 Gy. For the *in vitro* antimalarial activities from to extracts obtained with aerial materials part from directly irradiated seeds (M1), the effects observed with extracts from M1 were higher than those from M2. For M1 extracts plants, IC_{50} values were ranged between 1 $\mu\text{g/ml}$ and 2.96 $\mu\text{g / ml}$ and between 1 $\mu\text{g/ml}$ and 5.96 $\mu\text{g/ml}$ for M2 extracts plants for *P. falciparum* from to clinical isolates.

With *P. falciparum* K1 strain, the IC_{50} values were ranged between 1.31 – 4.08 $\mu\text{g/ml}$ for M1 and between 3.91 – 14.11 $\mu\text{g/ml}$ for M2 extracts. The best activities were observed with gamma-radiation doses exceeding 150 Grays (Gy).

Keywords: *Phyllanthus niruri* (*Phyllanthus odontadenius*), Gamma irradiation, secondary metabolites, Malaria, Antimalarial activity

1. INTRODUCTION

Human malaria is one of the most important health problems in tropical and subtropical regions. WHO estimates the number of clinical cases to be 216 million in 2010 approximately 40% of world's population is at risk of malaria and nearly 655,000 died from the disease, mainly children under 5, pregnant women and elderly (WHO, 2011 ; 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.

Plants produce more than thousands of different compounds through the secondary metabolism pathways. These secondary metabolites are often the keystone in the interactions between plants and their environment as for example phytoalexins, that are involved in plant defense. The properties of these molecules are used in traditional medicine, but also in modern allopathic medicine through the use of purified or derived components obtained from chemical hemisynthesis (Gravot, 2002).

Plants have been used medicinally throughout history, and the two best conventional antimalarial drugs, artemisinin from *Artemisia annua* and quinine from *Cinchona sp* (*Rubiaceae*), are both derived from traditional medicines. Traditional medicine using plant extracts continues to provide health coverage for 80% of the World's population, especially in the developing world (Subramanias *et al.*, 1971; Willcox and Bodeker, 2004).

Biosynthesis of secondary metabolites is dependent on some extrinsic and intrinsic constraints (Tourte, 2002) as some differences in quality and quantity of compounds within the same species are observed depending on regions of the world. This is one of the challenges in the production of secondary metabolites. To address this, the metabolic engineering approach is to modify the plant physiology to make it produce a protein molecule of economic interest. However, this approach has been facing many difficulties due to the implication of secondary metabolites in many regulations, relatively unknown, contributing to maintain plant homeostasis.

In recent years rapid procedures for obtaining transgenic roots have been developed using *Agrobacterium rhizogenes*, a soil pathogen which elicits adventitious, genetically (Ri T-DNA) transformed roots (Chabaud *et al.*, 2006 ; Hu and Du, 2006 ; Häkkinen *et al.*, 2005 ; Kim *et al.*, 2002 ; Bajaj and Ishimaru, 1999). As genetic variability is essential for any crop improvement programme, the creation and management of genetic variability becomes central base to breeding. Experimentally, induced mutations provide an important source for variability. A variety of ionizing radiation forms have been used as mutagens for mutation breeding including X rays, γ rays, neutrons and ion-beams in addition or no to chemical mutagens (Makeen and Babu, 2010 ; Nakagawa *et al.*, 2011).

Among chemical mutagens commonly used either are alkylating agents (N-methyl-N-nitrosoguanidine (MNNG), ethyl methane sulfonate (EMS) and sodium azide (NaN_3) for chemical mutagens, or ionizing radiation (γ -rays, X-rays, among many others) for physical mutagens. These often act on mutagenic chromosomal and molecular physiological level (Zryd 1988; Liakh and Lagron, 2002, 2005; Muthasamy *et al.*, 2005).

Radiation damages cell through the formation of reactive oxygen species (ROS). ROS induce oxidative damage of DNA, including strand breaks and base and nucleotide modifications (Nagagawa *et al.*, *Op. Cit*). In general, ionizing radiation predominantly induces the formation of radical by water radiolysis, and these then

attacks DNA and produce oxidative damage (Yoshihara *et al.*, 2013). The formation of 7,8-dihydro-8-oxo-2'-desoxyguanosine (8-oxo-dG) represents one of the most abundant and best characterized type of oxidative damage (Nakagawa *et al.*, 2011 ; Yoshihara *et al.*, 2013).

Phyllanthus odontadenius Müll.Arg. (synonym : *Phyllanthus bequaertii* Robyns & Lawalrée) is present in all the coastal countries of West Africa from Guinea-Bissau in the Congo, and from Rwanda, Kenya and Uganda to southern Africa. In Ghana, the leaves are eaten to get the hiccups. In Rwanda, the stem extract is used to treat diarrhea and cholera. Alcohol extracts of leaves and stems calmed induced diarrhea castor oil in mice. A water extract of the entire plant inhibited the DNA polymerase of hepadnaviruses (Oudhia, 2008 ; Fischer, 1993). Unander *et al.* (1991) reported that plant part unspecified used to treat earache in the northern Nigeria. The common names *geron-tsuntsaye*, *gero itache*, *ebógi* and *ebó zùnmaggi* are used for *P. amarus*, *P. odontadenius*, *P. pentandrus*, probably *P. urinaria* and probably also some *noo-Phyllanthus* species.

Some authors have published results on *Phyllanthus* species which ones by bad appellation have called *Phyllanthus niruri* that would probably *P. odontadenius* or *P. amarus*. For that, they reported that *P. niruri* primarily contains lignans, flavonoids, alkaloids, phthalic acid and tanins (Ishimari *et al.*, 1999). Polyphenols, coumarins, sterols and terpenes have also been found by some authors (Shimizu *et al.*, 1989 ; Veno *et al.*, 1988 ; Joa *et al.*, 1998 ; Gupita and Ahmed, 1984). It has been widely used in treating a number of traditional ailments (Unander *et al.*, 1991; Ishimaru *et al.*, 1999; Luyindula *et al.*, 2004) and has demonstrated *in vitro* antibacterial actions against *Staphylococcus*, *Micrococcus*, and *Pasteurella* bacteria as well as *in vivo* and *in vitro* anti-malaria properties.

Ion beams and γ -rays are utilized as models of high-linear energy transfer (LET) and low-LET radiations, respectively. It is known that high-LET radiations tend to confer greater biological effects, such as cell-killing and mutagenesis than low-LET radiations (Yoshihara *et al.*, 2013). Results obtained by Nakagawa *et al.* (2011) indicate that the G (guanine) to T (Thymine) transversion was greatly induced by γ -rays exposure. Moghaddam *et al.* (2011) reported that γ -rays to be the most efficient ionizing radiation of creating mutants in plants as they can induce high mutation numbers in plants.

The aim of the present study was to monitor the effects of γ -rays (^{137}Cs) 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.

1.1.2. Irradiation of seeds

Seeds of *P. odontadenius* obtained from drying fruits harvested on the Kinshasa university site were irradiated with gamma rays from Cesium 137 (Cs^{137}) source in the Conservatoire Lisa I Irradiator at the Department of Biochemistry, General Atomic Energy Commission (GAEC). The dose rate was 1.21 Gy/min (Javed *et al.*, 2000; Mahmoud and Al-Twaty, 2006) and the treatments ranged from 0 to 300 Grays (Gy). The first generation of irradiated seeds was designed as M1.

1.1.3. *In vitro* seeds germination

The irradiated material was germinated in Petri dishes with 3 replications. M1 seeds were disinfected with 70% (v/v) ethanol for 1 min, sterilized with 0.125% (w/v) HgCl_2 for 3 min, and washed with sterile distilled water. They were then handled with gibberellic acid (GA_3) 200 mg/L for 4 h and finally drained before being cultivated on modified Murashige and Skoog (MS) basal media without sucrose or phytohormone and supplemented with 0.8% agar (Zhao *et al.*, 2006. Jimenez *et al.*, 2007). The pH of the media was adjusted to 5.6 before autoclaving at 121°C for 15 min. Cultures were incubated at $25 \pm 1^\circ\text{C}$ under fluorescent light with 16 h photoperiod. Percentage of 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:

$$\text{Emergence reduction (\%)} = 100 - (\text{Average emergence in the dose} \times 100) / \text{average emergence in the control}$$

1.1.4. *In vitro* and *in situ* seedling transfer

After 2 months, M1 plantlets from Petri dishes were transferred to tubes containing MS basal media with 3% sucrose, without phytohormone supplemented with 0.8% agar for *in vitro* culture or 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. Rohmoser, 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).

2.1.5. Seedling growth and Plantlets survival

The length of vitroplants was shouted using a numeric apparatus PENTAX Optio w30 WATERPROOF and measured with Optimas6 software. The number of leaves per plant was counted manually.

The survival rate of plants was determined with following formula:
survival rate (%) = (number of the surviving plants x 100)/number of transferred plants

The number of mutants (albina, xantha and viridis) observed following the irradiation treatment in M1 was determined and the mutation frequency (Msd) was

calculated as follows (Maluszynski *et al.*, 2009): $Msd = [number\ of\ mutant\ (albina + xantha + viridis) \times 100] / N$ with N is the total number of seedlings obtained for a dose.

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.

1.2. *Culturing of second generation seeds*

The second generation (M2) was obtained from *in vitro* culture of M1 seeds using the same methods as for irradiated seeds. After *in vitro* germination, 50 plants of each irradiation dose treatment were sowing in row and plants were watered also three times a week. The same parameters such as diameter of collar, shoot length and branches number were determined. Fresh and dried biomass for aerial parts of M2 plants were equally measured after four months plants harvest.

2.3. *In vitro antimalarial activity*

Antimalarial activity assays were performed at the National Institute of Biomedical Research (NIBR) in Kinshasa/Gombe, DR Congo and at the UMR-MD3 laboratory, Institute of the Biomedical Research of the french Army - Antenne Marseille, France.

2.3.1. *Effects of plant drugs on clinical isolates of P. falciparum*

The stock solutions, 200 µg/ml for M1 extracts and 125 µg/ml for M2 extracts, 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 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 were 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 (125 to 0 µg/ml) of M1 or M2 extracts (Lekana-Douki *et al.*, 2011).

Plates were then maintained at 37°C in a humid atmosphere containing 5% CO₂. Quinine was used as control. After 48 h-incubation, thin smears were made and stained with GIEMSA 5% and parasitemia were determined with a Zeiss Primo Star microscope (GmbH/Germany) (Krogstad *et al.*, 1987, Trager *et al.*, 1976). Inhibition of parasitaemia (percent) was calculated as following:

Inhibition (%) = $(A - B/A) \times 100$, where A is the parasitaemia in the negative control and B, the parasitemia in the treated plates bucket. The IC₅₀ of each sample was obtained using the dose-response curves.

2.3.2. *Effects of plant drugs on chloroquine-resistant strain K1*

The chloroquine-resistant (IC₅₀ = 1010 ± 155 nM) strain K1 (Thailand, MR4-ATCC) was tested using the isotopic microtest method. For this the parasite was cultured according to Trager and Jensen (1976). Parasite culture was performed in RPMI 1640 medium (Gibco) supplemented with 25 mM HEPES, 50 mg/l hypoxanthine, 25 mM NaHCO₃, 20 mg/l gentamicin and 10% human serum at 37°C and in a standard gas mixture consisting in 10% O₂, 5% CO₂ and 85% N₂. Parasites were synchronized with sorbitol before use (Lambros and Vanderberg, 1979).

The *in vitro* susceptibility to extracts was determined by measurement of [³H] hypoxanthine incorporation into parasite nucleic acids using the method of [Desjardins et al. \(1979\)](#). Chloroquine was used as control. Extracts and chloroquine were placed in 96 wells microplates and serial dilutions were made in RPMI medium (final concentration ranging from 0 - 100 µg/ml for extracts). Synchronous culture with parasitaemia of 0.8% and 1.5% final hematocrit were distributed. Parasite growth was assessed by adding 1 µCi of [³H] hypoxanthine (specific activity of 14.1 Ci/mmol; Perkin-Elmer, Courtaboeuf, France) to each well at time zero. The plates were incubated for 42 h at 37°C in an atmosphere of 10% O₂, 5% CO₂ and 85% N₂ and then frozen at - 80°C. After thawing, the content of each well was collected onto filter plates (Filter Mate Cell Harvester, Perkin-Elmer) and 25 µl of scintillation fluid (Microscint O, Perkin-Elmer) was added to each well.

The level of parasite incorporation of radioactivity (in counts per minute) was measured with a scintillation counter (Top Count, Perkin-Elmer). Antimalarial activity was determined as concentration of drugs inducing 50% of growth inhibition (IC₅₀) by nonlinear regression analysis from the dose-response relationship as fitted by ICEstimator software ([Woodrow et al., 2013](#)).

2.4. Phytochemical analysis

2.4.1. 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.

2.4.2. Phytochemical screening

The chemical screening was carried out on all crude extracts. Alkaloids were detected with Draggendorff's and Mayer's reagents [[Harborne, 1998](#)]. Saponins were detected by frothing test [[Harborne, 1998](#); [Leconte et al., 1997](#)]. Presence of tannins was detected using Stiasny reagent and ferric chloride 2% [[Harborne, 1998](#); [Hagerman, 1988](#); [Peng and Jay-Allemand, 1991](#)]. Flavonoids were detected using Shinoda's reagent or aluminum chloride 5% [[Mabry et al., 1970](#)]. Anthraquinones were detected using Börtranger's reagent [[Harborne, 1998](#)]. Anthocyanins were identified using HCl 2N, heating and add iso-amyl alcohol ([Harborne, 1998](#)). 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 CHCl₃/MeOH/NH₄OH (9:2:0.5) and EtOAc/Iso-PrOH/NH₃ (85:15:5) as mobile phases and Draggendorff'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 to 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 Börtranger's reagent [[Harborne, 1998](#), [Wagner et al., 1984](#)].

264 *Statistical analysis*

265 Data were subjected to Analysis of Variance (Anova) using MSTAT-C Software
266 (Borzouei *et al.*, 2010) and compared to the software Statistica software with General
267 Linear and LSD test (Least Significant Difference) in order to identify differences
268 between treatments. Means of different treatments were separated with LSD at 5%
269 level of probability.

3. RESULTS

3.1. Effects of gamma-irradiation (^{137}Cs) on the growth of *P. odontadenius*

Results obtained in this work were mentioned in figures 1 (a-d), 2 (a-e) and 3 (a and b) and in the tables 1 to 5.

3.1.1. Seeds Germination and Growth of *in vitro* *P. odontadenius* seedlings

The Table 1 exhibiting the effects of Gamma-Ray (Cs^{137}) on *in vitro* irradiated seeds germination and seedling growth, shown that rate of *in vitro* seeds germination of M1 plants decreases with increasing irradiation doses. The highest germination rate ($43.85 \pm 11.17\%$) was observed for the control (0 Gy), while the lowest germination rate ($7.06 \pm 1.22\%$) was observed for 225 Gy irradiation treatment. A small effect on *in vitro* seeds germination was observed at doses of 25, 50, 75 and 100 Gy (Table 1).

The reduction of the emergence in M1 plants was higher after gamma-irradiation at 225 Gy (83.90% reduction when compared with the control) (Table 1). Low reduction of emergence was found following irradiation at 25, 50 and 75 Gy (19.77%, 19.73% and 16.33%, respectively). Following 100 Gy irradiation treatment, the reduction of the emergence of M1 plant was above 30% (Table 1).

The size and the number of leaves seedlings from *in vitro* cultured M1 were higher than those of the control (0 Gy) (Table 1). The size of leaves measured for the control plant was significantly different from that of M1 plants obtained after irradiation treatment from 150 Gy to 300 Gy. The number of leaves calculated for the control plant was significantly different from that of M1 plants obtained after irradiation treatment at 300 Gy.

Growth of *in situ* *P. odontadenius* plants

Results on growth of *P. odontadenius* plants *in situ* were presented in table 2 exhibiting Length, Collar diameter, seeds number per plant or seeds Weight (mg) and biomass per plants.

For plants which grew *in situ*, the size and collar diameter (14.01 ± 5.12 cm and 2.68 ± 1.07 mm, respectively) of M1 plants obtained after seeds irradiation at 50 Gy dose were significantly higher than those observed for the plant control (9.34 ± 4.38 cm and 2.01 ± 1.09 mm, respectively) and for seeds irradiated at 300 Gy (3.60 ± 0.28 cm and 1.10 ± 0.14 mm, respectively).

In *P. odontadenius* control plants, the number of seeds per plant was 234 ± 58 weighting 70.32 ± 17.30 mg. The largest number of seeds per plant was observed at 225 Gy with 715 ± 182 seeds per plant weighting 214.69 ± 54.72 mg while the most important quantity of biomass was obtained at 50 Gy (29.60 ± 9.19 g) and the lowest at 300 Gy (2.00 ± 0 g).

3.1.2. Morphological effects on *P. odontadenius* plants *in situ*

Phenotypic effects were also observed on *P. odontadenius* plants growing in the field. In particular, we observed changes on leaves of *P. odontadenius* plants whose seeds were irradiated at 50, 100, 125 and 225 Gy. In particular, at dose of 100 Gy, the change of plant leaf in purple color profoundly affects the individual plant who dies (**Fig.1c**). These effects are reflected to the roots which would furnish themselves too specific phenotypic traits (**Fig.2c and Fig.2d**).

Color defects in the roots are appeared blackish accompanied by white roots or blackish simply for plants obtained with seeds irradiated at 100 Gy (c and d). 50 Gy (b) has very long roots exceeding the size of the aerial part (Fig. 2).



1a:50 Gy 1b:50 Gy 1c: 100 1d: 100 Gy et 225 Gy
Fig.1(a – d): Morphomological effects of Gamma-ray on growth of *P. odontadenius* plants. 1a and 1b: plants from irradiated seeds with 50 Gy, 1c: plants



Fig.2 (a - e) : Effects of gamma irradiation on roots and plantlets of *P. odontadenius* obtained by gamma-irradiation (Cs137). 2a: control plant; 2b: 50 Gy irradiated plant; 2c and 2d: 100 Gy irradiated plant and 2e: 225 Gy irradiated plant

The frequency of mutations differs significantly depending on the doses used. The mutation frequency was the highest at 225 Gy with 22.22%, followed by 100Gy with 13.33%. Frequencies at doses of 50 and 125 Gy were respectively 8.33% and 9.09%. These percentage changes are an indication of what may be the changes in M2.

3.2. Effects of Gamma Irradiation (^{137}Cs) on *P. odontadenius* in M2

Control plants derived from seeds (T_0M_2) showed high values for the various parameters studied (size: 22.06 ± 6.91 cm, collar diameter with: 2.03 ± 0.53 mm, number of branches: 18.58 ± 7 and biomass per foot: 1.96 ± 0.76 g) and plants from 175 Gy ($T_{175}M_2$) showed low values for the average size (10.58 ± 4.23 cm), collar diameter (1.03 ± 0.31 mm) and branches number (10.04 ± 4.06).

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

Antimalarial activities of *P. odontadenius* crude extracts obtained after different irradiation treatment are reported in figures 3a and 3b.

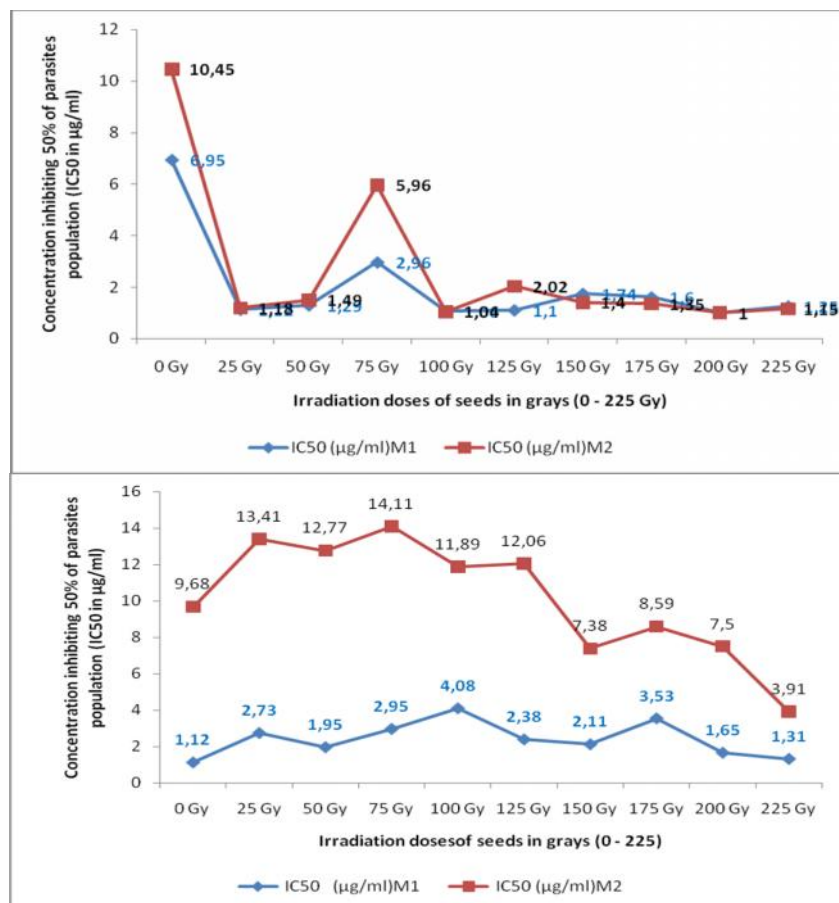


Fig. 3a: *In vitro* activities of *P. odontadenius* on isolates of *P. falciparum* K1

Fig. 3b: *In vitro* antiplasmodial activity on the *P. falciparum* K1 strain

When testing on *P. falciparum* clinical isolates, extracts obtained with plants from M₁ control (0 Gy) showed higher antimalarial activity than extracts obtained with plants from M₂ control. The *in vitro* antiplasmodial activities of *P. odontadenius* on *P. falciparum* isolates presented the IC₅₀ values ranged between 6.95 µg/ml for M₁ control (0 Gy) versus 10.45 µg/ml for M₂ control (0 Gy).

The antimalarial activity of crude extracts from M₁ plants and those from M₂ plants (samples) obtained after irradiation was greater than the antimalarial activity of M₁ and M₂ controls. The values of IC₅₀ were ranged between 1.06 µg/ml (100 Gy) to 2.96 µg/ml (75 Gy) for crude extracts from M₁ plants and between 1.00 µg/ml (200 Gy) to 5.96 µg/ml (75 Gy) for crude extracts from M₂ plants (Fig. 1). The lowest antimalarial activity was thus observed for M₁ and M₂ crude extracts obtained after seeds irradiation at 75 Gy.

The same results were obtained on the *P. falciparum* K1 strain. For crude extracts from M₁ plants, the IC₅₀ were ranged from 1.12 µg/ml (M₁ control: 0 Gy) to 4.08 µg/ml (100 Gy). For crude extracts from M₂ plants, the IC₅₀ were ranged between 3.91 µg/ml (225 Gy) and 14.11 µg/ml (75 Gy). Antimalarial activity of control (IC₅₀ = 9.68 µg/ml) did not differ significantly from the other except that of 75 Gy obtained in the second generation (IC₅₀ = 14.11 µg/ml) (P>0.05).

According to the WHO guidelines on malaria treatment and previous data, antimalarial activity of a drug or plant extract could be classified as follows in table 6 (Lekana-Douki *et al.*, 2011).

Table 6: IC₅₀ (µg/ml) of extracts from *P. odontadenius* plants obtained by gamma-rays (Cs¹³⁷) irradiation seeds on *Plasmodium falciparum* isolats and *P. falciparum* K1 strain

	High (IC ₅₀ < 5 µg/ml)				Promising (5 < IC ₅₀ < 15 µg/ml)				Moderate (15 < IC ₅₀ <50 g/ml)		Inactive IC ₅₀ > 50 µg/ml	
Doses (Gy)	M1-PfK1	M1-PfIs	M2-PfK1	M2-PfIs	M1 PfK1	M1-PfIs	M2 PfK1	M2-PfIs	M1	M2	M1	M2
0	1,12	-		-	-	6,95	9,68	10,45	-	-	-	-
25	2,73	1,12		1,18	-	-	13,41	-	-	-	-	-
50	1,95	1,29		1,48	-	-	12,77	-	-	-	-	-
75	2,95	2,96		-	-	-	14,11	5,96	-	-	-	-
100	4,08	1,06		1,04	-	-	11,89	-	-	-	-	-
125	2,38	1,01		2,02	-	-	12,06	-	-	-	-	-
150	2,11	1,74		1,4	-	-	7,38	-	-	-	-	-
175	3,53	1,59		1,35	-	-	8,59	-	-	-	-	-
200	1,65	1,00		1,00	-	-	7,5	-	-	-	-	-
225	1,31	1,25	3,91	1,15	-	-	-	-	-	-	-	-
250	2,78	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
275	0,92	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Legend: M1-PfK1 : M1 Extracts on *Plasmodium falciparum* K1 ; M1-PfIs : M1 Extracts on *P. falciparum* isolats
M2-PfK1 : M2 Extracts on *Plasmodium falciparum* K1 ; M2-PfIs : M2 Extracts on *P. falciparum* sauvage;

ND: no determined

Regarding the table 6, crude extracts obtained from *P. niruri* M1 showed high activity against both the *P. falciparum* K1 strain and *P. falciparum* isolates with IC₅₀ < 5 µg/ml (1.12 µg/ml- 4.08 µg/ml) or 5 < IC₅₀ < 15 µg/ml. Plant extracts of *P. niruri* M2 obtained with a single dose of irradiation (225 Gy) showed high antimalarial activity, IC₅₀ = 3.91 µg/ml. All doses gave promising antimalarial activities (5 < IC₅₀ < 15 µg/ml) on *P. falciparum* K1 whereas with *P. falciparum* isolates, control and 75 Gy extracts gave promising antimalarial activities with IC₅₀ values 10.45 µg/ml and 5.96 µg/ml, respectively.

3.4. Phytochemical analysis of *P. odontadenius*

Phytochemical analysis indicates that different types or chemical groups of secondary metabolites are more present in the second generation than in the first one (Tables 4 and 5). Except for tannins, anthocyanins and polyphenols that were detected in M1 and M2 plants control and the absence of anthraquinones in M1 and M2 control plants and those obtained after irradiation of seeds in any samples, the presence of other major chemical groups in both M1 and M2 generations varied depending of irradiation treatment. Alkaloids were not detected in M1 plants but were detected in M2 to plants from irradiated seeds at 25, 50 and 75 Gy. By contrast, saponins were found only in M1 plants obtained after irradiation at 25, 50, 75, 100, 150 and 250 Gy. Finally, compounds like free quinones, flavonoids, terpenes and steroids were more found in M2 than in M1 (Tables 4 and 5).

Table 1: Effects of Gamma-Ray (Cs¹³⁷) on *in vitro* Irradiated Seeds Germination and seedling Growth (n=6)

Treatments Parameters	0 Gy	25 Gy	50 Gy	75 Gy	100 Gy	125 Gy	150 Gy	175 Gy	200 Gy	225 Gy	250 Gy	275 Gy	300 G)	Lsd à 5%	CV (%)
Percentage of germination (%)	43.85±11.17 a	35.18±18.82 ab	35.20±4.80 ab	36.69±19.76 ab	30.34±7.25 abc	17.32±13.65 cde	24.44±9.59 bcd	15.24±10.82 cde	10.37±4.34 de	7.06±1.22 e	10.73±3.69 de	14.02±2.79 de	12.11±3.28 de	15.20	37.95%
Emergence Reduction (%)	0	19.77	19.73	16.33	30.83	60.50	44.26	65.25	76.35	83.90	75.53	68.03	72.38	-	-
length (cm)	6.36±2.37 a	5.88±2.69 a	5.09±2.33 ab	5.81±1.90 a	5.78±2.2 a	3.62±1.51 bc	3.00±0.51 c	2.75±0.75 c	3.60±1.68 bc	2.83±0.99 c	2.81±0.99 c	1.81±0.86 c	0 d	1.682	44.94%
Leaves number/plant e	5.6±2.1a	4.9±1.6 abc	5.0±2.6 ab	5.3±1.6 ab	5.2±0.9 ab	5.0±1.3 ab	4.6±1.7 abc	4.0±1.1 bcd	3.0±1.1 d	4.2±1.2 abcd	3.5±1.2 cd	3.0±1.1 d	0 e	1.43	35.68%

For the same parameter, values with identical letters were not significantly different at 5%

Table 2: Effects of gamma-ray (Cs¹³⁷) on *P. niruri* growth in M1 generation (n=6)

Treatments Parameters	0 Gy	25 Gy	50 Gy	75 Gy	100 Gy	125 Gy	150 Gy	175 Gy	200 Gy	225 Gy	250 Gy	275 Gy	300 G)	Lsd à 5%	CV (%)
Length (cm) for 4 months	9.34±4.38 bc	10.96±4.56 b	14.01±5.12 a	8.84±3.76 bc	9.31±3.65 bc	8.55±3.60 c	7.86±3.39 c	8.80±4.28 bc	9.01±4.33 bc	9.90±4.22 bc	7.94±3.42 c	7.61±3.42 c	3.60±0.28 d	4.741	15.18%
Collar diameter (mm) for 4 months	2.01±1.09 abc	2.23±1.24 ab	2.68±1.07 a	1.87±0.70 bc	1.95±0.87 abc	1.75±0.69 bcd	1.83±0.75 bcd	2.00±1.08 abc	1.83±0.75 bcd	1.93±0.83 abc	1.92±0.65 abc	1.39±0.61 cd	1.10±0.14 d	0.782	23.33%
Seeds Weight (mg) (Number/plts)	70.32±17.30 f (234±58)	166.1±24.60 b (554±62)	174.3±48.32 b (581±161)	115.2±25.78 de (384±86)	8107±2042 ef (270±68)	167.5±37.33 b (558±124)	159.9±13.14 bc (533±44)	125.4±35.14 cd (418±117)	137.0±16.37 bcd (457±55)	214.6±54.72 a (715±182)	26.75±7.78 g (89±26)	82.00±20.78 ef (273±69)	29.60±0.99 g (99±0)	39.51	22.33%
Biomass (g)	21.00±5.17 b	11.17±1.64 d	29.60±8.19a	3.43±0.7f	10.63±2.68 d	9.26±2.06 de	10.17±0.84 d	10.52±3.14 d	13.40±1.60 cd	17.00±4.34 bc	4.42±1.29 ef	10.87±2.76d	2.00±0.1 f	4.943	28.22%

For the same parameter, values with identical letters were not significantly different at 5%

Table 3: Effects of gamma-ray (Cs¹³⁷) on *P. niruri* growth in M2 generation (n=30)

Treatments Parameters	0 Gy	25 Gy	50 Gy	75 Gy	100 Gy	125 Gy	150 Gy	175 Gy	200 Gy	225 Gy	Lsd à 5%	CV (%)
Lenght (cm)	22.06±6.91 a	15.59±6.13 b	12.60±4.65 bc	12.58±4.50 bc	12.78±6.04 bc	13.28±6.45 bc	11.83±5.76 c	10.58±4.23 c	11.62±7.99 c	12.44±7.53 bc	3.633	42.78%
Collar Diameter (mm)	2.03±0.53 a	1.71±0.38 b	1.44±0.39 cd	1.59±0.39 bc	1.23±0.47 de	1.33±0.39 d	1.33±0.44 d	1.03±0.31 e	1.23±0.56 de	1.25±0.45 de	0.2979	38.98%
Number of branches	18.58±7.16 a	14.08±3.60 bc	12.04±5.33 bcd	14.27±4.68 b	12.31±4.61 bcd	10.27±3.32 d	11.31±4.32 cd	10.04±4.06 d	10.08±5.09 d	10.31±4.35 d	2.810	36.33%
Biomass (g)	1.96±0.76a	1.48±0.63b	1.16±0.73 bcd	1.37±0.61bc	1.07±0.77 cde	0.97±0.58 def	0.89±0.56 def	0.73±0.62 ef	0.68±0.73 f	0.85±0.69 def	0.389	55.64%

For the same parameter, values with identical letters were not significantly different at 5%

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

Chemical groups	Doses treatment (Gy)											
	0	25	50	75	100	125	150	175	200	225	250	275
Alkaloids	—	—	—	—	—	—	—	—	—	—	—	—
Saponins	—	+	+	+	+	—	+	—	—	—	+	—
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Anthocyanins	+	+	+	+	—	+	—	+	+	+	+	+
Free Quinones	—	—	+	—	—	—	—	—	+	—	+	+
Flavonoids	+	—	—	—	—	+	—	+	—	+	—	+
Terpenoids and Steroids	+	+	—	—	—	—	—	+	+	+	—	+
Anthraquinones	—	—	—	—	—	—	—	—	—	—	—	—
Polyphenols	+	+	+	+	+	+	+	+	+	+	—	+

+ : found; —: not found

Table 5 : Phytochemical screening of *P. odontadenius* crude extracts from M2 Plants

Chemical group	Doses treatments (Gy)											
	0	25	50	75	100	125	150	175	200	225	250	275
Alkaloids	—	+	+	+	—	—	—	—	—	—	—	—
Saponins	—	—	—	—	—	—	—	—	—	—	—	—
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Anthocyanins	+	+	+	+	+	—	—	—	—	+	+	+
Free Quinones	—	+	+	—	+	+	+	+	+	—	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids and Steroids	+	+	+	—	+	+	+	+	+	+	+	+

Anthraquinones	—	—	—	—	—	—	—	—	—	—	—	—
Polyphenols	+	+	+	+	+	+	+	+	+	+	+	+

+ : found; —: not found

DISCUSSION

On isolates *P. falciparum*, all extracts M1 from irradiated seeds have higher antimalarial activity against *P. falciparum* with the exception of those of control with 6.95 µg/ml who exceeded 5 µg/ml and has moderate antiplasmodial activity (Lekana-Douki *et al.*, 2011). In M2, except the control (0 Gy) and 75 Gy those have IC50 exceeding 5µg/ml with 10.45 and 5.96 µg/ml and were presented moderate antimalarial activity, all extracts from irradiated seeds presented higher antimalarial activities against *P. falciparum* with their IC50 low than 5µg/ml.

Absence or presence of one or another group of secondary metabolites in samples from M2 seeds could be explained by changes at the molecular level that would be induced by the energy of radiation given during the irradiation of the seeds. Some genes are sensitive and others were not sensitive. Certain genes would be sensible and the others never because of the sequence reparations on DNA damages or the presence of redundant genes can be explain this phenomena (Etienne *et al.*, 2006 ; Cain *et al.*, 2006 ; Bernot, 2001). It knows that punctual mutation request two respective cycles of replication for their definitive fixation. Their effectiveness was possible in the second cycle of replication in the moment that the incorrect pair of DNA produce definitive change in the sister molecules. (Maftah *et al.*, 2007).

From the above, the results obtained in this work confirm the hypothesis that secondary metabolites are stimulated by both abiotic (irradiation and various chemical compounds) and biotic elicitors groups (glycoproteins, molecules derived from microorganisms and polysaccharides) (Moghaddam *et al.*, 2011). The gamma rays interact with atoms and molecules to create free radicals which are capable of altering the major components of plant cells. These radicals affect morphology, anatomy, biochemistry and physiology of the plant according to the irradiation dose.

From 100 Gy dose, there is presumption of probable and obvious mutations or changes because emergency reduction from 100 Gy were upper 30% (Maluszynski *et al.*, 2009). Our results are consistent with those of Sakin and Sengar (2002) who obtained a high frequency of mutations with durum wheat (*Triticum durum* L.) to 100 Gy for Sofu cultivar.

Studies have shown that parameters such as the rate of seed germination, shoot length, root and seedling are affected by irradiation and chemical mutagen. For example, Dehpour *et al.*, (2011) showed that the rate of seed germination and plant height (stem or root) decreased when seeds of rice (*Oryza sativa* L.) were treated with gamma radiation and sodium chloride (NaCl). These observations were confirmed by Dhakshanammorthy *et al.*, (2010) on *Jatropha curcas* L. using mutagens (EMS and gamma rays). Khan *et al.* (2009) reported that sodium azide (NaN₃) affects the rate of seed germination, shoot length, root and delays germination. All the parameters decrease with increasing doses of irradiation or with increasing chemical mutagen concentrations. Then, as the survival of mature plants depends on the nature and extent of chromosomal damage (Koing *et al.* 2008; Borzouei *et al.* 2010), the increasing frequency of chromosomal damage associated with increased doses may be responsible for poor germination and reduced size and survival of plants.

Doses causing an emergency reduction of seeds or a reduction in size of plants by over 30% compared to the control are considered to be high for a large-scale program for mutation of plants with high probability to obtain mutants (Maluszynski *et al.*, 2009).

Survival rate of plants is significantly reduced and the lowest rate (10%) was observed at 300 Gy irradiation doses. Witness presented 100% survival of plants followed those of 125 Gy and 175 Gy 46.67% to 46.15%.

Effects of gamma irradiation varied according to whether they could be positive, increasing an existing character for such dose, or negative, decrease or disappear to a character that existed for such another dose. Akhar *et al.* (2011) show that the best plant survival of chickpea (*Cicer arietinum* L.) was observed at 180 Gy. Gray (1990) observed that the decrease in survival of irradiated plant material resulting in a physiological imbalance at various events affecting vital cellular macromolecules. Skoog (1935) and Smith and Kersten (1942) cited by Ramesh *et al.* (2012) noted that the lower survival rate of plants after gamma irradiation is attributed to the destruction of auxin.

In contrast to the stimulation of the size observed with increasing doses by Shah *et al.* (2008) for two varieties of chickpea (*Cicer arietinum* L.), *P. odontadenius* showed in this study, a decrease in size *in vitro* and *in situ*, in the second generation, when the plants are subjected to a continuous shade. Average plant height of *P. odontadenius* showed some higher values regardless of the growth rates *in situ*. Our results are consistent with those obtained by Al-Qurainy (2009) in the analysis of the effects of sodium azide (NaN₃) on growth and yield of *Eruca sativa* L. for the 30th and 45th day. Comparing our results with those obtained by the same authors in the 90th day, they do not correspond to those found with *E. sativa*.

In vitro control plantlets showed higher mean values for the rate of seed germination, rate of seedling survival and for the seedling average number of leaves than of all average rates of third parameters for plantlets from irradiated seeds. Parameters of *P. odontadenius* plant transferred *in situ* showed high average values of height, number of seeds per plant, collar diameter and biomass average for plants from irradiated seeds indiscriminately compared to control plants. Many authors agree that observed characteristics are also dependent on environmental factors (Khan *et al.*, 2005; <http://www.thecanadianencyclopedia.com/> July 28, 2011) because the phenotype is the sum of factors genetic and environmental.

The effects of size and collar diameter have been reported previously by Bounias (1972) on the primary and mutagenic effects of gamma irradiation on *A. thaliana* seeds. These observations confirm the theory that the first generation generally have a higher rate of heritable traits than the following for which the expression of these traits results from genetic and external conditions (Sakin and Sengar, 2002; Khan *et al.*, 2005). <http://www.thecanadianencyclopedia.com/> Op.Cit July 28, 2011).

However, the quantity of biomass per plant and fertility tend to decrease as the radiation dose increases. The decrease in biomass could be explained by 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). Ramesh *et al.* (2012) explains that the abnormal leaf growth (biomass) is due to disruption of pigments (e.g., chlorophyll), the chromosome aberration and inhibition of mitotic division.

Radiation with ionization of molecules especially water around the DNA molecule are responsible for the production of free radicals (ROS) attacking DNA molecules that could know the changes. These free radicals (RO •, HO •, H •, R •, ...) can damage or modify important components of plant cells and affect different morphology, anatomy, biochemistry and physiology of the structure plant cell

according to the irradiation doses ([Hopkins, 2003](#); [Hortathova et al., 2007](#); [Borzouei et al., 2010](#)).

Free radicals attack membranes that surround and protect cells, destabilizing and accelerating deterioration of cells and tissues. But others against, promote the formation of disulfide bonds in proteins by destabilizing conformations and structures of proteins, thus destroying their metabolic functions in the body responsible. As for DNA, free radical interact with the DNA molecule and alters their structure, it could well lead to mutations or death descendants of a lineage ([Gicquel, 2012](#)).

These disturbances are responsible for some of the negative effects expected by the treated plant material to gamma radiation ([Radfar et al., 2012](#); [Sarduie-Nasab et al., 2010](#); [Sakin, 2002](#)). They include changes in cell structure and metabolism of the plant, for example, dilatation of thylakoid membranes, alteration of photosynthesis or modulation antioxidant systems and accumulation of phenolic compounds ([Kovac's and Keresztes: 2002](#), [Kim et al., 2004](#)).

Effects of mutation were more pronounced in M1 than of M2 generation comparing to the control. Results obtained by [Shabnam et al. \(2011\)](#) assessment of changes in oil yield and oleic acid after gamma irradiation of Guizote (*Guizotia abyssinica* (L.f.) Cass) reported that M1 plants show considerable changes in their different characters. They showed that the composition of the seed oil guizote is 34.60% for the control, from 43.50 to 47.60% for M1 and 39.70 to 44.70% for M2. It is 28.10% for the control, from 43.72 to 58.21% for M1 and 43.10 to 53.34% for the M2 composition of oleic acid.

[Bhosle and Kothekar \(2010\)](#) confirm that seeds M1 of beans (*Cyamopsis tetragonoloba* (L.) Taub) produced more lethal and effects of pollen sterility while in M2, it are more thoroughly, the presence of various viable chlorophyll mutations. However, mutagenic effects increase both M1 in M2 with increasing doses of mutagenic ([Makeen and Babu, 2010](#)). [Ando and Montalván \(2001\)](#) show that sodium azide (NaN₃) gives more pronounced effects at 0.5 mM concentration than 0.1 mM only when the rice (*Oryza sativa* L.) is soaked in sodium azide. These results confirm the hypothesis that effects of irradiation are more pronounced in the first generation compared to the second ([Bhosle and Kothekar, op](#); [Shabnam et al. op](#)).

From the above, our results on phytochemical analysis confirm the hypothesis that secondary metabolites were stimulated by both abiotic (irradiation and various chemical compounds) and biotic (glycoproteins, molecules derived from microorganisms and polysaccharides) elicitors groups ([Moghaddam et al., 2011](#)).

Γ-rays interact with atoms and molecules to create free radicals which are capable of altering the major components of plant cells. These radicals affect morphology, anatomy, biochemistry and physiology of the plant according to the irradiation dose. [Hopkins \(2003\)](#) give four main groups of secondary metabolites in plants including terpenoids for the first group, phenolic compounds for the second group, Saponins, cardiotonic heterosids and cyanogenic heterosids and glucosinolates for the third group and alkaloids the fourth. These four groups are found in varying amounts in plants of *P. odontadenius* derived from irradiated seeds.

Flavonoids and other phenolic compounds (tannins, free quinones, anthocyanins and polyphenols) were prominently reported previously. [Kähkönen et al. \(1999\)](#) cited by [Zhang et al. \(2010\)](#) reported that phenolic compounds have antioxidant activities, allowing them to protect plants against damage caused by radiations. Flavonoid synthesis can be explained by the activity of phenylalanine amino-lyase (PAL), which, as a response to radiation (gamma and UV) in alleviating the damage caused by it ([Moghaddam et al., 2011](#)).

With the presence of flavonoids in all samples of M2, our results confirm those of [Lois \(1994\)](#) cited by [Moghaddam et al., 2011](#) which showed that irradiation of the Arabet plant (*Arabidopsis thaliana* of the Brassicacaceae family) increased the level of flavonoids which are accumulated in the aerial parts of the plant. Besides flavonoids, our results show that the level of alkaloids, free quinones, terpenes and steroids and anthocyanins increased.

Presence of alkaloids in M2 and their absence to M1 generation could be explained by the effect of gamma radiation on cellular proteins that release amino acids such as lysine, phenylalanine, tyrosine, tryptophan and ornithine which are the starting point for the biosynthesis of alkaloids ([Hopkins, 2003](#)). It could be the same even for terpenoids and steroids derived from acetyl-CoA pathway, this latter come from to a disorder of the Embden-Meyerof way.

Our results on antimalarial activity obtained in this work, M1 and M2 appear low values of IC_{50} in all, to say that high antimalarial activities, compared to those obtained by [Soh et al. \(2009\)](#) on the antimalarial activity of *P. niruri* (certainly *P. odontadenius* or *P. amarus*) collected at three different sites in the Democratic Republic of Congo (DRC). The best antimalarial activity was the one with the aqueous extracts from stems of Kisantu's *P. niruri* with IC_{50} value of $11 \pm 2 \mu\text{g/ml}$, whereas we obtained $IC_{50} < 11$ except for doses of 25, 50, 75, 100 and 125 Gy in M2.

In vitro antimalarial activities from crude extracts of *P. odontadenius* with high doses of gamma rays can be explained by the increase in total phenolics and specific compounds such as coumarin, p-coumaric acid and salicylic acid unlike what [El-Hamid \(2010\)](#) found on the biosynthesis of phenolic compounds in seedlings culantro gamma irradiated at low doses.

Control M1 crude extract showed good antiplasmodial activity on *P. falciparum* chloroquino-resistant exceeding all samples. This exceptional antiplasmodial activity can be explained by original compounds found in *P. odontadenius* or by phenolic compounds produced during storage which give place on enzymatique reactions ([Macheix et al., 2005](#), [Cimanga et al., 2004](#)). It known that effects of mutation were more pronounced in M1 than of M2 generation comparing to the control [Shabnam et al. \(2011\)](#).

CONCLUSION

Radiation of seeds of *P. odontadenius* induced a reduction of the size, collar diameter, number of leaves and biomass in M1 and M2 plants grown *in vitro* and *in situ*. These parameters decrease linearly with increasing irradiation doses with a more pronounced effect in the first generation than in the second one. Synthesis of secondary metabolites increase in the second generation compared to the first one with a more important synthesis in phenolic compounds (including flavonoids) than in alkaloids and terpenoids.

Antimalarial activities of M1 crude extracts of *P. odontadenius* were higher than that of M2 crude extract. For M1 crude-extracts, the highest activity was observed at 100 Gy while for M2 crude-extract, the best antimalarial activities were observed at 50, 175, 200 and 225 Gy.

In conclusion, gamma-irradiation of *P. odontadenius* seeds at dose of 50 and 100 Gy seem the best choice with regard to the development of the plant and the production of active secondary metabolites. We need to test, however, whether the plant extracts from these doses would also provide high antimalarial activities and

lower toxicity on human cells. These studies are essential for plant breeding of *P. niruri* L. with antimalarial activities improving.

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