Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org

doi: 10.4103/2221−1691.354430

Impact Factor: 1.51

Antiglycation, antioxidant, antiacne, and photoprotective activities of crude extracts and triterpene saponin fraction of *Sapindus saponaria* L. fruits: An *in vitro* study

Regildo M. G. Silva[1,2✉], Gustavo R. Martins[1], Laura M. B. Nucci[1], Filipe O. Granero[2], Célia C. M. Figueiredo[2], Patrícia S. Santiago[3], Luciana P. Silva[4]

[1]*Laboratory of Herbal Medicine and Natural Products, Department of Biotechnology, Faculty of Sciences, Humanities and Languages, São Paulo State University, Assis, São Paulo, Brazil*

[2]*Institute of Chemistry, São Paulo State University, Araraquara, São Paulo, Brazil*

[3]*São Paulo State University, Campus de Registro, Registro, São Paulo, Brazil*

[4]*Fundação Educacional do Município de Assis, Assis, São Paulo, Brazil*

ABSTRACT

Objective: To evaluate the photoprotective, antioxidant, antiglycation, and antiacne activities of crude extract (CESs) and triterpene saponin fraction (TSSs) of *Sapindus saponaria*.

Methods: HPLC-MS purification was performed on a Symmetry TM C18 column. The saponins were identified by a UV detector. Antioxidant activity was evaluated by DPPH and O_2^- radicals scavenging, and FRAP and TBARS assays. Glycation activity was assessed by relative electrophoretic mobility and inhibition of advanced glycation end products (AGEs) formation. Additionally, antiacne activity was determined by inhibition of *Cutibacterium acnes*, and photoprotective effect was evaluated by Mansur's method.

Results: Most of the triterpene saponins detected in the fraction by HPLC-MS analysis were hederagenin as the aglycon. CESs and TSSs presented varying antioxidant activity in DPPH (CESs: 75.69% and TSSs: 83.65%), FRAP (CESs: 425.39 μM TE/g DW and TSSs: 649.36 μM TE/g DW), TBARS (CESs: 42.96% and TSSs: 52.16%) and O_2^- radicals scavenging (CESs: 61.33% and TSSs: 86.69%) tests. CESs and TSSs also exhibited antiglycation activity comparable to bovine serum albumin treated with aminoguanidine. In addition, CESs and TSSs showed inhibition of AGE formation (34.48% and 61.85%, respectively). Antiacne activity against *Cutibacterium acnes* was observed with a minimum inhibitory concentration equal to minimum bactericidal concentration (CESs: 36.11 μg/mL and TSSs: 18.34 μg/mL). In photoprotective assays, CESs and TSSs showed maximum absorbance of 1.42 to 0.20 and 2.80 to 1.30, respectively, in the wavelength range of 260 to 400 nm. Furthermore, CESs and TSSs showed sun protection factors of 8.89 and 14.89, respectively.

Conclusions: *Sapindus saponaria* fruit extracts show strong antioxidant potential and antiglycation activity against bovine serum albumin glycation and AGE formation. Besides, they presented antibacterial activity against *Cutibacterium acnes* and photoprotective effect against UV-A and UV-B.

KEYWORDS: Photoprotective; AGE formation; Free radical; Antioxidant; Triterpene saponin; *Sapindus saponaria*

1. Introduction

Sapindus saponaria (*S. saponaria*) L. belongs to Sapindaceae family

Significance

Sapindus saponaria is a species-rich in bioactive compounds mainly saponins; however, it has been poorly studied. This study shows *in vitro* antioxidant, antiglycation, antiacne and photoprotective activities of the crude extract and triterpene saponin fraction of this plant.

✉To whom correspondence may be addressed. E-mail: regildo.silva@unesp.br

How to cite this article: Silva RMG, Martins GR, Nucci LMB, Granero FO, Figueiredo CCM, Santiago PS, et al. Antiglycation, antioxidant, antiacne, and photoprotective activities of crude extracts and triterpene saponin fraction of *Sapindus saponaria* L. fruits: An *in vitro* study. Asian Pac J Trop Biomed 2022; 12(9): 391-399.

Article history: Received 13 May 2022; Revision 28 July 2022; Accepted 12 August 2022; Available online 1 September 2022

and it is popularly known as 'saboeiro' or 'Sabão-de-soldado'. It is a tropical tree commonly found in the central west, north, and northeast areas of Brazil[1]. Its fruits contain high levels of saponins that are used to treat ulcers, skin sores, and inflammations[2]. The hederagenin saponin is the most abundant aglycon in the fruit pericarp, which makes this plant a source of large amounts of triterpenoic acid and triterpene saponins. Consequently, great interest has been given to this species and its active compounds[3].

Among the possible pharmacological activities of *S. saponaria* fruit extract and its triterpene compounds are the antioxidant, antiglycation, antiacne and photoprotective activities[4]. Studies have demonstrated that antioxidants from natural sources can be used to prevent diseases related to oxidative stress. This process is promoted by high production and accumulation of free radicals and reactive oxygen species (ROS). They are associated with cellular and metabolic injuries causing premature aging, inflammations, cardiovascular and neurodegenerative disease, and cancer[5]. Currently, different studies have shown that plants rich in triterpenoid compounds presented several pharmacological activities, mainly the scavenging of free radicals and inhibition of ROS formation, thus highlighting an important source of antioxidants[6].

Glycation is a process that involves a non-enzymatic addition of reducing sugars in free amino groups of proteins, lipids, or nucleic acid[7]. Non-enzymatic glycation causes complications in molecules, cells, tissues, and organs, mainly by generating and/or aggravating oxidative processes. The result of glycation is a class of modified compounds known as advanced glycation end products (AGEs)[7]. AGEs affect structural cell components and they are related to alterations in signal transduction pathways that involve ligands in extracellular matrix and/or in the level of soluble signals such as free radicals, cytokines, and hormones *via* interactions with cell receptors[8]. They are also associated with secondary complications such as atherosclerosis, neuropathy, cataracts, retinopathy, nephropathy, and especially vascular diseases and diabetes. Studies have shown that plant extracts may have inhibitory activities on AGE formation due to their large amount of antioxidant compounds since free radicals are involved in this process[9].

The skin is perhaps the most vulnerable part of the human body. It is a fact that daily skin exposure to the external environment causes problems such as acne, pigmentation, and sunburn marks. Acne is a well-known skin condition mainly observed in people aged between 15-25 years and it results from an overproduction of sebum accompanied by a proliferation of *Cutibacterium acnes* (*C. acnes*)[10]. The common acne treatments focus on decreasing *C. acnes* population and sebaceous gland activity and producing an anti-inflammatory effect. Antibiotics and hormones have been used for years for the treatment of acne; however, they may cause severe side effects and drug resistance. Considering that inflammatory conditions are related to oxidative stress, plant materials are good candidates due to their antioxidant activity[11]. Thus, phytotherapeutic approaches have been studied as an alternative to providing high antibacterial activity with no or fewer side effects[12].

Another fact is that it is not possible to avoid skin exposure to the sun and, consequently, sun protection is needed. However, a regular and continuous application of sunscreens can be damaging due to their side effects such as dermatitis, melanoma, and skin cancers[13]. Sunscreens are also unable to totally protect skin from ultraviolet rays (UV-R) radiation; therefore, sunscreen formulations containing bioactive compounds from plants are being increasingly studied. The UV-R absorbed by the skin produce oxidative agents, such as free radicals and/or ROS, which can induce premature aging and skin cancer. Plant extracts contain many phytoconstituents and these compounds, with antioxidant activity are involved in the photoprotection mechanism[14]. Sunscreens containing natural compounds such as polyphenols, phenolic acids, and triterpenoids may neutralize oxidative agents and prevent negative effects of UV-R. Besides, these compounds also present the potential to stimulate skin blood circulation and remove dead cells, demonstrating an antiaging activity[15].

Considering the abovementioned beneficial activities of plants rich in antioxidant compounds, this study aimed to demonstrate the *in vitro* antiglycation, antioxidant, antiacne, and photoprotective activities of the crude extract and triterpene saponin fraction of *S. saponaria*.

2. Materials and methods

2.1. Plant material

Fruits of *S. saponaria* were collected from specimens in the São Paulo State University (UNESP-Assis, Brazil) (22°32'20''S and 50°22'60''W). A voucher specimen (741) has been deposited in the Herbarium Assisense, Assis, Brazil. Fruits were pulped, frozen (−18 ℃), lyophilized, and grounded to obtain a dry mass.

2.2. Preparation of methanolic fruit extract

A total of 300 g dry mass of fruits was extracted using 1 500 mL methanol by mechanical maceration for 24 h in the dark at room temperature. The extracts obtained were concentrated in a rotary evaporator to 2 000 mL.

2.3. Preparation of triterpene saponins fraction

Briefly, 1 000 mL methanolic extract and 1 500 mL 5% sulfuric acid in methanol (*v/v*) were mixed and heated at 60 ℃ under reflux for 3 h. After hydrolysis reaction, pH was adjusted to 6 by addition of 5% potassium hydroxide (KOH) in methanol (*v/v*). Activated charcoal (100 g) was added to the resulting mixture and it was heated under reflux for 30 min at 60 ℃. The resulting solution was filtered to obtain the crude triterpene saponins fraction[2].

2.4. Determination of total triterpenoid content (TTC)

TTC was determined by the vanillin-perchloric acid assay described by Song *et al*[16]. Results were expressed as μg oleanolic acid equivalent (OAE) per g dry weight (DW) and determined by a standard calibration curve (y=16.005x+0.025 6, R^2=0.998 7).

2.5. Evaluation of triterpenoids by high–performance liquid chromatography and mass spectrometry (HPLC–MS)

HPLC-MS was used to evaluate the triterpenic content of the triterpene saponins fraction. HPLC purification was performed on a Symmetry TM C18 column using acetonitrile/deionized water (0-30 min, 2∶3; ~30 min, 3∶2) at 40 ℃ and a flow rate of 0.2 mL/min. The saponins were identified by a UV detector at a maximum absorption wavelength of 210 nm. Afterward, the purified fraction was ionized by electrospray ionization in positive mode with the scan range of *m/z* 700-1 500 for MS analysis.

2.6. Evaluation of antioxidant activity

2.6.1. DPPH radical scavenging test

Antioxidant activity was determined by DPPH methodology described by Rufino *et al*[17]. Solutions containing 1 mL acetate buffer (pH 5.5, 100 mM), 1.25 mL ethanol, 250 μL DPPH solution (500 μM), and 50 μL of crude extract or triterpene saponin fraction of *S. saponaria* were mixed and kept at room temperature in dark for 30 min. The absorbances were read at 517 nm in a spectrophotometer UV-Vis. Gallic acid was used as a positive control at 100 μg/mL.

2.6.2. Ferric ion reducing antioxidant power (FRAP) test

FRAP test was performed following the methods described by Benzie and Strain[18] with some modifications. Briefly, 2.7 mL FRAP, 270 μL ultrapure water, and 90 μL of the samples were mixed and this solution was heated at 37 ℃ for 30 min. Absorbances were read at 593 nm and results were expressed as μM Trolox equivalent (TE) per gram of dry weight (DW).

2.6.3. Lipid peroxidation inhibition test

Thiobarbituric acid reactive species (TBARS) assay was used to evaluate lipid peroxidation inhibition by the method described by Guimarães *et al*[19]. Dried egg yolk was diluted (1%, *w/v*) in phosphate buffered saline (20 mM, pH 7.4). Then, 1 mL of this solution was sonicated and mixed with 0.1 mL of samples or positive control (Trolox 140 μg/mL). Lipid peroxidation was stimulated by the addition of 0.1 mL AAPH (0.12 M) at 37 ℃ for 30 min. After cooling to room temperature, 0.5 mL thiobarbituric acid (0.67%) and 0.5 mL trichloroacetic acid (15%) were added and this mixture was heated for 15 min at 97 ℃. Then, samples were centrifuged for 10 min at 1 200 rpm and the absorbance of supernatant was read at 532 nm. Results were expressed as a percentage of TBARS inhibition.

2.6.4. Superoxide (O_2^-) radical scavenging test

Scavenging potential on superoxide (O_2^-) radical generated by hypoxanthine-xanthine oxidase (XO) was performed according to the method of Kirby and Schmidt[20] with some modifications. A total of 20 μL Na_2EDTA, 5 μL of sample diluted in methanol, and 145 μL buffer were mixed and transferred to 96-well microplates (Falcon). Subsequently, 50 μL XO solution diluted in buffer (1∶10) was added to the mixture. The reaction mixture was incubated at 25 ℃, and the absorbances were measured each 20 s for 5 min at 570 nm, using an ELISA plate.

Control was 5 μL methanol and (+)-catechin was used as the positive control. Results were expressed as percentage of inhibition calculated by the equation: Inhibition (%) = [(control rate–sample reaction rate)/control rate]×100.

2.7. Evaluation of antiglycation activity

2.7.1. Preparation of glycation reaction mixture

To evaluate the antiglycation activity, glycation reaction mixtures were prepared following the methods described by Wijetunge and Perera[21] with some modifications. Bovine serum albumin (BSA) (2 mg/mL) was added to phosphate buffered saline (10 mM, pH 8.0) and ribose (1 mol/L) in the presence or absence of aminoguanidine (AMG, 40 mM) or samples (10 mg/mL). For the relative electrophoretic mobility (REM), the reaction mixtures were incubated for 7 d at 37 ℃. For the inhibition of AGE formation assay, they were incubated for 15 d at the same temperature.

2.7.2. REM evaluation

REM was performed as described by the method of Cyr *et al*.[22] and Wijetunge and Perera[21], with some modifications. SDS-PAGE was prepared using 5% stacking gel and 8% separation gel. An aliquot of the glycation reaction mixture was added to an equal volume of sodium dodecylbenzene sulfonate sample buffer (20 mg/mL), 30% glycine, 0.25 M Tris-HCl buffer (pH 6.8), and this solution was boiled for 3 min. From this solution, 15 μL was applied to the stacking gel and the electrophoresis was run at 30 mA. After the process, the gel was stained with Coomassie-Blue (R-250). BSA (0.3 mg/mL) with AMG (40 mM) was used as positive control.

2.7.3. Inhibition of AGE formation

The glycation reaction mixture and OPA reagent were mixed to obtain a 1 mL solution that was incubated at room temperature for 2 min. Fluorescence excitation and emission maximum were read respectively at 360 and 460 nm to determine AGE formation. The results were expressed as a percentage of inhibition of AGE formation.

2.8. Evaluation of antiacne activity

Antiacne activity was performed using broth dilution methodology described by Patil *et al*.[23] and Jantarat *et al*.[11] to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). *C. acnes* were grown in Brain Heart Infusion (BHI) broth under anaerobic conditions at 37℃ for 72 h. The turbidity of bacterial suspension was adjusted to about 1×10^8 CFU/mL using McFarland No. 0.5. Crude extract and triterpene saponin fraction (0.05 g) was diluted in ultrapure water and this solution was adjusted to 100 mL to acquire a 500 μg/mL stock solution. Then, it was filtered in a 0.45 μm membrane and diluted with BHI broth in a two-fold dilution series in glass test tubes at different concentrations. Tetracycline was diluted in series to obtain different concentrations and used as the positive control. Then, 50 μL *C. acnes* suspension was added to each sample solution (1 mL). Then, solutions were incubated under anaerobic conditions at 37℃ for 48 h. MIC was determined by the lowest concentration with a clear culture. Negative control was prepared following the same procedure used for the samples excluding *C. acnes*.

To determine MBC, the dilution that represents MIC and three of the more concentrated sample dilutions were inoculated on BHI agar and incubated under an anaerobic environment at 37℃ for 48 h. Positive control was determined using one of the less concentrated sample dilutions. MBC was determined by the lowest concentration that did not present visible growth.

2.9. Evaluation of photoprotective activity

Photoprotective activity was evaluated according to Mansur *et al*.[24] to determine sun protection factor (SPF) of the crude extract and triterpene saponin fraction. Samples were diluted in methanol to obtain concentrations of 250, 500, and 1000 μg/mL for crude extract and 10, 50, and 100 μg/mL for triterpene saponin fraction. Sample absorbances were measured at UV-A and UV-B wavelength range (260-400 nm), with 5 nm increments, and three determinations were carried out at each point. SPF was calculated by the Mansur equation: $SPF=CF\times\sum_{290}^{320} EE_\lambda\times I_\lambda\times Abs_\lambda$ where CF represents correction factor (constant value equal to 10), EE_λ is erythemal effect of solar radiation at each wavelength λ; I_λ is solar intensity at wavelength λ; Abs_λ is sample absorbance at each wavelength λ. The values of EE×I are constant and previously determined by Sayre *et al*.[25]. Octyl methoxycinnamate (OMC – sunscreen) (10 mg/L) was used as positive control.

2.10. Statistical analysis

The data obtained in this study were expressed as mean±standard deviation (SD) and statistically analyzed by Analysis of Variance (ANOVA) followed by Tukey's *post hoc* test using BioEstat software version 5.1. $P<0.05$ was considered significantly different.

Table 1. Determination of total triterpenoid content in crude extract and triterpene saponin fraction of *Sapindus saponaria* fruits (μg oleanolic acid equivalents/g dry weight).

Concentration (μg/mL)	Crude extract	Triterpene saponin fraction
25	305.16	998.47
50	423.65	1123.54
75	598.33	2098.78
100	659.81	3589.41
250	702.88	6457.02
500	787.14	7115.23
1000	847.52	8023.89

3. Results

3.1. Determination of TTC

Table 1 shows TTC observed in crude extract and triterpene saponin fraction of *S. saponaria* fruits at different concentrations (25 to 1000 μg/mL). In this study, the crude extract and triterpene saponin fraction showed increased TTC at increasing concentrations. TTC in crude extract ranged from 305.16 to 847.52 μg OAE/g DW. Meanwhile, triterpene saponin fraction presented the highest TTC and it ranged from 998.47 to 8023.89 μg OAE/g DW.

3.2. Evaluation of triterpenoids by HPLC–MS

Table 2 shows molecular weight, CID spectrum, suggested structure, and number of peaks detected in triterpene saponin fraction of methanolic extract of *S. saponaria* fruits by HPLC-MS analysis. Most of the triterpene saponins detected in the fraction were hederagenin as the aglycon. This could be concluded from the fact that MS presented ion ($[aglycon]^-$) at *m/z* 471 as the product of most of the precursor ions recorded by $[M-H]^-$. Saponins derived from oleanolic acid (*m/z* 455) were minority in variety and quantity. On the other hand, bisdesmosidic saponins were identified in trace amounts. Based on the interpretation of CID-MS data, possible sugar sequences and the presence of acylation were proposed (*e.g.* 132 Da of arabinose or xylose, and 146 Da of rhamnose). Sugar sequences in saponins were determined by sugar loss fragmentation; although, internal sugar loss was poorly detected and thus it was not considered. The most frequent sugars identified in carbohydrate chain of the saponins were arabinose (ara) and rhamnose (rha); however, glycose (glc) and xylose (xyl) were also identified.

3.3. Antioxidant activity

3.3.1. DPPH, FRAP and TBARS tests

Table 3 shows the activity of crude extract and triterpene saponin fraction of *S. saponaria* fruits in different antioxidant *in vitro* assays (DPPH, FRAP, and TBARS). In DPPH test, the highest antioxidant activities of the crude extract were presented at 500 (66.23%) and 1000 μg/mL (75.69%), which were not statistically different from

Table 2. Molecular weight, CID spectrum, suggested structure, and number of peaks detected in triterpene saponin fraction of methanolic extract of *Sapindus saponaria* fruits.

$[M-H]^-$ (*m/z*)	CID spectrum	Suggested structure	Number of peaks detected
879	879, 751, 609, 471	Hed-ara-rha-ara or Hed-ara-rha-xyl	2
925	879, 861, 752, 609, 471	Hed-ara-rha-ara or Hed-ara-rha-xyl w/ 1 OAc	3
964	921, 903, 881, 861, 849, 752, 609, 471	Hed-ara-rha-ara or Hed-ara-rha-xyl w/ 2 OAc	3
752	609, 581, 471	Hed-ara-rha	1
947	752, 609, 471	Hed-ara-rha-glc	1
956	912, 893, 752, 609, 471	Hed-ara-rha-glc w/ 1 OAc	1
993	950, 937, 909, 873, 893, 752, 609, 471	Hed-ara-rha-glc w/ 2 OAc	2
868	731, 591, 455	OAc-ara-rha-xyl or OAc-ara-rha-ara	2
905	865, 858, 731, 587, 455	OAc-ara-rha-xyl or OAc-ara-rha-ara w/ 1 OAc	4
952	912, 889, 863, 844, 733, 589, 455	OAc-ara-rha-xyl or OAc-ara-rha-ara w/ 2 OAc	4

Hed: Hederagenin; OAc: Oleanolic acid; ara: Arabinose; rha: Rhamnose; glc: Glycose; xyl: Xylose; CID: Collision-induced dissociation.

gallic acid (positive control) (87.43%). These results showed a dose-dependent response. Similar results were observed for the triterpene saponin fraction that presented the highest antioxidant activities at 50 and 100 µg/mL (78.58% and 83.65%, respectively) and they also did not differ from the positive control. In FRAP test, the greatest ferric reducing antioxidant power was observed at the highest concentration of both crude extract (425.39 µM TE/g DW) and triterpene saponin fraction (649.36 µM TE/g DW). In TBARS test, the highest inhibition of lipid peroxidation activity was observed for crude extract at 1000 µg/mL (42.96%) and for triterpene saponin fraction at 100 µg/mL (52.16%), which were not significantly different from Trolox (positive control) (49.59%).

3.3.2. XO inhibition test

Figure 1 presents the results of inhibition of O_2^- formation by the crude extract and triterpene saponin fraction of *S. saponaria*. All treatments presented at least 20% inhibition of O_2^- formation at all concentrations, and the greatest inhibition was observed for the triterpene saponin fraction at 100 µg/mL (86.69%) and for the crude extract at 1000 µg/mL (61.33%), which was not significantly different from the positive control (64.23%).

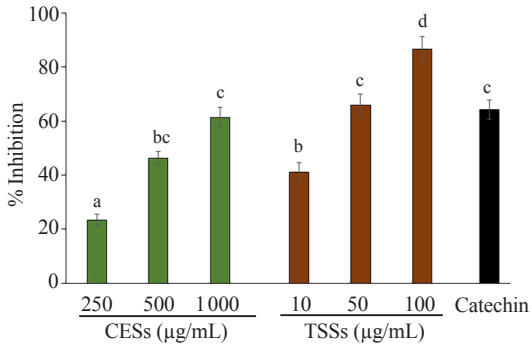

Figure 1. Inhibitory activity of O_2^- formation of the crude extract (CESs) (250, 500, and 1000 µg/mL) and triterpene saponin fraction (TSSs) (10, 50, and 100 µg/mL) of *Sapindus saponaria* fruits. Catechin (10 µg/mL) was used as positive control. Different letters indicate significant difference (P<0.05).

Table 3. Antioxidant activity of crude extract (CESs) and triterpene saponin fraction (TSSs) of *Sapindus saponaria* fruits by DPPH, FRAP, and TBARS tests.

Treatment	DPPH[1]	FRAP[2]	TBARS[3]
CESs (µg/mL)			
25	3.23±0.02^a	221.57±12.44^a	1.23±0.03^a
50	8.36±0.89^b	236.63±10.24^a	1.79±0.17^a
75	14.55±1.01^c	242.79±11.02^a	2.23±0.23^a
100	22.46±1.25^c	263.03±10.23^a	6.44±0.96^a
250	35.66±2.36^d	289.36±10.22^a	8.43±0.87^a
500	66.23±2.11^e	349.17±15.31^b	22.48±1.23^b
1000	75.69±3.02^e	425.39±11.46^b	42.96±2.38^c
TSSs (µg/mL)			
10	35.04±2.45^d	457.44±10.89^b	41.23±3.02^c
50	78.58±3.52^e	623.63±13,81^c	45.66±2.94^c
100	83.65±2.98^e	649.36±10.88^c	52.16±2.45^c
Gallic acid (100 µg/mL)	87.43±0.20^e	-	-
Trolox (140 µg/mL)	-	-	49.59±0.00^c

Results are expressed as mean±standard deviation (SD). [1]% antioxidant activity, [2]µM Trolox equivalent (TE)/g dry weight (DW), [3]% TBARS inhibition. Results followed by different letters in the same column were significantly different (P<0.05).

3.4. Evaluation of antiglycation potential

3.4.1. REM test

REM test was performed using the crude extract at 250, 500, and 1000 µg/mL (Lane 1, 2, and 3, respectively) and triterpene saponin fraction at 10, 50, and 100 µg/mL (Lane 4, 5 and 6, respectively). BSA (A), BSA+ribose (B), and BSA+ribose+AMG (C) were used as standard protein, glycated protein, and antiglycation standard, respectively. As shown in Figure 2, the crude extract and triterpene saponin fraction of *S. saponaria* presented protection against protein glycation. BSA treated with the crude extract or triterpene saponin fraction showed an electrophoretic profile similar to BSA treated with AMG (C).

3.4.2. Inhibition of AGE formation

In the evaluation of inhibition of AGE formation, a concentration-dependent activity was observed (Figure 3). The crude extract of *S. saponaria* showed 34.48% inhibition at 1000 µg/mL, which did not significantly differ from the triterpene saponin fraction at 10

μg/mL (39.23%). However, the greatest inhibitory activity of AGE formation was observed for the triterpene saponin fraction at 100 μg/mL (61.85%).

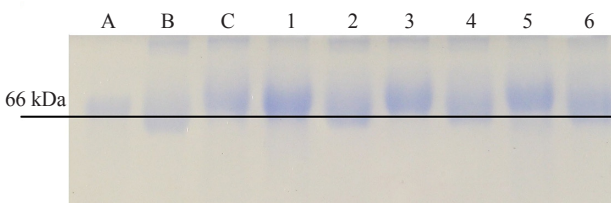

Figure 2. Relative electrophoretic mobility test for the evaluation of antiglycation potential. A=BSA; B=BSA+ribose; C=BSA+ribose+AMG; 1=BSA+ribose+CESs (250 μg/mL); 2=BSA+ribose+CESs (500 μg/mL); 3=BSA+ribose+CESs (1 000 μg/mL); 4=BSA+ribose+TSSs (10 μg/mL); 5=BSA+ribose+TSSs (50 μg/mL) and 6=BSA+ribose+TSSs (100 μg/mL). BSA: bovine serum albumin, AMG: aminoguanidine.

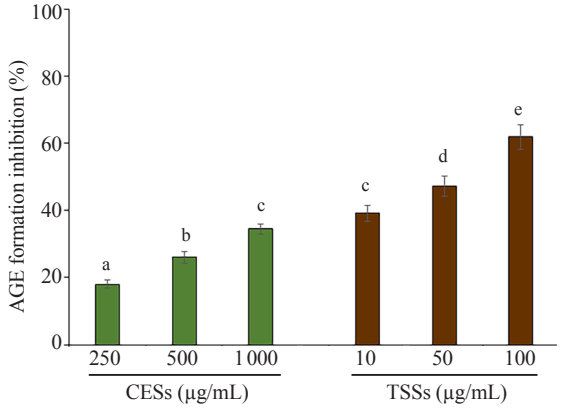

Figure 3. Inhibitory activity of the CESs and TSSs on AGE formation. Different letters indicate significant difference (P<0.05). AGE: advanced glycation end product.

3.5. Antiacne activity

The crude extract and triterpene saponin fraction showed antiacne activity as the MIC against *C. acnes* was equal to the MBC. The crude extract presented MIC of 36.11 μg/mL, whereas the triterpene saponin fraction presented MIC of 18.34 μg/mL. The activities of the crude extract and triterpene saponin fraction were approximately 400-fold and 200-fold lower than the positive control (MIC=0.09 μg/mL), respectively.

3.6. Photoprotective activity

Figure 4A shows the spectrophotometric profiles of the crude extract and triterpene saponin fraction of *S. saponaria* compared to OMC, used as the positive control. The crude extract showed the maximum absorbance of 1.42 at 1 000 μg/mL at 260 nm and reached 0.20 at 400 nm, while triterpene saponin fraction demonstrated the maximum absorbance of above 2.80 at 100 μg/mL at 260 nm and reached 1.30 at 400 nm. The results of triterpene saponin fraction at 100 μg/mL were similar to the maximum absorbance presented by OMC within the range evaluated (260 to 400 nm). Figure 4B shows the SPF of CESs was 7.78 at 1 000 μg/mL, which showed no significant difference from triterpene saponin fraction at 10 μg/mL (8.89); however, the greatest SPF of triterpene saponin fraction was obtained at 100 μg/mL (14.89).

4. Discussion

Triterpenes are commonly found in different plant parts such as peels, leaves, stems and barks, flowers, and mainly fruits and

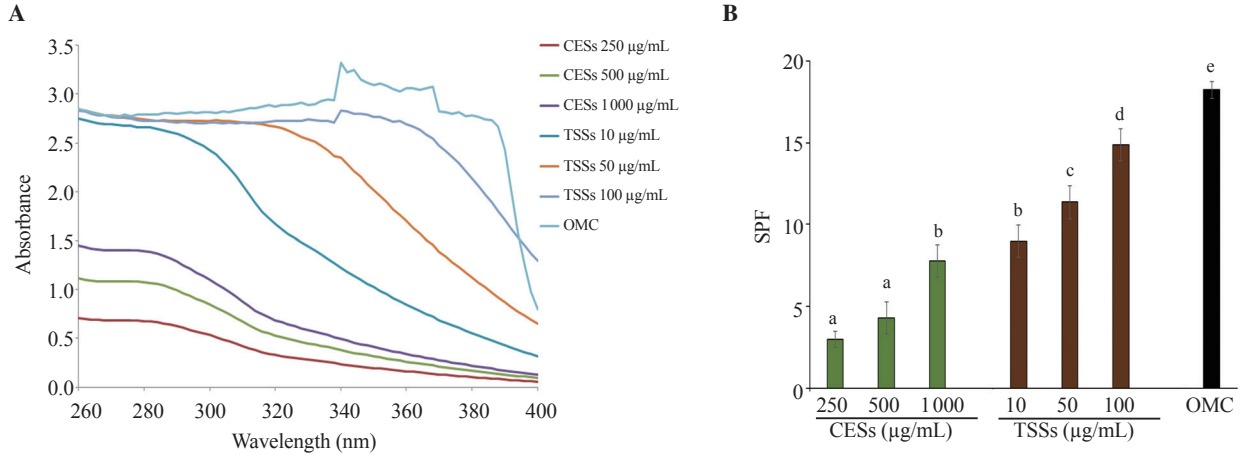

Figure 4. Photoprotective activity of the CESs and TSSs. (A) UV-visible absorption spectra and maximum wavelength of CESs and TSSs of *Sapindus saponaria* fruits at different concentrations (CESs: 250, 500, and 1 000 μg/mL; TSSs 10, 50, and 100 μg/mL) compared to the positive control octyl methoxycinnamate (OMC). (B) Spectrophotometric determination of sun protection factor (SPF) of the CESs and TSSs. Different letters indicate significant difference (P<0.05).

seeds; however, *S. saponaria* is one of the species that present high triterpenoid content in its fruits. TTC obtained in this study was similar to results reported in the previous study performed by Gasca *et al*[26].

The presence of different triterpene saponins (hederagenin and oleanolic acid) was identified in the fraction of methanolic extract of *S. saponaria* fruits by HPLC-MS. These results corroborate the results of previous studies conducted by Murgu and Rodrigues-Filho[27] that performed dereplication of glycosides from *S. saponaria* using LC-MS and observed the presence of saponins in the fruit extract of this species. These results also corroborate the studies performed by Rodríguez-Hernández *et al*.[2] and Gasca *et al*.[26] that showed the presence of triterpene saponins equal and similar to those observed in the present study; however, they evaluated different extracts and plant parts.

The present study showed high antioxidant potential of CESs and TSSs in DPPH radical scavenging, iron ion chelation, and lipid peroxidation inhibition assays, highlighting that TSSs presented the highest activities at 100 µg/mL. The results of the antioxidant tests are similar to the results of studies carried out by Singh and Kumari[28] and Bhuvaneswari *et al*.[29] that evaluated extracts from leaves and fruits of other species of *Sapindus* genus.

In the present study, XO inhibitory activities of the crude extract and triterpene saponin fraction of *S. saponaria* fruits were evaluated using the *in vitro* enzymatic hypoxanthine-xanthine oxidase (HX–XO) system. A recent study performed by Wippich *et al*.[30] reported that XO converts HX or xanthine to uric acid, H_2O_2, and O_2^-, which can cause hyperuricemia. Bou-Salah *et al*.[31] demonstrated that XO enzyme catalyzes uric acid formation, which, if in excess, can lead to an increase in superoxide (O_2^-) and ROS during the catabolism of purines, and consequently cause oxidative stress, mutagenesis, and cancer. Studies carried out by Quy *et al*.[32], and Ye *et al*.[33] reported that natural products present XO inhibitory activity and inhibition of O_2^- formation. According to Cicero *et al*.[34], O_2^- generated by the HX-XO system reacts with nitroblue tetrazolium (NBT) to produce formazan (NBT reduction), which can be identified and measured by a spectrophotometer. The authors report that compounds that lower O_2^- concentration and do not affect uric acid formation are considered superoxide selective scavengers. On the other hand, compounds that lower both O_2^- and uric acid are considered XO inhibitors.

According to the antiglycation results observed in REM test, the electrophoretic profiles of treatments with extract and/or fraction are similar to the treatment with AMG (antiglycation standard). Previous studies report that reducing or mitigating the production and accumulation of glycation products is associated with the prevention and treatment of diseases such as diabetes and different types of cancers[35]. Study carried out by Guguloth *et al*.[36] demonstrated *in vivo* antidiabetic effects of *S. saponaria* leaf extracts. On the other hand, studies performed by Sachin *et al*.[4] and Stadlbauer *et al*.[37] showed the effect of other species of genus *Sapindus* on the damage caused by diabetes. Thus, the antiglycation results presented in this study may be directly correlated with the inhibition of protein glycation and a decrease in physiological, cellular, and molecular damages caused by diabetes.

Moreover, both CESs and TSSs were able to inhibit AGE formation. Studies and reviews performed by Luo *et al*.[6], Vatner *et al*.[7] and Mehdi *et al*.[5] have suggested that oxidative processes play a major role in accelerating the formation of AGEs. In addition, AGEs are involved in auto-oxidation reactions producing free radicals and other reactive intermediates and stimulating generation of ROS. Inhibitors of AGE formation decrease the production and release of free radicals in the body, protecting against damages caused by oxidative stress, preventing premature aging, and presenting therapeutic effects on different degenerative diseases such as diabetes, cancer, and Alzheimer's. Thus, the results of the inhibitory activity of CESs and TSSs on AGE formation provide important information for future studies on the damage caused by high AGE production in diabetic patients and premature aging.

C. acnes is assumed to be responsible for the development of inflammation and acne. According to Thinh *et al*.[38], MIC threshold is commonly attributed to the value of 10 µg/mL; however, MIC values between 10 and 100 µg/mL are considered good activity. Thus, both crude extract and triterpene saponin fraction of *S. saponaria* fruits can be considered good antimicrobial agents against *C. acnes*. These results corroborate the study carried out by Garcia *et al*.[39] that demonstrated antimicrobial activity of extracts from different parts of *S. saponaria*. Similarly, Wei *et al*.[40] demonstrated that *Sapindus mukorossi* presented antibacterial potential, highlighting its bactericidal activity against *C. acnes*.

In the present study, CESs and TSSs showed photoprotective activity against UV-B. A recent study performed by Li *et al*.[41] investigated the photoprotective effect of natural products and observed that plant extracts can protect the skin from UV-B radiations due to the presence of compounds with UV absorbing capacity and pharmacological properties such as antioxidant activity. A previous study performed by Gasca *et al*.[26] demonstrated that the extracts and fraction of *S. saponaria* present high triterpene saponins content, which was also reported by Nafiunisa *et al*.[42] that evaluated other species from Sapindaceae family. Diaz-Barradas *et al*.[43] demonstrated that different triterpenes from plant extracts presented photoprotective activity.

This study presents important information about the photoprotective, antioxidant, antiglycation, and antiacne potential of the crude extract and triterpene saponins fraction of *S. saponaria* fruits, a species of the genus *Sapindus* that has been poorly studied so far. The results showed promising antioxidant potential of the crude extract and triterpene saponins fraction in different assays.

Moreover, they demonstrated antiglycation potential in inhibiting BSA glycation and AGE formation. In addition, the results revealed that the crude extract and triterpene saponins fraction showed antibacterial action against *C. acnes* and photoprotective action against damages caused by UV radiation (A and B). HPLC-MS analysis demonstrated that the main active compounds of the plant extract and fraction are triterpene saponins, mainly hederagenin and oleanolic acid, which latter may be responsible for the antioxidant and antiglycation activities, as demonstrated by Franco *et al*.[44] and Song *et al*.[45] that observed that triterpene saponins obtained from other plant species presented these activities.

The limitation of this study is that these results were obtained in *in vitro* assays. Therefore, *in vivo* studies are needed to evaluate its clinical use and provide a possible pharmaceutical and/or cosmetic application.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

This study was supported by the São Paulo Research Foundation Grant - FAPESP No. 2013/10039-1.

Authors' contributions

RMGS and GRM conceived and designed the study. LMBN and CCMF performed the experimental work. LPS and PSS analyzed the results. FOG and RMGS wrote the manuscript. All authors read and approved the manuscript.

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