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Research Article

PRELIMINARY INVESTIGATION ON THE CHEMOPREVENTIVE PROFILE OF *GARCINIA XANTHOCHYMUS*

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ABSTRACT

Garcinia species have been reported to possess compounds with antibacterial, antioxidant, apoptotic, hepatoprotective and others biological effects. The *Garcinia xanthochymus* Hook. f. (Clusiaceae) is a native plant from some Asian countries and is the most cultivated species in Brazil. Also, has been described to possess some *in vitro* anti carcinogenic compounds. Because of these characteristics, in the present study it was assessed the ability of extracts, fractions and a biflavonoidmorelloflavone, obtained by bio guided assay from *G. xanthochymus,* in their chemo-preventive role by quinona reductase 1 (QR1) induction and genotoxic/antigenotóxic damage. To evaluate the chemo-preventive profile of compounds extracted from *G. xanthochymus,* the quinone reductase assay and Comet assay were performed. It was observed a doubling of the quinone reductase enzyme activity by ethyl acetate and butanolic fractions, and morelloflavone ($p < 0.001$). However, by the Comet assay was observed the genotoxicity of morellofavone, ethyl acetate and butanolic fractions. Regarding anti-genotoxicity, the same fractions and morelloflavone caused DNA damage in post-treatment. Our results suggest that although there is induction of quinone reductase enzyme from morelloflavone, ethyl acetate and butanolic fractions isolated from *G. xanthochymus*, these have genotoxic profile in some concentrations, showing that the widespread use of the plant could bring harm. Additional tests are needed to evaluate the toxicity power of the *G. xanthochymus*.

Keywords: Chemoprevention; *Garcinia xanthochymus;* morelloflavone; quinone reductase 1; Comet assay*;* bio guided study.

INTRODUCTION

Garcinia xanthochymus Hook. f. (Clusiaceae) is a medicinal plant native to the some Asian countries.[1,2] Currently, is cultivated in some tropical countries of Africa, South America and Australia. The trees have large shiny dark green leaves and edible acidic yellow fruits which have been used widely as a traditional medicine for diarrhea and dysentery.[3,4] Previous investigations based phytochemical studies of leaves, fruits, seeds, bark and stems on *G. xanthochymus* have resulted in the isolation of benzophenones, flavonoids, triterpene, xanthones, ester and bioflavonoids,[4-6] with some of these with biological activities. *Garcinia* species have been reported to possess compounds with antibacterial,[7] antioxidant,[8] apoptotic,[9] hepatoprotective,[10] and antiviral activity.[11] Further, it was demonstrated the ability to inhibit aromatase,[12] α-glucosidase,[13] and pro-inflammatory mediators synthesis via interruption of NF-κB and MAPK pathways[14] and promote the cancer chemoprevention.[7,15] Originally proposed by Sporn *et al.*,[16] the classical definition of cancer chemoprevention is the use of natural, synthetic or biological chemical agents to reverse, suppress or prevent either the initial phase of carcinogenesis or the progression of neoplastic cells to cancer. As a whole, cancer chemoprevention definition is the use of pharmacological interventions to treat or reduce the risk of developing cancer.[17] Considering cancer as the end stage of a chronic disease process called carcinogenesis, it is of extreme interest to find natural compounds that could intervene on the carcinogenesis progress and causes none or minimum damage to healthy cells. Thus, the present study assesses the potential chemo-preventive activity from the leaves and fruits of *G. xanthochymus*, by bio guided study.

MATERIALS AND METHODS

Isolation and purification

Dried powdered leaves and fruits were extracted by maceration using n-hexane (1.8 L x 3) and ethanol (2.7 L x 3) at room temperature. The extract was filtered and the hexane and ethanol solvents were evaporated under vacuum to yield residues 8.0 g and 90.0 g, respectively. The concentrated ethanol extract (EtOH*e*) was solubilized with H_2O:MeOH (H_2O:MeOH*f*) (6:4), EtOAc and n-butanol which were then concentrated under reduced pressure to yield the EtOAc fraction (EtOAcf) (24.6 g) and BuOH fraction (BuOHf) (15.1 g). The EtOAcf (1.5 g) was chromatographed by gel permeation over Sephadex LH-20 column eluted with methanol to afford 45 fractions, which were combined after comparison of their TLC profile [Silica gel 60, PF254, EtOAc:H_2O:CH_3CO_2H:HCO_2H (100:27:11:11)] in sixteen (A1-A16) fractions. The fraction A15 (269.3 mg) was purified by prep. RP-HPLC [MeOH:H_2O:CH_3CO_2H (75:24.5:0.5), UV detection at 254 nm; flow rate 10 ml/min] to afford morelloflavone (100 mg).

Cell culture and treatments

Murine hepatoma cells Hepa-1c1c7 (ATCC® CRL-2026™, Rockville, MD) were maintained in Minimum essential medium, alpha (α-MEM), (Sigma, MO, USA) supplemented with 10 % heat inactivated fetal bovine serum (FBS) (Cultilab; Brazil) and 100 U/ml penicillin, and 0.1 mg/ml streptomycin (Invitrogen, CA, USA). Human Hep G2 cells (ATCC®HB-8065™, Rockville, MD were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % FBS, and 100 U/mL penicillin, 100 µg/mL

streptomycin, 600 µg/mL l-glutamine (*In vitro* gen, CA, USA). Bovine serum albumin (BSA), flavin adenine dinucleotide (FAD), glucose-6-phosphate (G6P), β-nicotine amide dinucleotide phosphate (NADP), thiazolyl blue tetrazolium bromide (MTT), glucose-6-phosphate dehydrogenase (G6PD), menadione, Tween-20, dimethyl sulfoxide (DMSO), Tris–HCl and β-naphthoflavone (β-NF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The cells were maintained in humidified 5 % CO_2 at 37°C and subcultured every three or four days. The cells were plated in 96 and 24-well culture plate at 1×10^4 and 1×10^5 cells/well for NAD(P)H:quinone reductase 1 (QR1) and Comet assay, respectively. Aftercell attachment, approximately 24 hours, the tests compounds extracted from the *G. xanthochymus* (1.0 – 120.0 µM) were added with fresh media and the cells were incubated for 48 hours. *G. xanthochymus* compounds were dissolved in DMSO, the final concentration of DMSO to cells was no more than 0.5 %. Also, the cells were washed twice with phosphate-buffered solution (PBS) before being subjected to QR1 and Comet assay.

NAD(P)H:quinone reductase 1 activity in Hepa-1c1c7 cell culture

NAD(P)H:quinone reductase 1 activity was evaluated as described previously, with some modifications.[18] Briefly, Hepa-1c1c7 cells were treated with tests compounds extracted from the *G. xanthochymus*. 4'-bromoflavone (4'BF) was reported to be strong QR1 inducer[19] and was adopted as positive control. DMSO was used as solvent control. After removal medium with PBS, the cells were per-meabilised with 0.8 % digitonin in 2 mM EDTA at pH 7.6. For measurement of QR1 activity, the assay solution (25 mMTris–HCl, pH 7.4, 1 mMG6PD, 50 mMmenadione, 30 mM NADP, 5 mM FAD, 0.07 % (w/v) BSA, 0.03 % (w/v) MTT, 0.01 % (v/v) Tween-20, and 1 unit/mL of yeast glucose-6-phosphate dehydrogenase) was added to each well. QR1 activity was measured as the reduction of menadione to menadiol, this being coupled to the non-enzymatic reduction of MTT by a blue formazan. The reaction generated to a blue color, which was measured after 5 minutes incubation at room temperature on orbital shaker. Readings were made at 595 nm using iMark Micro plate Reader (Bio-Rad Laboratories, Hercules, CA, EUA). Simultaneously to the QR1 assay, the protein was measured by crystal violet staining of an identical set of test plates. The cultured medium was removed and adherent cells were stained with 0.2 % crystal violet dissolved in 2 % ethanol solution. The absorption was measured at 595 nm, and the IC_{50} values were determined. The results are expressed as means ± standard error from triplicate bioassays for each test isolate.

Single cell gel electrophoresis assay (Comet assay)

The Hep G2 treatment protocol assay was performed by following the protocol of Scolastici *et al*.[20] Briefly, in the pre-treatment, after seeding and cell adhesion the medium was removed and cells were treated for 1 h with extracts, fractions and morelloflavone at different concentrations (according to QR1 assay results). Cells were then washed with PBS and treated with H_2O_2 (0.1 mM) for 10 minutes. After treatments with the compounds and mutagen, the cells were washed with PBS, trypsinized, centrifuged at 180 g for 3 minutes and re-suspended into fresh medium. Regarding the post-treatment, after seeding and cell adhesion the medium was removed and cells treated with H_2O_2 (0.1 mM) for 10 minutes. Cells were then washed with PBS, and compounds were added in medium for 1 h at 37 °C. After this period, cells were washed with PBS, trypsinized, centrifuged at 180 g for 3 minutes, and re-suspended into fresh medium. Comet assay of Hep G2 cells was performed under alkaline condition following method of Singh *et al*.[21] with some modifications. After the both treatments, cells were collected separately and washed with PBS by centrifuging at 180 g for 5 minutes in cold centrifuge at 4°C. Slides were initially coated with a layer of normal melting point agarose (0.75 % in PBS). After, cells were mixed with 1 % low melting point agarose and layered in slides. The cells were immediately covered with cover glass and the slides submitted at 4°C for 5 minutes to allow solidification of the agarose. The cover glass was removed and the slides were immersed in lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mMTris, 1 % Triton X-100, 10 % DMSO, pH 10) at 4°C for an hour in the dark. After lysis, the slides were placed in the alkaline buffer (300 mM NaOH, 1 mM EDTA pH 13) in an electrophoretic chamber for 30 minutes to allow DNA unwinding. The electrophoresis was carried out under 25 V and approximately 300 mA for 20 minutes at 4°C. All of the steps described were conducted under the dark to prevent additional DNA damage. Subsequently, the slides were immersed in neutralization buffer (0.4 M Tris–HCl pH 7.5) for 5 minutes, dried, fixed in 100 % ethanol for 10 minutes, and stored at 4°C until analysis. Slides were stained with ethidium bromide; the slides were analyzed at 400× magnification using a fluorescence microscope (ZEISS®, Germany) equipped with a 515-560-nm excitation filter and a 590-nm barrier filter. Image of 100 randomly selected cells was analyzed from each sample. All experiments were repeated in an independent test. Measurement was made by image analysis TriTek CometScore™ version 1.5, determining the mean tail moment (product of tail DNA/total DNA by the tail center, in arbitrary units).

Statistical analysis

Statistical analysis was carried out using Graph Pad Prism® Version 5.01 (Graph Pad Software, San Diego, CA, USA). All data were expressed as mean ± SEM and generated from three independent experiments. Groups of data were compared with the analysis of variance (ANOVA) followed by Tukey's multiple comparison tests and Kruskal Wallis followed by Dunn's pos test. Values of $P < 0.05$ were regarded as significant.

RESULTS

NAD(P)H:quinone reductase 1 induction in *G. xantochymus* compounds

In the effort to search a novel chemopreventive agents, the QR1 assay was used to identify detoxification enzyme inducers from the *G. xantochymus* compounds. Regarding the treatment of Hepa-1c1c7 cell line with EtOAcf isolated from *G. xanthochymus* leaves and fruits, it was observed that there was potential induction of the enzyme quinone reductase 1 at 20 µg/mL (Table 1). The treatments with higher concentrations showed no increase in the potential induction of EtOAcf, and a significant increase in cytotoxicity (data not shown). The treatment of Hepa-1c1c7 cell line with The BuOHf and morelloflavone from leaves suggesting that both has some chemopreventive activity and not cytotoxic (Table 1). There was no significant induction of QR1 at to 20 µg/mL with the other extracts and fractions from leaves or fruits of *G. xanthochymus*. In Table 1, we can observe the levels of enzyme induction and cell viability of the obtained compounds from *G. xanthochymus* leaves and fruits in the range from 1.25 to 20 µg/mL.

Aspects genotoxic and antigenotoxic from *Garcinia xanthochymus* compounds

The Comet assay was performed in order to characterize the genotoxic potential of compounds obtained from *G. xanthochymus* as well as the potential antigenotoxic, as proposed by Scolastici *et al*.[20] Evaluation of the genotoxicity/antigenotoxicity was performed on human hepatocarcinoma cell line (Hep G2) and the concentrations used in the assay were pre-established according to the QR1 induction test, taking into consideration the cell viability. Results obtained by Comet assay are presented in Table 2 and Table

3, showing the DNA damage (tail moment) in Hep G2. It was observed that after treatment with EtOAcf from the leaves, It was capable of cause DNA damage at concentrations from 5 to 30 µg/mL (Table 2). EtOAcf and BuOHf obtained from the fruits of *G. xanthochymus* were also genotoxic at 5 µg/mL. The morelloflavone was genotoxic from 1.25 to 5 µg/mL, including the inducer concentration of QR1 (Table 2). The protective effect of *Garcinia sp.* compounds against H_2O_2-induced DNA damage was also evaluated using the alkaline single cell electrophoresis assay (Comet). Evaluation of antigenotoxicity was performed in Hep G2 and treatments were performed from non-genotoxic concentrations obtained by the Comet assay genotoxicity protocol. The EtOAcf from the leaves of *G. xanthochymus* demonstrated in pre-treatment that concentrations from 0.156 to 0.625 µg/mL presented an intensification of DNA damage compared to the positive control. In the post-treatment, only the tested concentration of 0.625 µg/mL showed significant difference compared to the positive control (Table 3). The EtOAcf and BuOHf from fruits, showed in the pre-treatment, intensified DNA damage (Table 3). In the post-treatment, the EtOAcf showed that in concentrations from 2.5 µg/mL to 0.625 µg/mL was observed a significant difference when compared to the positive control (Table 3). In the analysis of post-treatment with BuOHf, was observed no statistical significance in relation to the positive control at concentrations of 1.25 and 0.625 µg/mL, and significant difference when compared to positive control at a concentration of 0.312 µg/mL (Table 3). In evaluating antigenotoxicity of morelloflavone, it was observed in pre-treatment that the concentration of 0.312 µg/mL there was no statistically significant difference from the positive control. In the post-treatment, it was observed that at concentrations of 0.15 and 0.075 µg/mL no significant difference were observed when compared to the positive control, demonstrating that these concentrations may have to morelloflavone repair capacity.

Table 1: Quinone reductase 1 induction ratio value of extracts, fractions and morelloflavone obtained from leaves and fruits of *Garcinia xanthochymus* in Hepa-1c1c7 cell line

	Garcinia xanthochymus	Quinone-reductase 1 induction IR	CV (%)
Leaves	Morelloflavone	2.5 ± 0.0028	101 ± 7.49
	EtOHe	1.8 ± 0.1247	85.0 ± 4.33
	Hexe	1.5 ± 0.0577	59.4 ± 12.23
	EtOAcf	2.3 ± 0.404	87.6 ± 5.01
	H_2O:MeOHf	0.5 ± 0.0666	75.0 ± 6.94
	BuOHf	2.2 ± 0.0866	71.4 ± 7.06
Fruits	EtOHe	1.8 ± 0.0882	91.6 ± 1.4
	EtOAcf	2.4 ± 0.0796	62.1 ± 4.32
	H_2O:MeOHf	1.2 ± 0.0960	79.2 ± 8.0
	BuOHf	1.3 ± 0.0296	81.6 ± 4.12
4'-bromoflavone		2.9 ± 0.218	125 ± 5.6

IR: Induction ratio of quinone reductase 1 (QR1); CV: Cell viability, percentage of live cells at the highest concentration tested (20 µg/mL), 4'-bromoflavone (positive control)

Table 2: Genotoxicity presented by *Garcinia xanthochymus* fractions and morelloflavone

	Controls	**Morelloflavone**	**EtOAcf- leaves**	**EtOAcf- fruits**	**BuOHf- fruits**
	TM ± SEM	TM ± SEM	TM ±SEM	TM ±SEM	TM ±SEM
Untreatedcells	10.60 ± 1.61				
H_2O_2	66.92 ± 6.28***				
DMSO	11.40 ± 2.78				
0.312 µg/mL		11.56 ± 2.53	14.12 ± 1.87	16.53 ± 1.80	13.84 ± 2.69
0.625 µg/mL		7.89 ± 2.31**	20.44 ± 2.67***	14.24 ± 2.16	12.09 ± 1.87
1.25 µg/mL		16.13 ± 1.19*	22.18 ± 3.56***	24.13 ± 3.59***	17.68 ± 2.72*
2.5 µg/mL		16.05 ± 1.69*	21.94 ± 3.24***	20.58 ± 3.17***	16.54 ± 3.10*
5 µg/mL		26.87 ± 3***	29.56 ± 2.98***	29.62 ± 2.86***	22.44 ± 2.26***
10 µg/mL		¶	28.28 ± 3.70***	¶	26.30 ± 3.43***
20 µg/mL		¶	21.01 ± 3.47**	¶	40.38 ± 4.74***
30 µg/mL		¶	47.80 ± 5.19***	¶	¶

TM: Tail Moment; Kruskal Wallis analysis with post-test Dunn (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$. ¶Untested concentrations

Table 3: Antigenotoxicity presented by *Garcinia xanthochymus* fractions and morelloflavone

	Controls	**Morelloflavone**		**BuOHf- fruits**	
		TM±SEM		TM±SEM	
		Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Untreatedcells	8,70 ± 1,35				
H_2O_2	66,92 ± 6,28***				
DMSO	11,40 ± 2,78				
0,075µg/mL		155.22 ±8.34***	49.24 ± 6.91**	¶	
0.156µg/mL		100.24 ± 7.25**	37.25 ± 6.32***	¶	
0.312µg/mL		86.60 ± 6.76	55.48 ± 6.35	¶	
0.625µg/mL		¶		137.12 ± 8.32***	18.72 ± 4.7**
1.25µg/mL		¶		139.67 ± 7.94***	40.72± 5.2***
2.5µg/mL		¶		116.8 ± 8.78***	58.18 ± 9.2***

TM: Tail Moment; Kruskal Wallis analysis with post-test Dunn (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$. ¶Untested concentrations

Table 3 (Continuation): Antigenotoxicity presented by *Garcinia xanthochymus* fractions and morelloflavone

	Controls		EtOAcf- leaves	EtOAcf- fruits
	TM±SEM		TM±SEM	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Untreated cells				
H2O2				
DMSO				
0,075µg/mL	¶		¶	
0.156µg/mL	184.72 ± 4.88***	92.21± 8.2	¶	
0.312µg/mL	156.3 ± 6.20***	80.56 ± 7.5	¶	
0.625µg/mL	120.32 ± 4.45***	57.05± 9.1***	145.8 ± 7.67***	143.17 ± 8.4***
1.25µg/mL	¶		137.43 ± 8.23***	53.67 ± 7.9***
2.5µg/mL	¶		119.38 ± 6.49***	31.40±4.6***

TM: Tail Moment; Kruskal Wallis analysis with post-test Dunn (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$. ¶Untested concentrations

DISCUSSION

Several studies *in vitro* and *in vivo* indicate that the reduction of quinones by electrophilic QR1 is an important detoxification pathway in which quinones are converted into hydroquinones, demonstrating that the enzyme QR1 is a potential biomarker for the detection of chemo-preventive agents against the initial phase of cancer.[22,23] The induction of QR1 can help in chemoprevention against cancer and chemical toxicity with natural or synthetic compounds.[24] The various properties of flavonoids as antioxidant, metal binding capacity, ability to affect the endocrine system and the ability to prevent enzymatic activation of carcinogens, are consistent with a protective role against malignant disease. The requirements for structure/activity to the antioxidant properties of the flavonoid aglycones have been studied in detail using a variety of assay systems.[25] We found no previous studies involving the chemoprevention potential by NAD(P)H:quinone-reductase 1 activity in Hepa 1c1c7 cell culture from the *G. xanthochymus* fractions or morelloflavone after literature review. Nevertheless, in the present study, we found in this study promising results about induction of QR1 by the morelloflavonebiflavonoid. This substance is described in the literature to possess some *in vitro* anti-carcinogenic activity.[26]

Rice-Evan *et al.*[27] showed in a study of structure-activity the importance of 3',4'-dihidroxil in B ring and a double linkage 2,3 in C ring, as a major determinants of antioxidant activity. Thus, the structure of morelloflavone could favor its antioxidant activity. It is known that the flavonolquercetin is an inducer of phase II enzymes and detoxification of carcinogens.[28,29] Prestera *et al.*[30] proposed that anti-carcinogenic diet can inhibit cancer initiation acting on the balance between activation of phase I enzymes (such as cytochrome P450) and phase II (detoxification enzymes such as GST, QR1 and UDP-glucuronyltransferase). Factors that suppress or inhibit phase I enzymes or induce phase II enzymes are likely to have a protective role against cellular damage. A biflavonoid from *Garcinia sp.*, the kolaviron, have been established as an inducer of phase II detoxification enzymes and inhibition of stress response proteins[15], suggesting that morelloflavone can act the same way. Although the studies about the biflavonoids are rare when it is the role of induction of detoxification enzymes, the role of flavonoids in inducing QR1 is strongly reported[31]. It has been reported that apigenin flavonoid, a flavone as the luteolin unit from morelloflavone, is capable of inducing QR1 1.6 times[25]. The taxifolin, flavanonol which differs from flavanone by the presence of a hydroxyl group in the C ring, it is also capable of inducing QR1.[32] The effect of supplemental feeding of naringenin in rats demonstrated through QR1 assays that the flavanone can promote QR1 activity in the liver from these animals under oxidative stress conditions.[33] In our study, we observed similarity to the data presented in the literature review, where the induction QR1 by morelloflavone was 2 times higher in concentration (2.5 µg/mL), with no cell death at this concentration by crystal violet, showing the importance of their role in the chemoprevention. Similarly in the context of cytotoxity, Lin *et al.*[34] describe the morelloflavone showed moderate activity with IC_{50} to 82 uM, while volkensiflavona (naringenin I-3-II-8 apigenin) showed a weak activity with inhibition at 200 uM in HIV-1 infected PBMC. In the study, the authors describe the biflavonoids constructed units linked flavanone, flavone I-3 II-8, as in the case of morelloflavone which presents moderate to weak cytotoxicity activity against PBMC HIV-1 positive. Other biflavonoids linked by I-3 II-8 as GB1a and GB2a, are moderately active, while biflavanones linked by A ring from two units of naringenin (rhusflavanone and succedaneaflavanone) were inactive. The study strongly suggests that the hydroxyl groups and at least one unit of the flavones in the biflavonoids are required for inhibitory activity of HIV-1 infected PBMC. A connection I-3 II-8 is required to display the biflavanones activity, and active compounds become inactive when hydroxyl groups are methylated. The biflavonoid, 3″,4′,4‴,5,5″,7,7″-heptahydroxy-3,8-biflavanone, known as GB1[35] was also shown not to be toxic (at 50 µg/mL) against some tumor cell lines.[36] Importantly, the concentrations tested in different studies cited above were higher than those tested by our group (2.5 µg/mL), suggesting that the morelloflavone concentration tested, not have any cytotoxic profile. The profile induction of QR1 from extracts and fractions of *Garcinia sp.* not exists, and in this work they did not show the ability to duplicate the activity of the enzyme. In the context of non-tumor lines, Matsuo *et al.*[37] reported the cytotoxicity of the units forming the morelloflavone separately, namely the flavonoid naringenin, teolin in human cells line, TIG-1 e HUVEC. Data showed a high variation between the IC_{50} cell lines, and also among other flavonoids studied.[37] These data suggest that the structure-activity relationship of flavonoids and biflavonoids with human and murine cells is still unclear, and its toxicity differs depending on the cell type. Protection against DNA damage is an important chemo-preventive property which can prevent the initiation phase of cancer. Flavonoids having antioxidant activity and anti-radicals are capable of quenching free radicals, which can promote DNA damage and mutations. The alkaline Comet technique allows identifying the presence of breaks in single strands of DNA and is used to verify ownership chemo-preventive protection against certain DNA damage.[38] The chemo-preventive activity or lack of protective action and DNA damage can also be assessed, respectively, by anti-genotoxicity and genotoxicitytests using the Comet assay[21]. Studies conducted in HepG2 cell line with the objective of evaluating the protective effect of α-hederine isolated from plants (saponintriterpenic) against DNA damage caused by H_2O_2, the authors observed that the compound showed no protective action (antigenotoxic). *In vivo* studies demonstrated that ascorbic acid (vitamin present in many types of fruits and vegetables) have a protective action against the DNA damage induced by ethanol in embryonic cells of rat hippocampus, and in glial cells from human

brain.[39] The kolaviron obtained from *G. kola* at concentrations 30-90 μM decreased DNA breakage induced by H_2O_2 in human lymphocytes and mouse liver cells[7]. The structure of kolaviron shows the existence of a hydroxyl radical in the C ring, which possible drives its antioxidant to the chemo-preventive activity. We found no previous studies involving the chemoprevention potential of the *G. xanthochymus* compounds after literature review. Nevertheless, studies about the protective effects of the plant should be intensively evaluated, since this can induce the activity of the enzyme quinone-reductase 1.

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