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Research Article

Correlation between Antioxidants and Antiradical Activities with *In Vitro* Antimalarial Activity of *Phyllanthus odontadenius*

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Abstract

This work aims to bring out the correlation between antioxidants and antimalarial activities of secondary metabolites of Po M2 plants (flavonoids, tannins and phenolic compounds contents) and their *in vitro* antimalarial activity by antioxidant activities justification. Field experiments were conducted both.

Seeds from to one plant of *Phyllanthus odontadenius* were irradiated with 150 Gy dose (M1 seeds) and cultured first *in vitro* following transfer of M1 plants in field. Seeds from M1 plants were cultured for obtaining M2 plants which are used as biological material in this work after their harvest. In addition, phytochemical screening was carried out using reagent methods combined with spectrophotometric methods revealed the presence of flavonoids, tannins and phenolic compounds. The *in vitro* antimalarial activity assays on isolate *Plasmodium falciparum* were determined using microscopic method revealed that the highest in vitro antimalarial activity was extracted from 1503 plants (1.43 ± 0.17 µg/mL) justified by the highest of tannin content (0.76 ± 0.13 mg/g) and ratio between IC_{50} from DPPH and flavonoids quantity (15.52%) and that from DPPH and total phenolic compounds (5.17%). However, ratio between IC_{50} from DPPH or ABTS radicals and total phenolic contents were low than these of IC_{50} from DPPH or ABTS and flavonoid contents for each plant extract. Evaluation of extracts antioxidant activity was determined using DPPH and ABTS as radicals.

This work confirm that there is correlation between *in vitro* antimalarial activity and antioxidants and antiradical activities of secondary metabolites of P.o. M2 plants (flavonoids, tannins and total phenolic compounds). When IC_{50} for DPPH test is high, *in vitro* antimalarial activity is also high, and, in opposite, when IC_{50} for ABTS is low, *in vitro* antimalarial activity is high.

Keywords: Antioxidant Activity; Antiradical Activity; Antimalarial Activity; Phyllanthus Odontadenius; Irradiation; Gamma Rays

Introduction

Historically, plants have been source of inspiration for new drug compounds. Thus, plant-derived medicines have made an important contribution to human health. According to the World Health Organization (WHO) in 2008, over 80% of the world's population relies on traditional medicine for their primary health care needs (Pierangeli and Rivera, 2011). Many different natural substances have been identified and many of them have been used in traditional medicine for the prophylaxis and treatment of diseases.

However, the evaluation of phytotherapeutic properties as an antioxidant remains an interesting and useful task, especially for plants of rare or less frequent use or not known in medicine and medicinal traditions. Oxidative stress is the disproportion between

oxidants and antioxidants in favor of oxidants potentially leading to damages. Reactive Oxygen Species (ROS) are a class of compounds that are formed from oxygen metabolism. These molecules such as, hydroxyl radical ($OH^.$), peroxide ($ROO^.$) and superoxide radicals ($O_2^.$), can cause severe damage to cells and tissues during various diseases which are linked to heart diseases, carcinogenesis and many other health issues [1,2].

The use of synthetic antioxidant molecules such as BHA (butylated hydroxyl anisole), PG (propyl gallate) and BHT (butylated hydroxyl toluene) is currently being questioned because of toxicological potential risks [2]. Now, new plant sources of natural antioxidants are sought [3,4]. Indeed, polyphenols are natural compounds widely used in the plant domain and which have an increasing importance thanks to their beneficial effects on human health [5]. Their role as natural antioxidants is generating increasing interest in prevention and treatment of cancer, inflammatory diseases and cardiovascular diseases (Suhaj, 2006). They are also used as additives in food, pharmaceutical and cosmetic products [3].

Phyllanthus odontadenius is one of the most used plants in traditional medicine both in the Democratic Republic of the Congo and elsewhere. Phytochemical studies performed on its total extracts revealed presence of many chemical compounds such as: polyphenols; flavonoids; coumarins, tannins, etc. [6,7]. Several phytochemical and pharmacological studies attest to its success in therapy: extracts from roots, twigs and leaves reveal antispasmodic, anti-cancer, antiplasmodial, anti-dysenteric, antifungal, analgesic and other properties [8].

Plants from irradiated seeds by gamma radiation (Cs-137) showed an improvement of antimalarial activities than wild plants due to changes of secondary metabolites [9]. However, no study has been conducted on the antioxidant activity of *P. odontadenius* plants obtained with seeds irradiated by gamma rays. Hence, this work was conducted to correlate the *in vitro* antimalarial activities and the antioxidant activities of *P. odontadenius* plants with their secondary metabolites.

The objective of this work is firstly to carry out chemical screening of *P. odontadenius* plants in order to establish a relationship between secondary metabolites found (i.e. total phenolic, flavonoids, tannins, etc.) and their pharmacological properties, such as antimalarial activity. Secondarily to correlate the antioxidant activity of extracts by DPPH and ABTS radicals' method and to quantify plant compounds obtained.

Materials and Methods

Plant materials

Phyllanthus odontadenius plant extracts from irradiated seeds by gamma rays (Cs-137) were used as biological materials. Seeds from of one feet of *P. odontadenius* plant were irradiated with gamma rays from Cesium – 137 (Cs-137) source in the Conservatome Irradiator Lisa I at the Department of Biochemistry in the CGEA/CREN-K. The dose rate was 1.21 Gy/min [10-12]. and the treatment doses ranged from 150 to 200 grays (Gy) with 25 Gy of step. Thus, seeds obtained from irradiated seeds were designed as M1. The *in vitro* germination of seeds was conducted in Petri dishes after seeds treatment respectively in ethanol solution (70%) for 1 min, Mercuric chloride solution (0.125%) for 3 min and washed with sterile distilled water. Seeds were then handled with gibberellic acid (GA3, 200 mg/mL) and finally cultivated on Murashige and Skoog (MS) basal media without sucrose or growth regulators and supplemented with 0.8% agar [13,14]. After germination, rate of seed germination was determined [12] and plantlets from Petri dishes were transferred in polyethylene bags containing some grams of soil for *in situ* growth in the ground in randomized complete block [15]. The plantlets were watered three times a week and harvested after four months.

Seeds from M1 generation plants were used in the same way for obtaining of second generation (M2) plants. M2 plants from 150 Gy (Po-150) used exactly as biological material for phytochemical screening, antiplasmodial activity and antioxidant activity.

Phytochemical analysis

Preparation of crude extracts

In each case, powdered air-dried aerial plant material was extracted with methanol. Ten% maceration in methanol (10 g/100 mL) for 48 h at room temperature was performed. After filtration, the filtrate was evaporated completely to dryness in an oven (40°C). A solution of Ten mg/mL of each extract were prepared for thin layer chromatography (TLC) qualitative assays, whereas 1 mg/mL solutions for quantitative assays and 10% aqueous and chloroform solutions for chemical screening by solution reactions.

Phytochemical screening

The chemical screening was carried out to identify chemical constituents of all crude extracts. Alkaloids were detected with

Bouchardât, Draggendorff's and Mayer's reagents [16]. Flavonoids were detected using Shinoda's reagent or cyanidine reagent [17]. Saponins were detected by the foam test [16,18]. Presence of tannins was detected using ferric chloride 1% reagent, Stiasny reagent and the Bate-Smith test [16,19,20]. Anthraquinones were detected using Bornträger's reagent [16]. Anthocyanins were identified using HCl 20%, heating first before addition of isoamylic alcohol [16]. Steroids and terpenoids were identified using Liebermann-Bouchard's reagent [16].

The presence of flavonoid and terpen-steroid was confirmed by TLC performed on silica gel plates 60F254 10 x 10 cm. Flavonoids were detected using AcOEt/CH3COOH/HCOOH/H_2O (100/11/11/27) as mobile phase with NEU's reagent (1% diphenylboric acid ethanolamine complex, methanolic solution 1%). Steroids and terpenoids were detected using chloroform/ethyl acetate mixture (20/30, v/v) and n-Hexane/MeOH (9/1) as mobile phases and Liebermann-Bouchard's reagent. After drying, the TLC plates heated at 105°C for 10 min to intensify the spot colors [16,21].

Quantitative analysis of compounds

The content of flavonoids in plant extracts was determined spectrophotometrically according to Quettier., *et al.* [22]. Methanolic solutions (1 mg/mL) for each plant extracts were added with 1 mL methanolic solution of AlCl3 (2%) and the whole was well stirred. After the incubation for 1 h at room temperature and protected from light, absorbance was determined by the spectrophotometer at 415 nm on a spectrophotometer *Thermo Genesys* 10S UV-VIS. Mixtures were prepared in triplicate for each analysis and average values are reported. The same procedure was done for the standard solution of quercetin in nine different dilutions (ranging from 0.4 to 200 µg/mL). Quercetin solutions allowed establishment of the calibration line establishment. The blank solution was prepared with 1 mL of MeOH. The content of flavonoids in each extracts was expressed in mg equivalent of quercetin per gram of the dry extract of corresponding plants.

Polyphenol assays were performed using method described by Gutfinger [23] using Folin-Ciocalteu reagent. To 0.5 mL of each methanolic extract (1 mg/mL), 5 mL of distilled water and 0.5 ml of Folin-Ciocalteu reagent were added. After 3 minutes, 1 mL of saturated solution of Na_2CO_3 (20%) was added. The mixture was then stirred and incubated at room temperature and protected from light for 1 h. Absorbances were read at 725 nm on a spectrophotometer Thermo Genesys 10S UV-VIS. Each assay was repeated three times. The same procedure was followed for different dilutions of gallic acid solution (ranging from 5 to 150 µg/mL) in order to get the calibration line. For white, the plant extract was replaced by MeOH. The quantity of total polyphenols is expressed in mg equivalent of gallic acid per g of dry extract of corresponding plants.

Tannins were determined using the method of Dawra., *et al.* (1988) reported by Soetan [24] using the SPECTRONIC 21D MILTON ROY as spectrometer. 0.2 g of each plant powder was weighed and soaked with solvent mixture (80 ml of acetone and 20 ml of glacial acetic acid) for 5 h to extract tannin. The filtrates were removed and the samples were filtered through a double layer filter paper to obtain the filtrate. A set of standard solution of tannic acid was prepared ranging from 0 to 10 ppm. The absorbances of the standard solution as well as the filtrates were read at 720 nm.

In vitro antimalarial activity

Antimalarial activity assays were performed at the National Institute of Biomedical Research (NIBR) in Kinshasa/Gombe (DR Congo). Solutions of *P. odontadenius* extracts prepared in 1% DMSO and diluted successively two fold in succession to have control concentrations. Clinical isolates of *P. falciparum* were obtained to Hospital of Marble Palace Center in Kinshasa/Ngaliema from symptomatic malaria children (0-5 year old) with high parasitaemia; relative abundance of parasites estimated at more than ten trophozoites by microscopic field or four crosses (++++) according to WHO and the National Malaria Control Program (NMCP). Blood sample was taken from, children who did not receive antimalarial treatment in the previous three weeks.

Venous blood samples (5 mL) were collected in EDTA tubes, and centrifuged for 5 min at 3000 rpm in Thermo Scientific HERAEUS LABOFUGE 200 for separation of plasma and erythrocytes. Isolates of *P. falciparum* was maintained in culture following Trager and Jensen [25] method in RPMI 1640 medium containing 25 mM HEPES, 25 mM sodium bicarbonate and 10% of pooled human serum.

After homogenization, 50 µg of suspension were distributed in each well of a spot plate containing decreasing concentrations of half extracts. Plates were then maintained at 37°C in humid atmosphere containing 5% CO_2 for 48 h. Quinine was used as negative control. Thin smears were made and stained with GIEMSA 5%. After 48 h of incubation, under laminar flow, the thick drops are prepared and dried for 24 h then, they are examined at the 100x objec-

tive with binocular microscope OLYMPUS CX21 [26,27]. Inhibition of parasitaemia (in percent) was calculated as follow:

Inhibition (%) = (A – B/A) x 100, where A is the parasitaemia in the negative control and B, the parasitaemia in the treated plate bucket. The IC_{50} of each sample was obtained using the dose-response curves.

Evaluation of antioxidant activity

DPPH assay

The scavenging activity of DPPH free radical by methanol extracts was evaluated by TLC and spectrophotometrically.

Qualitative evaluation

5 µL of each plant extract (10 mg/mL) and 2 µL for reference substances (1 mg/mL methanol solutions) such as rutin (RUT), caffeic acid (K), chlorogenic acid (C), quercetin (QT) and hyperosid (HYP) were spotted on TLC plate. The used mobile phase was: AcOEt/CH3COOH/HCOOH/H_2O (100: 11: 11: 27; v/v) as eluent. After migration, the air-dried plates were spread with with a 0.02% DPPH solution. Appearance of yellow or purple spots in light of day reflects anti-radical activity of extracts against DPPH.

Quantitative evaluation

The radical DPPH (2,2-diphenyl-1-picrylhydrazyl) is colored violet. The addition of antioxidants will reduce this radical and cause the discoloration of the mixture, which is measured by spectrophotometry at 517 nm and is proportional to the concentration of antioxidants. This method is generally standardized with compounds such as caffeic acid, gallic acid, quercetin etc. [28,29].

To a 0.1 mL of each methanolic extract was added to 1 mL of the methanolic solution of DPPH (100 µM). The mixture was then kept away from light at room temperature for 30 min and the absorbance was measured at 517 nm, using MeOH as blank. Preparations of the samples and the control (quercetin) are carried out under the same operating conditions. 3 repeats are made for each concentration. The decrease in absorbance is measured spectrophotometrically and the% IP (inhibition pourcentage) is calculated according to the following formula.

Pourcentage of inhibition = [1- (Ax/Ac)] × 100; where Ax: Absorbance of the ABTS radical in the presence of the extract and Ac: Absorbance of the ABTS radical in the presence of methanol (Control) [30].

ABST test

The blue-green colored ABTS + • was produced by mixing ABTS diammonium salt (0.35 mL, 7.4 mmol/L) with of potassium persulfate (0.35 mL, 2.6 mmol/L). The mixture was kept in the dark at room temperature for 16 h to allow completion of radical generation, and then diluted with methanol (about 1:60) so that its absorbance at 734 nm was 0.70 ± 0.02 measured on a spectrophotometer Thermo Genesys 10S UV-VIS.

The addition of an antioxidant compound causes the reduction of the radical ABTS +• in ABTS. The antioxidant activity was determined by the discoloration of the solution and is expressed as inhibition percentage (PI) of the absorbance at 734 nm, wavelength at which the radical ABTS + • has a characteristic absorption band [31,32]. Decolorization of the radical is measured spectrophotometrically at 734 nm and is proportional to the concentration of antioxidants [3,6,33].

Briefly, 0.3 mL of each plant extract and 0.3 mL of MeOH as a blank were added 1 mL of a dilute solution of ABTS. The absorbance was recorded at 734 nm after 30 min of incubation in the dark at room temperature. The measurement was done in triplicate for each concentration. The reducing power of the extract is compared with that of quercetin and the percentage inhibition is calculated using the following formula:

Pourcentage of inhibition = [1- (Ax/Ac)] × 100; where Ax: Absorbance of the ABTS radical in the presence of the extract, Ac: Absorbance of the ABTS radical in the presence of methanol (Control). These two tests make it possible to highlight the anti-radical power of a pure antioxidant or an antioxidant extract.

The data were analyzed statistically using Microsoft Excel and Origin 6.1 software.

Results

Development characters of irradiated plants

In vitro seeds germination of *P. odontadenius*

The rate of germinated seeds observed after 3 weeks is illustrated in figure 1

Seeds from Po.1504 showed high rate of germination (40.74 ± 13.26%) compared to the control seeds (0 ± 0%), Po.1503 (14.51 ± 7.57%) and Po.1505 (18.56 ± 12.07%). Using T-student test, the rate of Po.0 differs significantly with Po.1504 ($P < 0.05$) and

germination percent of Po.1504 differs significantly with those of Po.1503 and Po.1505 ($P < 0.05$). But no significant difference was observed between Po.0 and Po.1503, and between Po.0 and Po.1505.

Weight of M2 plant seeds

The weight of 100 M2 plant seeds obtained after harvesting is illustrated in figure 2.

Figure 1: Rates of M2 seeds *in vitro* germination (Po.0: Plants from unirradiated seeds, Po.1503, Po.1504, Po.1505: M2 seeds from M1 plant number (3, 4 and 5) out coming seeds irradiated at 150 Gy doses.

Figure 2: Weight of 100 seeds of M2 plants.

In Figure 2, Po.1503 showed high weight of 100 seeds in comparison to Po.1504 and Po.1505. But, no significant difference was observed statistically using F test ($P < 0.05$, F-Cal (0.20) < F-Tab (3.58)) between respective values of weight for 100 seeds (Po.1503 = 31.4 ± 4.98 mg, Po.1504 = 27.6 ± 4.04 mg and Po.1505 = 29.0 ± 4.18 mg).

Biomass of M2 *P. odontadenius* plants

The fresh biomass (g) after harvesting plants is shown in the figure 3.

Figure 3: Weight of fresh biomass of M2 Plants.

The Biomass (g) of *P. odontadenius* plants showed high value for Po.1504 (7.38 ± 3.13 g) follow by Po.1505 (6.52 ± 2.21 g) and Po.1503 (4.57 ± 1.55 g). Statistically, no significant difference was observed between these values of biomass ($P < 0.05$) using F test.

Phytochemical analysis

The phytochemical analysis includes qualitative and quantitative aspects.

Phytochemical screening

The results of Phytochemical screening of M2 *P. odontadenius* plants are illustrated in table 1.

TLC phytochemical analysis

TLC profiles of the flavonoid analysis are given in figures 4a and 4b; Rutin (Rf = 0.4) and quercetin (Rf = 0.99) were used as standards. The phytochemical study also revealed the presence of terpenes - steroids in chloroformic plant extracts, as shown in Figure 4c.

Tannins contents

Figure 5 show quantities of tannins in plant extracts obtained spectrophotometrically.

Figure 4: TLC developed in the mixture AcOEt/CH3COOH/HCOOH/H_2O (100/11/11/27) for flavonoids analysis and revealed by the Neu reagent; a: plate examined in the day light and b: plate examined under UV lamp at 366 nm; c: TLC made in the chloroform/ethyl acetate mixture (20/30, v/v), visible light evaporation and UV lamp 366 nm, after vaporization of the sulfuric anisaldehyde reagent on the plate, followed by heating in oven at 105 °C for 3 min.

Chemical groups	1503	1504	1505
Alkaloids	+	+	+
Flavonoids	+	+	+
Gallic Tannins	+	+	+
Catechic Tannins	-	-	-
Quinons	-	-	-
Anthocyanins	+	+	+
Leucoanthocyanins	-	-	-
Diterpenoids	+	+	+
Triterpenoids	+	+	+
Saponins	-	-	-

Table 1: Chemical screening of M2 *P. odontadenius*

Legend: + = present, - = absent

Regarding the table 1, no difference between plant groups was observed, the same phytochemical compounds were obtained. Alkaloids, flavonoids, gallic tannis, anthocyanins and terpenoids were presents. Catechic tannins, quinons, leucoanthocyanins and saponins were absents in all plants.

Po.1503 with 0.76 ± 0.13 mg/g showed higher tannic contents than Po.1504 (0.57 ± 0.07 mg/g) and Po.1505 (0.56 ± 0.04 mg/g). Statistically, no significant difference was observed between these values of treatment ($P < 0.05$) using F test [F-Cal (0.29) < F-Tab (4.39)].

Figure 5: Quantities of tannins in extracts from M2 *P. odontadenius* plants.

Flavonoid and total phenol contents

Figure 6 present flavonoid and total phenolic contents of methanolic extracts of M2 *P. odontadenius* from seeds of M1 plants and those from natural *P. odontadenius* plants (Po. Nat.). The calibration curves obtained with quercetin at 415 nm and gallic acid at 725 nm both used as standards, were respectively: $y = 0.016x + 0.324$ with R2= 0.994 and $y = 0.006x – 0.013$ with R2 = 0.996.

Figure 6: Flavonoid and total phenolic contents of methanolic M2 *P. odontadenius* extracts.

In figure 6, we can find out that Po.1505 with 108.93 ± 0.03 mg equivalent Quercetin per g of dry extract presented an higher flavonoid content than Po. Nat - the natural plant- (98.75 ± 0.03), Po.1503 (93.03 ± 0.06) and Po.1504 (90.17 ± 0.03 mg equivalent quercetin per g of dry extract). The Student test reveals that Po.1505 values differ significantly ($p<0.05$) from all the other values but the Po. Nat value differs significantly from Po.1504 but not from Po.1503; and this last doesn't significantly differ from Po.1504.

Concerning the total phenolic content, the natural *P. odontadenius* (Po. Nat) with 369.87 ± 0.12 mg equivalent Gallic acid per g dry of extract exhibited higher value in comparison to other values (Po.1503 = 297.08 ± 0.12, Po.1504 = 301.14 ± 0.04 mg/g and Po.1505 = 309 ± 0.07 mg/g). Based on T-Student test, it appears that value of Po. Nat differs significantly from all values ($p<0.05$); on the other hand, Po.1505 differs significantly from Po.1503 but not from Po.1504.

The rate (% W/W) of flavonoids in total phenolic content were respectively 26.70% for the sample from natural plants (Po. Nat), 33.33% for Po.1503, 29.94% for Po.1504 and 35.23% for Po.1505.

In vitro Antiplasmodial activity of aqueous extracts

The *in vitro* antiplasmodial test on M2 *P. odontadenius* extracts exhibited high antimalarial activity and the figure 7 illustrate the different values.

Figure 7: *In vitro* antimalarial activity of M2 *P. odontadenius* extracts on isolates of *P. falciparum.*

In figure 7, Po.1503 (1.43 ± 0.17 µg/ml) exhibited high antimalarial activity than Po.1504 (1.45 ± 0.64 µg/ml) and Po.1505 (2.47 ± 0.32 µg/ml). Statistically, no significant difference ($P<0.05$) was observed between Po.1503 and Po.1504. On the other hand, Po.1505 differs significantly with Po.1503 but not with Po.1504.

In vitro Antioxidant and antiradical activities

The assessment of the antioxidant potential was determined by DPPH and ABTS tests. The results are expressed by the IC_{50} values of M2 *P. odontadenius* extracts in comparison to standards. Figures 9 and 10 give respectively inhibition in percents and the antioxidant potential of radicals.

DPPH and ABTS

The inhibition percentages (%) of DPPH and ABTS radicals by methanolic extracts of M2 plants and those of natural plants extracts is illustrated in figure 8.

Figure 8: a. Percentage inhibition (%) of DPPH as function of different extracts concentrations of M2 *P. odontadenius* and natural plants, b: Percentage inhibition (%) of ABTS as function of different extracts concentrations of M2 *P. odontadenius* and natural plants.

In figure 8, natural plant extracts presented high inhibition percentages than all M2 plant extracts (Po-1503, Po-1504 and Po-1505) against both DPPH and ABTS radicals.

Quantification of antioxidant potential

The results of the quantification of antioxidant potential of M2 *P. odontadenius* extracts, are presented in figure 9 together with those of the natural plant extract and the used standard.

Figure 9: Quantification of antioxidant potential of M2 *P. odontadenius* Extracts.

In figure 9, it's noteworthy that Po-1503 (14.44 ± 1.26 µg/ml) showed higher IC_{50} value for DPPH radical reduction than all tested plant extracts, thus expressed low antioxidant activity than all extracts. In contrarily, natural *P. odontadenius* plant extracts showed low IC_{50} (3.61 ± 0.15 µg/ml) following Po-1504 (7.22 ± 0.53 µg/ml) followed by Po-1505 (7.22 ± 0.87 µg/ml) extracts, consequently, high antioxidant activity. Statistically, significant difference ($p<0.05$) has been observed between quercetin and Po-1503 with all the other values, but no significant difference ($p<0.05$) has been recorded between Po-1504 and Po-1505. These last extracts showed however significant difference ($p<0.05$) with Po-1503 and Po-Nat.

Concerning ABTS radical, natural plant and Po-1503 extracts showed similar IC_{50} value i.e. 5.68 ± 0.88 µg/ml and 5.68 ± 1.28 µg/ml respectively and presented higher antioxidant activity than both Po-1504 (11.36 ± 1.31 µg/ml) and Po-1505 (11.36 ± 1.63 µg/ml). Statistically, no significant difference ($p<0.05$) could be pointed out between quercetin, natural plant and Po-1503. On the other hand, Po-1504 and Po-1505 exhibited similar value as shown above and significant difference ($p<0.05$) was observed between their value and those of the standard, the natural plant and Po-1503.

The effects of secondary metabolites on DPPH and ABTS radicals are illustrated in figure 10.

Figure 10: a. Ratio between secondary metabolites (flavonoids and phenolic contents) and antioxydant activities. b: Ratio between secondary metabolites (tannin contents) and antioxydant activities comparing to in vitro antimalarial activities.

In regard of figure 10, Po-1503 showed high value for ratio (15.52%) between IC_{50} from DPPH and flavonoid quantity and high value ratio (6.11%) between IC_{50} from DPPH and total phenolic compounds. The low ratio (1%) from IC_{50} DPPH between total phenolic value for natural plant extracts. However, ratio between IC_{50} from DPPH or ABTS radicals and total phenolic contents were lower than these of IC_{50} from DPPH or ABTS and flavonoid contents for each plant extract. In the other hand, the ratio between DPPH IC_{50} and tannin content was directly proportional to the *in vitro* antimalarial activity, ie when the DPPH IC_{50}/Conc. tannins is high, *in vitro* antimalarial activity is also high. In contrast, the ratio of ABTS IC_{50} to tannin content is inversely proportional to the *in vitro* antimalarial activity (i.e., when ABTS IC_{50}/Conc. of tannins is high, the *in vitro* antimalarial activity is low).

Discussion

P. odontadenius is an interesting plant used as medicinal plant, but its natural low seed germination is a major handicap. Some studies showed that gibberellic acid is an activator for the germination of *P. odontadenius* seeds Jimenez., *et al.* [13] reported by Kikakedimau., *et al.* [9,12,14]. This solution is sometimes fleeting when the domestication of this plant could be obtained by seeds from exclusively of one of *P. odontadenius* plant. Sometimes, seeds from M1 plants do not germinate to give M2 plants. This was also observed by Kikakedimau [9] where M2 seeds for one of control plants didn't germinate. A similar event was observed in this work where control seeds didn't germinate.

The domestication of plants is one of the methods used to obtain homozygous individuals with recessive alleles, which are sometimes deleterious because of the inbreeding. This would explain the lack of germination of some control seeds of *P. odontadenius* plants [34]. In our work, this problem was not observed with irradiated seeds of *P. odontadenius* that is a reason why gamma rays is used in plant breeding [35]. The utility of any mutagen in plant improvement depends not only on mutagenic efficacy; but also it depends on the relationship between mutation frequency and irradiation dose. In addition, it depends also to mutagenic efficacy and the production of desirable changes without association with non-desired mutagenic changes.

The changes of germination rate of M2 *P. odontadenius* seeds could be explain by the effect of gamma ray doses on DNA molecule. This molecule are affected differently such as in the cell damages caused often through the formation of reactive oxygen species (ROS) [36,37]. Others changes such as these on seeds weight and plant biomass could be explain by the same effect. However, no significant difference is observed on these parameters although some differences were observed between individual plants. It could be explained that gamma rays have a fleeting effect on the genes cod-

ing these traits. This effect was observed by Kikakedimau [8] on *P. odontadenius* plants from irradiated seeds at 150 and 200 Gy. On the other hand, Shabnam., *et al.* [38] reported that the effects of mutation were more pronounced in M1 than M2 generation in the comparison to the control plants.

Moreover secondary metabolites from M2 plants did not showed also any difference between all the plant groups. The same secondary metabolites (alkaloids, flavonoids, tannins, anthocyanins, terpenoids and steroids) were found on the different plant groups analyzed. Our results corroborate with those reported previously by Luyindula., *et al.* [39]. However, the present study did not reveal anthraquinons and saponins contrarily to the results reported by Ravikumar., *et al*. [40] and Chaphalkar., *et al.* [41] which revealed saponins in *P. emblica*. The high *In vitro* antimalarial activities (i.e. IC_{50} < 5 µg/mL) according to the WHO classification [42] could be explained by the presence of these different compounds [43].

The IC_{50} is conversely related to the antioxidant capacity of compounds because it expresses the amount of antioxidant required to decrease by 50% concentration of the radical. The lower values of IC_{50} indicate the effectiveness of the extract and thus a stronger antioxidant capacity [44]. High concentrations of total phenolic, flavonoid and tannin contents could explain the high antioxidant activity found in the samples from M2 plants (Figure 9 and 10). *P. odontadenius* extracts on DPPH or ABTS radicals showed high antioxidant activities in comparison to results obtained by Stangeland., *et al.* [45] on different extracts from three Ugandan medicinal plants (*H. rubrostipulata, V. adoensis* and *Z. chalybeum*). Values obtained in this work for DPPH radical varied between 2.31 µg/mL (IC_{50} of vitamin C) and 7.96 µg/mL (IC50 for vitamin E), two natural antioxidants reported by Arina and Rohman [30], except those of Po-1503. These results corroborate those of *Phyllanthus urinaria* (IC50 5.74 µg/mL) reported by the same authors [46,47].

This work show that the antioxidant activities are directly related with total phenolic compounds whoever radical used, especially DPPH (% < 2.5) except for Po-1503 where the report between IC_{50} of DPPH and total phenolic value (5.17%) exceeded those between IC_{50} of ABTS and total phenolic content value (2.04%) (Figure). This exception could be explained by biosynthesis of specific substances other than observed in natural plants, Po-1504 and Po-1505. These compounds could also explain the high *in vitro* antimalarial activity obtained with extracts of Po-1503.

Conclusion

The present study aimed to see the correlation between the type of secondary metabolites from plants obtained by gamma-ray irradiation of seeds and their antioxidant activity.

The investigation carried out showed that the control M2 seeds have did not germinate only irradiated M2 seeds did with respective values of 14.53% for Po-1503, 40.74% for Po-1504 and 18.56% for Po-1505. Natural *P. odontadenius* presented high value of phenolic content but less for flavonoid contents than plants from irradiated seeds. Correlation between DPPH IC_{50} and flavonoid contents could explain the *in vitro* antimalarial activities conversely with ABTS IC_{50} and the same conclusion can be made with the tannin contents.

Conflicts of Interest

None declared

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