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Research Article

Salvia extracts: Unraveling phenolic compounds and assessing their antiglycation, anti-inflammatory, and cytotoxic properties

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Abstract

Introduction: This study aimed to identify compounds present in the methanolic extract (ME) of four *Salvia* species *S. aegyptiaca* (SAE), *S. verbenaca* (SVE), *S. barrelieri* (SBA), and *S. argentea* (SAR), using HPLC ESI-QTOF MS/MS, and to evaluate their antiglycation, anti-inflammatory, and cytotoxic effects for both methanolic and decoction extracts (DE). The research focused on exploring the phytochemical profile and biological activities of Salvia species, which are known for their medicinal properties.

Materials and Methods: HPLC ESI-QTOF MS/MS was employed to identify phenolic compounds in the extracts. Antiglycation activity was assessed using a model system, while cytotoxicity was evaluated using two cell lines: mouse fibroblast cells (3T3) and human cervical cancer cells (HeLa).

Results: Four major phenolic compounds were identified in all four plants: caffeoyl-O-hexoside (glucoside), rosmarinic acid, derivatives of apigenin, and an isomer of p-coumaroylquinic acid. Additionally, luteolin-7-O-glucoside was detected in all extracts except SAE. All extracts demonstrated significant antiglycation efficacy, with inhibition efficiency exceeding 69% at 2 mg/mL. Notably, the methanolic extract of *S. barrelieri* (ME SBA) exhibited the highest activity, achieving an IC50 of approximately 35 µg/mL. Cytotoxicity testing revealed weak and insignificant effects for decoction extracts on 3T3 cells, whereas slight proliferation was observed with methanolic extracts. Similarly, most extracts showed no toxicity toward HeLa cells, except for the decoction extract of *S. verbenaca* (ED SVR), which exhibited some cytotoxicity.

Discussion: The presence of common phenolic compounds across the studied Salvia species highlights their potential as sources of bioactive molecules. The observed antiglycation activity suggests these extracts could be beneficial in preventing glycation-related diseases. However, the cytotoxicity results indicate that further optimization may be required to enhance their therapeutic potential.

Conclusion: This study successfully identified key compounds in Salvia species and demonstrated their notable antiglycation properties. While cytotoxic effects were minimal, the findings underscore the potential of these plants as natural remedies for specific health conditions, warranting further investigation into their pharmacological applications.

Graphical abstract

Keywords

Salvia species; AGE inhibition; Anti-inflammatory; cytotoxic effect; HPLC-ESI-QTOF

Introduction

Diabetic individuals who continue to have high blood sugar might develop serious problems such as nephropathy, retinal disease, neuropathy, and atherosclerosis. The development of glycoltoxines that address the glycation problem alleviates these consequences (AGE: advanced glycation end products). These latter ones are heterogeneous compounds that were produced non-enzymatically and by oxidation as a result of their reactions with reduced sugars, and they can alter proteins, lipids, and nucleic acids (Chinchansure et al. 2015) One of the aging processes, along with protein glycation and AGEs (advanced glycation end products), is thought to exist. The development of AGEs, which are thought to play a role in the pathophysiology of diabetes and aging-related problems, are caused by a sequence of non-enzymatic interactions between the carbonyl group on reducing sugars and the amine group on proteins (Chinchansure et al. 2015; Rahbar and Figarola 2002). Several studies have demonstrated the inhibition of AGEs using natural products such as propolis (Xavier et al. 2017), bee venom (Behroozi et al. 2014), bacteria (Prastya et al. 2019), fungi (Yap et al. 2018), and medicinal plants (Agawane et al. 2019; Deo et al. 2016; Mahomoodally et al. 2019).

Inflammatory responses, ROS, macrophages, and human neutrophils are tightly related. In order to promote their use in the treatment of chronic inflammatory disorders, there was rising interest in natural antioxidants found in medicinal plants that can serve as scavengers by lowering the impact of ROS and free radicals. Neutrophils and macrophages are crucial for the control of the inflammatory response in addition to the defence against bacteria and other pathogens. Superoxide was produced in enormous quantities by stimulated neutrophils' NADPH oxidase, a precursor to hydrogen peroxide and other reactive oxygen species such as hypochlorous acid, which was a highly microbicidal species produced by myeloperoxidase (Winterbourn et al. 2016).

Algeria, one of the richest Arab countries, contains 3164 plant species, making it one of the largest collections of medicinal plants. Locals and traditional healers utilize these plants to treat a variety of diseases (Benarba 2016). Salvia genus plants have been used for food and traditional medicine for a very long time. A screening of *S. aegyptiaca*, *S. verbenaca*, *S. barrelieri* and *S. argentea* revealed that the majority of them showed potent anti-inflammatory, anticancer, antibacterial, and antioxidant properties (Imanshahidi and Hosseinzadeh 2006; Mamache et al. 2020; Tohamy et al. 2016).

In vitro techniques were used in this work to evaluate the phytochemical composition and potential of two distinct extracts of the aerial parts of *S. aegyptiaca*, *S. verbenaca*, *S. barrelieri*, and *S. argentea* as antiglycan and anti-inflammatory regulating agents.

Materials and Methods

Plant material collection

During the months of May and June, the wilayas of Batna, Sétif, Jijel, and Borj BouArreridj, respectively, harvested the medicinal herbs: *S. aegyptiaca* (SAE, Voucher number 104 SO 27/4/16 BAT/SA/HL), *S. verbenaca* (SVE, 103 SV 7/5/16 SET/SA/HL), *S. barrelieri* (SBA, 102 SB 7/5/16 JIJ/SA/HL), and *S. argentea* (SAR 105 SA 7/5/16 JIJ/SA/HL). Prof. Laouer H. (University of Sétif 1) identified the plants after the harvest. The plants were dried for 10 days in the open and in the dark to guarantee their proper preservation. SAE, SVE, SBA and SAR were then processed via an electric grinder to create a fine powder, which was subsequently kept in sterile bags.

Preparation of extracts

By macerating the plant powder in 85% methanol at a rate of 15% (W/V) for 7 days at room temperature, the methanolic extracts were produced. The filtrate was concentrated in a rotary steamer of the BUCHI type under vacuum after filtering through muslin and filter paper. After that, the resulting extracts were fully dried (Markham 1982).

According to Perera et al. (2008), the process of producing the aqueous extracts involves decocting the plant powder in distilled water at a rate of 3% (W/V). A heated plate was used to set the combination, and it was allowed to boil there until the volume of the solution was reduced to 1/8th of its original size. The filtrate was thoroughly dried after being filtered through muslin and filter paper.

HPLC-QTOF MS/MS Analysis

Salvia extracts were reanalysed chromatographically using HPLC-QTOF-MSMS, using an Agilent Poroshell 120 EC-C18 type column (3.0 x 50 mm, 2.7 m) with an Agilent 3000 infinite series chromatograph (Agilent Technologies, Santa Clara, CA, USA) connected to a Dionex thermoscientific ultimate 3000 mass spectrometer (QTOF/MSMS). The mobile phase was made up of a combination of the two solvents A (demineralized water) and B (methanol) in a gradient that goes from 100% A for the first ten minutes to 10% A for the next fifteen minutes and 90% B for the last fifteen minutes. The volume of the injection was 10 µL. The column temperature was 30°C, and the solvent flow rate was 0.5 mL/min. The HPLC-QTOF/MSMS equipment uses a 4500 V capillary voltage with H-identification and a negative (multi-range MCP detector) ionization mode with nitrogen gas flowing at a rate of 8.0 mL/min.

Antiglycation activity

The AGE fluorescent dye dosing method mediated by fructose was used to investigate the antiglycation activity of the study extracts (Matsuda et al. 2003). DMSO-dissolved plant extract (2 mg/mL), human serum albumin (10 mg/mL), 0.5 M fructose, and phosphate buffer 0.1 M (pH 7.4) with 0.1 M sodium azide as an antibacterial agent were combined in a 96-well microplate. Seven days were spent heating the reaction mixture to 37 °C. Following that, a microplate reader was used to measure the fluorescence between 330 and 440 nm (SpectraMax M2, Molecular Devices, USA). Rutin was employed as the benchmark. Calculating the % inhibition was done as follows (formula 1):

$$\%\,Inhibition = \frac{SampleFluorescence}{ControlFluorescence}\ x\ 100 \quad (1)$$

Using Ez-fit software, the extract with an activity greater than 50%was was tested again to determine the IC_{50} (Perrella Scientific, USA).

Anti-inflammatory activity (inflammatory Burst)

The procedure outlined by Helfand et al. (1982) was followed for conducting the luminol-mediated chemiluminescence experiment. Briefly, 25 µL of diluted human blood (collected from volunteers who were free from any diseases and had not consumed any medication for at least three weeks) in $HBSS^{++}$ media (Hanks medium including $MgCl^2$ and $CaCl^2$). UDC was incubated with 25 µL of various test solution concentrations on a white surface microplate (Costar, NY, USA). The reaction mixture was incubated for 15 minutes at 37° C in a thermostat luminometer chamber (Labsystems, Helsinki, Finland). After incubation, 25 µL of intracellular ROS detector (Luminol) and 25 µL of opsonized zymosan serum were added to each well except from the blank, which contains just $HBSS^{++}$. For 50 minutes, the luminometer measures the various ROS production levels in the cells in terms of relative light units. Ibuprofen as a typical anti-inflammatory drug was used as a standard.

Cytotoxicity on cell line

Using the colorimetric test with the MTT standard (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl- tetrazolium bromide), the cytotoxic activity of the various plant extracts was assessed in 96-well flat-bottomed microplates (Mosmann 1983). In order to accomplish this, 3T3 and HeLa cells (mouse fibroblast and human cervical cancer cells, respectively, obtained from the Panjwani Center for Molecular Medicine and Drug Research) were cultured in modified medium (Dulbecco Eagle) with 5% fetal bovine serum, 100 IU/mL penicillin, and 100 g/mL streptomycin in a 75 cm^2 flask and maintained in an incubator at 5% CO^2 and 37 °C. The cultivated cells were gathered, measured with a hemocytometer, diluted in their own media at a concentration of 5.10^4 cells/mL, and then added to microplates at a rate of 100 µL for each well. The media in the wells was withdrawn after an overnight incubation and replaced with 200 µL of brand-new medium containing various amounts of plant extracts. Each well received 200 µL of MTT (0.5 mg/mL) after 48 hours, which was then added and incubated for a further 4 hours. The addition of 100 µL of DMSO stopped the process. Using a microplate reader, the rate of MTT to formazan reduction was measured spectrophotometrically at 570 nm (Spectra Max plus, Molecular Devices, CA, USA). The formula 2 used to determine the cytotoxicity.

$$\%Inhibition = \frac{100 - ((mean\ Abs\ test\ solution - mean\ Abs\ negative\ control\ solution)}{mean\ Abs\ positive\ control\ solution - mean\ Abs\ negative\ control\ solution} x\ 100 \quad (1)$$

Software called Soft-Max Pro was used to determine the findings (% inhibition) (Molecular Device, USA).

Statistical Analysis

Each sample was analyzed three times. Using GraphPad Prism Version 8.0.2, the results are given as means with standard deviation (SD) and evaluated using one-way analysis of variance (ANOVA) and Tukey's test (GraphPad Software, Inc, USA). Significant differences were determined to exist when the p values were > 0.05.

Results

HPLC-QTOF MS/MS analysis

The chromatography analysis of the methanolic extracts of the aerial parts of SAE, SVR, SBA and SAR plants obtained by the LC ESI-QTOF MS/MS method showed the presence of several products, 42 of which were identified. All these compounds were identified by interpreting their MS and MS/MS spectra obtained using literature and online databases. These compounds shown in Table 1. The identification of biological compounds made it possible to observe the great diversity of these species in these products. Indeed, four major phenolic compounds in common had been identified for the four plants studied: caffeoyl-O-hexoside (glucoside) (m/z: 341, Rt 0.8 to 0.85 min), rosmarinic acid (m/z: 359.03, Rt: 8.69 at 8.74 min), derivatives of apigenin (m/z: 269.01, Tr: 10.70 at 10.79 min), and an isomer of p-coumaroylquinic acid (m/z 339.192, Rt: 14.66 at 14.95 mins). In addition, traces of palmitic acid (m/z 255) were observed in a retention time range of 14.24 to 14.31 min. Another compound luteolin-7-O-glucoside (m/z: 4.77) was observed in the retention time of 8.30 to 8.65 min for all the extracts of the plants studied except the SAE extract, whereas luteolin-4-O-glucoside (m/z: 477.04) and methyl luteolin glucuronide (m/z: 517) were observed only in SVR and SAR at 9.21 and 9.187 min, respectively.

Table 1. Bioactive compounds identified by QTOF ESI-MS/MS in methanolic extract of Salvia species Statistical indicator

N	Rt (min)	m/z	SAE	SAR	SVR	SBA	Name of Compound
			Fragments (%)	Fragments (%)	Fragments (%)	Fragments (%)	
1	0.80 SAE 0.84 SAR 0.80 SVR 0.85 SBA 1.34 SBA	341,086	179.036 (25.57) 341.068 (100) 342.072 (10.16)	179.036 (25.57) 341.068 (100) 342.072 (10.16)	179.036 (25.57) 341.068 (100) 342.072 (10.16)	179.036 (29.28) 341.068 (100) 342.072 (11.78)	Caffeoyl-O-hexoside
2	7.04 SAE	639,04	301.001 (44.44) 464.034 (23.67) 477.09 (87.75) 639.041 (98.01)	Abs	Abs	Abs	Quercitin-3-O-glucoronide-hexose or Isorhamnetin-3, 7-di-O-glucoside
3	7.60 SVR	539,05	Abs	Abs	161.006 (100) 179.014 (51.95) 359.034 (30.22) 519.094 (90.67) 537.024 (72.42)	Abs	Rosmarinic acid glucoside (Isomer 3)
4	7.89 SAE 7.88 SBA	717,06	339.01 (45.51) 475.045 (100) 519.020 (42.24)	Abs	Abs	339.01 (32.27) 475.045 (100) 519.020 (54.45) 717.06 (6.88)	Salvianolic acid B (Isomer 1)
5	8.18 SAE	719,07	161.005 (12.41) 339.01 (46.79) 365.025 (18.39) 475.045 (100) 519.020 (65.91) 719.068 (10.31)	Abs	Abs	Abs	Salvianolic acid B (Isomer 1)
6	8.30 SAR 8.50 SAR 8.65 SAR 8.58 SVR 8.31 SBA	447.0 4461.02		285.001 (51.61) 447.038 (100)	285.001 (100) 461.015 (12.51)	285.001 (52.03) 447.038 (100) 448.42 (20.40)	Luteoline-7-O-glucoside
7	7.48 SAE	593,08	369.015 (5.04) 473.50 (9.22) 593.076 (100) 594.80 (31.91)	Abs	Abs	Abs	Apigenin-6,8-di-C-glucoside
8	8.74 SAE 8.74 SAR 8.69 SVR 8.72 SBA	359,03	161.006 (100) 179.014 (20.01) 197.023 (54.14)	161.006 (100) 179.014 (21.15) 197.023 (52.10)	161.006 (100) 179.014 (21.15) 197.023 (52.10)	161.006 (100) 179.014 (21.20) 197.023 (49.83)	Rosmarinic acid
9	8.95 SAR 9.18 SAR	475,03	Abs	161.001 (8.64) 299.021 (100) 300.024 (12.72) 475.028 (11.97)	Abs	Abs	Quercétine-O-glucuronide
10	8.96 SAR 8.99 SBA	741,05	Abs	179.015 (9.73) 359.034 (7.43) 381.013 (100) 382.016 (13.56) 579.035 (6.85) 741.050 (13.09)	Abs	179.015 (12.18) 359.034 (6.32) 381.013 (100) 382.016 (16.41) 579.035 (7.74) 741.050 (7.72)	Rosmarinic acid derivative
11	9.05 SAE	361,12	161.006 (100) 179.014 (16.52) 197.023 (53.17)	Abs	Abs	Abs	Rosmarinic acid isomer
12	9.06 SVR	431,05	Abs	Abs	269.015 (15.85) 431.05 (100)	Abs	Apigénine 7-O-glucoside
13	9.15 SBA	447,04	Abs	Abs	Abs	269.014 (100) 285.001 (35.65) 447.038 (20.32)	Apigénine glucose
14	9,18 SAR	517,04	Abs	285.00 (5.82) 299.02 (100) 300.24 (14.86) 413.03 (9.57)	Abs	Abs	Hispdilin glucose
15	9.21 SVR	447,04	Abs	Abs	269.014 (11.08) 285.001 (100) 447.038 (13.36)	Abs	Luteoline-4'-O-glucoside
16	9,62 SAR	300	Abs	283.998 (5.82) 299.02 (100) 300.024 (16.08)	Abs	Abs	Hispiduline, or Diosmétinee

Table 1. Bioactive compounds identified by QTOF ESI-MS/MS in methanolic extract of Salvia species Statistical indicator (ending)

N	Rt (min)	m/z	SAE	SAR	SVR	SBA	Name of Compound
			Fragments (%)	Fragments (%)	Fragments (%)	Fragments (%)	
17	10.03 SAR 10.05 SVR 10.02 SBA	582,19	Abs	342.105 (28.35) 462.147 (100) 463.149 (28.74) 582.187 (67.16)	342.105 (28.35) 436.171 (9.37) 462.147 (100) 463.149 (30.83) 582.188 (64.85)	342.105 (23.96) 462.147 (100) 463.149 (24.08) 582.188 (72.96)	Tri-coumaroyl spermidine
18	10.17 SAR 10.16 SBA	285,01	Abs	285.007 (100)	Abs	285.007 (100) 286.10 (16.40)	Lutéoline
19	10.29 SAE	717,05	339.009 (47.57) 359.034 (6.38) 519.020 (100)	Abs	Abs	Abs	Salvianolique acid E
20	10,39 SAR	517,04	Abs	283.998 (6.92) 299.02 (82.20) 300.024 (14.96) 457.021 (100) 458.024 (27.71) 517.034 (22.11)	Abs	Abs	Hispiduline ou diosmétinee Derivative
21	10.79 SAE 10.71 SAR 10.72 SVR 10.70 SBA	269,01	269.014 (100) 270.017 (14.84)	269.014 (100) 270.017 (14.84)	269.014 (100) 270.017 (14.84)	269.014 (100) 270.017 (14.57)	Apigenin derivative
22	11.61 SAE	503,28	503.276 (100) 504.279 (26.55)	Abs	Abs	Abs	Madecassic Or hypericine
23	12,08	283,03	Abs	Abs	Abs	268.006 (68.01) 269.009 (11.25) 283.027 (100)	Genkawanin ou Cerimaritin
24	12.24 SAE	503,28	503.276 (100) 504.279 (26.55)	Abs	Abs	Abs	Madecassique acid (98.79%) masse 345.345 hypericin 99%
25	12.75 SAE 12.99 SAR	487,28	485.268 (7.71) 487.283 (100) 488.286 (28.37)	283.117 (9.84) 485.268 (14.28) 487.283 (100) 488.286 (32.44)	Abs	Abs	Pygenic acid B b
26	13.20 SAE	487,28	485.268 (7.71) 487.283 (100) 488.286 (28.37)	Abs	Abs	Abs	Pygenic acid B
27	13.76 SAE 13.52 SVR	471,29	469.275 (100) 470.278 (30.34) 471.289 (81.89) 472.293 (25.51)	Abs	469.275 (8.30) 471.289 (100) 472.293 (28.40)	Abs	Pygenic acid Ab or Corosolic acid
28	14.01 SAE 13.98 SPJ	369,20	163.095 (15.73) 369.198 (100) 370.202 (25.93)	Abs	163.095 (16.41) 193.101 (11.54) 369.198 (100) 370.202 (27.56)	163.095 (15.98) 193.101 (11.91) 337.176 (5.27) 369.198 (100) 370.202 (26.94)	Rhodomyrtone
29	14.72 SAE 14.69 SAR 14.66 SVR 14.65 SBA	339,192	163.094 (45.48) 339.192 (100) 340.195 (31.08)	163.094 (45.48) 339.192 (100) 340.195 (31.08)	163.094 (42.04) 339.192 (100) 340.195 (25.49)	163.094 (43.04) 339.192 (100) 340.195 (25.18)	p-Coumaroyl quinnic acid isomer
30	15.05 SAE 15.25 SAR 14.81 SVR 15.03 SVR	455	455 (100)	455 (100)	453.281 (100) 454.284 (28.56)	Abs	Oleanolic acid or Ursolic acid
31	15.71 SAE 15.73 SAR 15.72 SVR	279,20	279.20 (100) 280.203 (21.35)	279.20 (100) 280.203 (21.35)	279.20 (100) 280.203 (23.21)		Linoleic acid
32	16.31 SAE 16.28 SAR 16.27 SVR 16.24 SBA	255.203.	255.203 (100) 256.206 (18.66)	. 255.203 (100) 256.206 (18.66)	255.203 (100) 256.206 (19.88)	255.203 (100) 256.206 (17.73)	Palmitic acid or Isopalmitic
33	16.69 SAR 16.69 SVR 16.65 SBA	281,22	Abs	281.215 (100) 282.219 (20.13)	281.215 (100) 282.219 (20.98)	281.215 (100) 282.219 (16.73)	Trans vaccinic acid or Oleic acid

Note: SAE – S. aegyptiaca; SAR – S. argentea; SBA – S. barrelieri; SVR – S. verbenaca

On the other hand, apigenin-7-O-glucoside (m/z: 431) and apigenin glucose (m/z: 477) were observed at 9.06 and 9.15 min, respectively, for SVR and SBA. On the other hand, apigenin-6,8-di-C-glucoside (m/z: 593, Rt: 7.48) was eluted only with SAE. A characteristic molecule of the genus *Salvia*, salvianolic acid B (m/z: 717, Rt: 7.89) and its isomer (m/z: 719, Rt: 8.18) were observed only in SAE and SBA. Unlike other extracts, SAE was characterized by the presence of a few minor compounds such as isorhamnetin-3,7-di-O-glucoside (m/z: 639, Rt: 7.04 min), the isomer of rosmarinic acid (m/z: 361.12, Rt: 9.05), salvianolic acid E (m/z: 717.05, Rt: 10.29), and madecassic acid (m/z: 503.28, Rt: 12.24 min).

Inhibition of glycation

The inhibition of glycation of the different extracts studied at 2 mg/mL was significant and almost comparable for the two types of extract for each plant ($P > 0.05$) and varied between 69.2 and 81.8% (Fig. 1). The efficacy of the extracts used at this dose (2 mg/mL) remained lower than that of rutin with 99.6% ($P > 0.0001$).

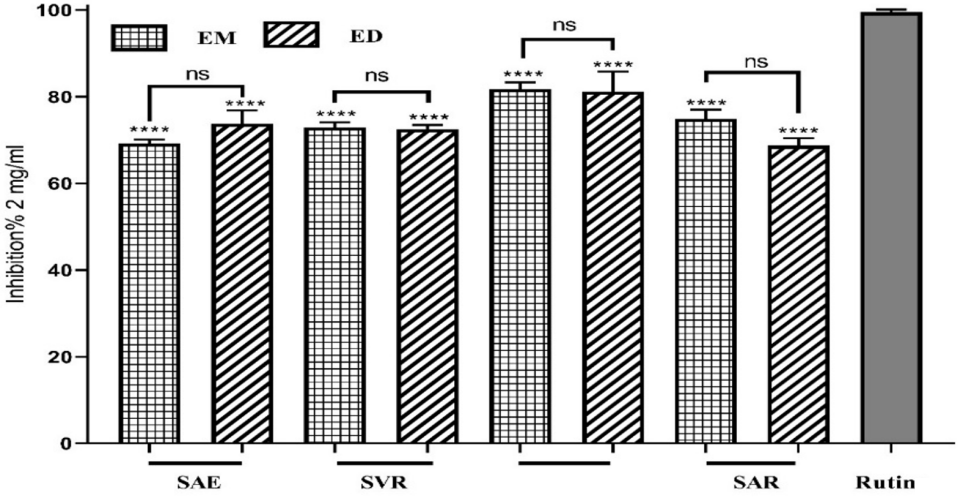

Figure 1. Antiglycation activity of different plant extracts of genus Salvia at 2 mg/mL. ***Note:*** SAE – *S. aegyptiaca*; SAR – *S. argentea*; *SBA – S. barrelieri*; SVR – *S. verbenaca*; Me – methanolic extract, DE – decocted extract. Results were represented as mean of % ± SD (n=3) (**** $P \leq 0.0001$) vs Rutin as standard; ns – not significant.

In terms of IC_{50}, the metanol extracts of the plants studied always showed a high activity in comparison with the extracts obtained by decoction ($P>0.0001$). Indeed, the most effective inhibition was observed with SBA extracts with IC_{50} values of 70.0 ± 0.1 and 90.1 ± 0.5 µg/mL for ME SBA and DE SBA, respectively, followed by the different extracts according to this order: ME SVR > ME SAE > DE SVR > ME SAR > DE SAE. The recorded IC_{50} values were 100.0 ± 0.1, 110.0 ± 0.001, 170.0 ± 0.6, 190.0 ± 0.3 and 210.0 ± 4.0 µg/mL, respectively. DE SAR shows the lowest activity (360 ± 0.4 µg/mL). None of the extracts showed significant activity in comparison with rutin (IC_{50}: 20.0 ± 0.4 µg/mL, $P \leq 0.0001$) (Fig. 2).

Anti-inflammatory activity (inflammatory Burst)

Anti-inflammatory activity was monitored by measuring the luminescence produced following oxidation of luminol by reactive oxygen species generated by immune cells. The inhibition of immune cells by the different extracts studied leads to a reduction in the light emitted. Indeed, the extracts prepared from SAE, SBA and SAR showed very significant activities and even comparable with that of ibuprofen used as a standard (IC_{50}: 12.0 ± 0.003 µg/mL, $P > 0.05$) (Fig. 3). The ME SAE and ME SBA extracts (IC_{50} 39.5 ± 4.6 and 35.4 ± 3.1 µg/mL) showed a significant and comparable effect with the same extracts obtained by decoction with the following IC_{50} 38.1 ± 0.8 and 38.1 ± 3.1 µg/mL. The most remarkable inhibitory activity was obtained with ME SAR with an IC_{50} of 28.8 ± 1.6 µg/mL. On the other hand, the weakest effects were obtained with the EM SVR and ED SVR extracts with IC_{50} values of the order of 88.6 ± 0.5 and 261.54 ± 22.81 µg/mL.

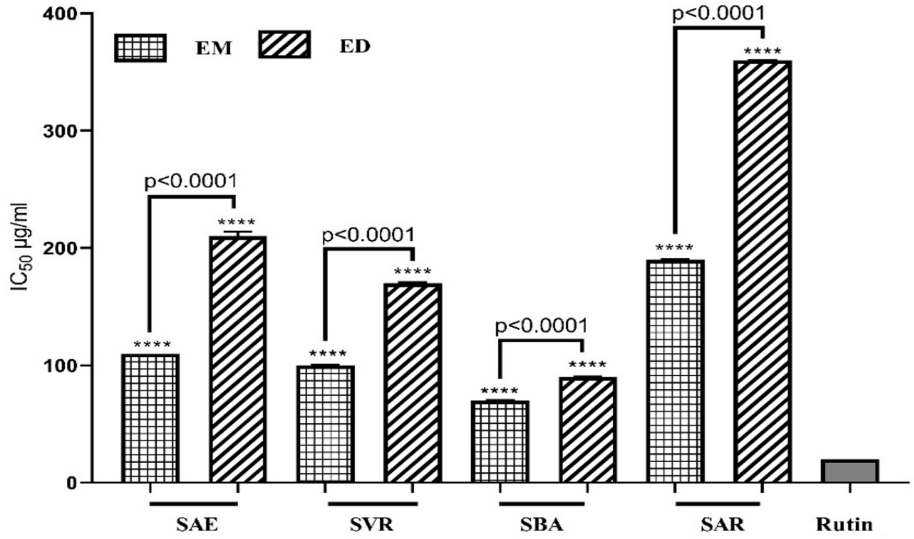

Figure 2. Antiglycation activity of different plant extracts of the genus Salvia. ***Note:*** SAE – *S. aegyptiaca*; SA –: *S. argentea*; SBA – *S. barrelieri*; SVR – *S. verbenaca*; Me – methanolic extract, DE – decocted extract. Results are represented as a mean of % ± SD (n=3) (**** P≤ 0.0001) vs Rutin as standard; ns – not significant.

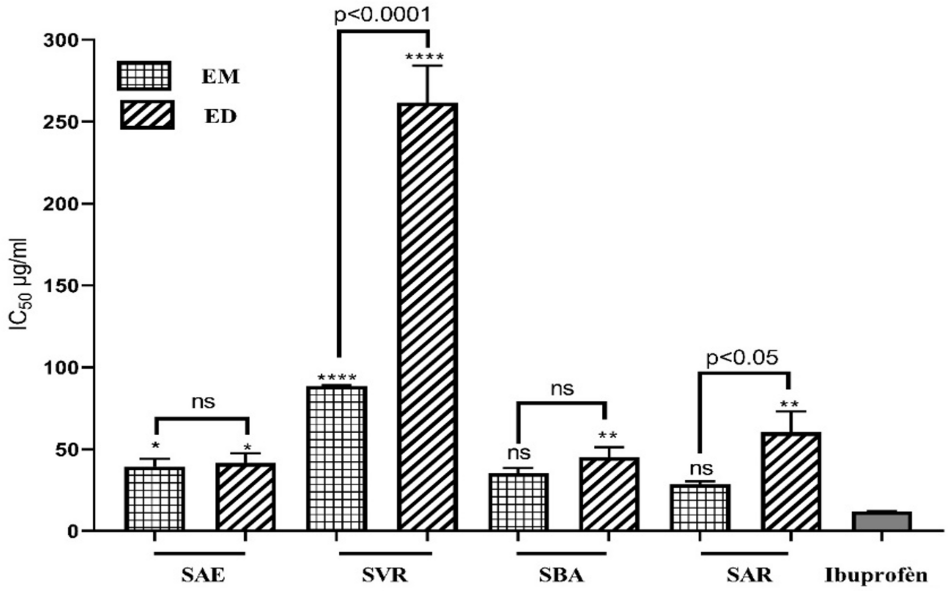

Figure 3. Anti-inflammatory activity (respiratory outbreak) of different plant extracts of the genus Salvia. ***Note:*** SAE – *S. aegyptiaca*; SAR – *S. argentea*; SBA – *S. barrelieri*; SVR – *S. verbenaca*; ME – methanolic extract, DE – decocted extract. Results are represented as mean IC50 ± SD (n=3) (*P≤0.05, P≤0.002, **** P≤ 0.0001) vs Ibuprofen as standard; ns – not significant.

Cytotoxicity test

Two cell lines 3T3 and HeLa were used to test the toxicity of different plant extracts of the genus *Salvia*. Indeed, the results showed that all the decocted extracts had too weak and non-significant cytotoxic effects (P>0.05) **(**Fig. 4). Toxicity rates vary between 3.47 and 20.47%. However, a slight proliferation was observed in the presence of methanolic extracts with rates between 7.76 and 13.2% (P>0.05). Similarly, the different extracts studied did not show any toxicity towards the HeLa cell line except for DE SVR which has a toxicity of 32.5 ± 7.61%. On the other hand, a proliferation of 33.8 ± 5.82 was observed in the presence of DE SAR. A slight and insignificant proliferation was observed with the other extracts (P>0.05) (Fig. 5).

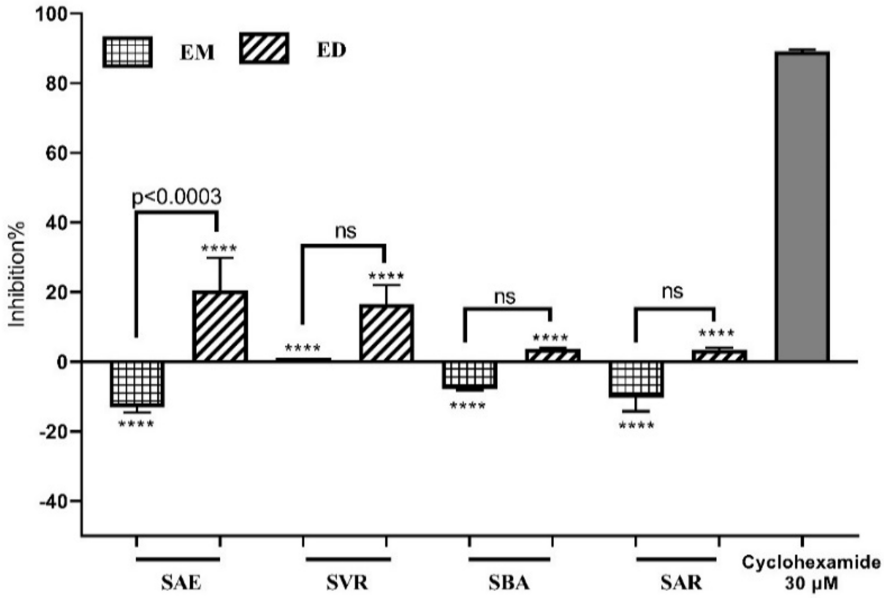

Figure 4. Cytotoxicity on the 3T3 cell line of different plant extracts of the genus Salvia. *Note:* SAE – *S. aegyptiaca*; SAR – *S. argentea*; SBA – *S. barrelieri*; SVR – *S. verbenaca*; ME – methanolic extract, DE – decocted extract. Results are represented as mean of % ± SEM (n=3) (**** P≤ 0.0001) vs Cyclohexamide as standard; ns – not significant.

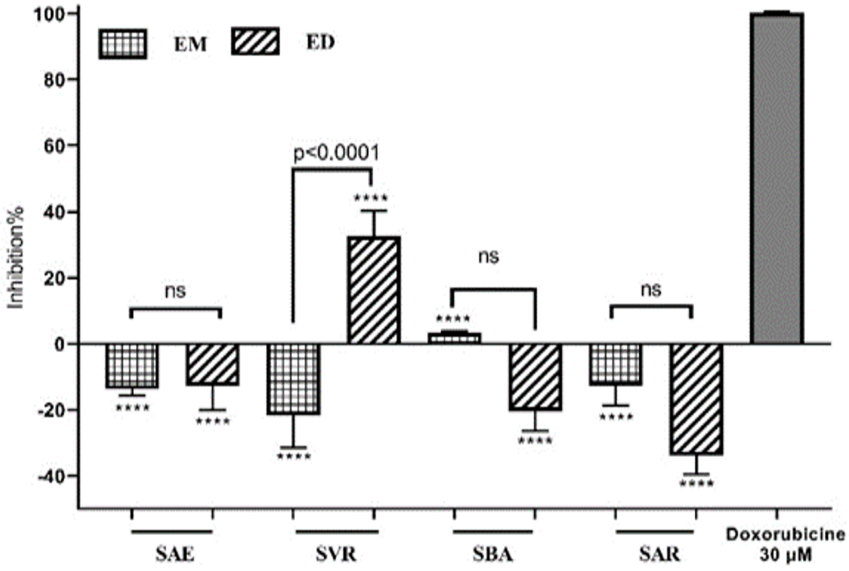

Figure 5. Cytotoxicity on the HeLa cell line of different plant extracts of the genus. Salvia. ***Note:*** SAE – *S. aegyptiaca*; SAR – *S. argentea*; SBA – *S. barrelieri*; SVR – *S. verbenaca*; ME – methanolic extract, DE – decocted extract. Results are represented as mean of % ± SEM (n=3) (**** P≤ 0.0001) vs Doxorubicin as standard; ns – not significant.

Discussion

LC-ESI-QTOF-MS/MS analysis (negative mode) identified several compounds responsible for several biological activities. The first compound in common with the four methanolic extracts was eluted after the 0.8th minute with an m/z 341.086. This compound was characterized by a fragment of m/z 179.036, which corresponds to half of the caffeic acid resulting from the loss of half glucoside (m/z: 161); this compound was identified as caffeoyl-O-glucoside (Ben Said et al. 2017; Hossain et al. 2010; Katanić

Stanković et al. 2020; Tang et al. 2020). At the 7.04th min, a compound was detected specifically for SAE with m/z 639. This compound contains a fragment of m/z 301 specific to quercetin and probably resulting from the loss of two glucose moieties (m/z 174) and glucoside (162). The attempted identification according to MoNA identified quercetin-3,4'-di-o-glucoside. However, the same compound with these m/z moieties can be identified as isorhamnetin-3,7-di-O-glucoside (Ben Said et al. 2017; Brito et al. 2014; Šulniūtė et al. 2017). A peak seen only in SVR with a mass of 539.05, the corresponding component was identified as a glucoside isomer of rosmarinic acid (Katanić Stanković et al. 2020). The peaks of a compound (m/z: 717.06 and 719.07) were observed in SAE at 7.89 and 8.18 min, respectively, while it was observed in SBA at 7.88th min; this molecule was identified as an isomer of Salvianolic acid B (Afonso et al. 2018; Ul Haq et al. 2020; Yang et al. 2015). Another type of Salvianolic acid was eluted at the 10.29th min with m/z 717.05 (339, 359 and 519); this compound was identified as Salvianolic acid E by Oliveira-Alves et al. (2017); Toplan et al. (2017); Yang et al. (2015), while it was identified as an isomer of Salvianolic acid B by Afonso et al. (2018). Except ME SAE, a peak was observed at the 8.30th to 8.65th min with an m/z 447.04, characterized by a fragment of 285 characteristic of luteolin resulting from the loss of a half of hexoside (glucoside, m/z 162); the molecule corresponding to this spike was identified as luteolin-7-O-glucoside (Afonso et al. 2018; Hossain et al. 2010; Katanić Stanković et al. 2020; Koutsoulas et al. 2019; Toplan et al. 2017; Ul Haq et al. 2020). However, the peak of luteolin alone was observed in SAR and SBA with its characteristic fragment (m/z 285). Another phenolic compound characteristic of SAE only with a mass of 593.08 and these fragments (353.22, 369.015, 383.38, 473.50, 503.33, 593.076) was identified as apigenin-6,8-di-C-glucoside (Afonso et al. 2018). The second component in common with the methanolic extracts of SAE, SVR, SAR and SBA was eluted in a time interval between 8.68 to 8.74 min with an observed mass of 359.03. This compound was characterized by fragments of m/z 161.006 and 179.014 characteristic of the glucoside and caffeic acid moieties respectively, which constitute rosmarinic acid, a major constituent of the Lamiaceae family and the genus *Salvia* (Afonso et al. 2018; Hossain et al. 2010; Katanić Stanković et al. 2020; Kontogianni et al. 2013; Koutsoulas et al. 2019; Oliveira-Alves et al. 2017; Šulniūtė et al. 2017; Toplan et al. 2017; Ul Haq et al. 2020; Yang et al. 2015). Two peaks of the same molecule were observed at the 8.95th and 9.18th min for EM SAR only with a mass of 475.03 with a major fragment 299.01; the attempt to identify this molecule with the MoNA and Meu databases made it possible to obtain 2S,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[5-hydroxy-2-(4-hydroxyphenyl)-6-methoxy-4-oxochromen-7-yl]oxyoxane acid -2-carboxylic acid and/or quercetin-O-glucuronide respectively, two compounds with great similarity. At the 9.06th min, a minor compound was detected in SVR with a mass of 431.05, characteristic by two fragments 269.015 specific to apigenin (Dehkordi et al. 2020) (also eluted at the 10.70th min in all the extracts), resulting from the loss of half of the glucoside acid, this molecule was identified as apeginin-7-O-glucoside (Afonso et al. 2018; Katanić Stanković et al. 2020; Kontogianni et al. 2013; Ul Haq et al. 2020). The HPLC analysis also made it possible to identify a compound present in the SAR at the 9.18th and 9.62nd min with m/z equal to 475.031 and 300 respectively with two fragments of 299.021 and 283.99 characteristic of hispidulin or diosmetin (Afonso et al. 2018; Ben Said et al. 2017; Koutsoulas et al. 2019; Toplan et al. 2017; Ul Haq et al. 2020), while the first compound was identified as hispidulin-7-O-glucoronide (Li et al. 2016). Another compound derived from hispidulin or diosmetin was observed at the10.39th min for the same extract because of these common fragments with the compounds identified previously (283, 299 and 300).

However, a compound was identified in the same time interval (8.21 min), but in SVE only with a mass of 447.04; the attempt to identify this compound with MoNA yielded luteolin-4 '-O-glucoside with 100% similarity. A minor compound was observed in SAR, SVR and SBA only at the 10.3rd min with a mass (m/z) 582.19 and then identified as a derivative of coumaric acid (Tricoumaroyl spermidine) using the base Meu with an 87% similarity. SBAwas characterized by the presence of a compound with a mass of 283.03, according to the literature and the MoNA database, this compound can be identified as genkwanin (Katanić Stanković et al. 2020; Koutsoulas et al. 2019; Šulniūtė et al. 2017). A major compound in common was eluted from the 14.66th to the 14.97th min (m/z: 339); this compound was characterized by the presence of fragment 163 characteristic of the p-coumaric acid moiety, resulting from the loss of moiety of quinnic acid, suggesting that it was an isomer of p-coumaroylquinnic acid (Baeza et al. 2016; Clifford et al. 2006; Zhang et al. 2018). A peak was observed at the 14.81st to the 15.03rd min in the methanolic extract except that of SBA with a mass of 455.03, similar molecules have been identified

as uleanolic acid (Koutsoulas et al. 2019; Kumar et al. 2017) or ursolic acid (Kontogianni et al. 2013; Koutsoulas et al. 2019). In addition to several molecules identified by the literature, compounds have been detected and identified by the MoNA and Meu database such as pygenic acid (m/z: 487), 6,8-dihydroxy-2,2,4, 4-tetramethyl-7-(3-methylbutanoyl)-9-(2-methylpropyl)-9H-xanthene-1,3-dione (m/z: 369) and madecassic acid (m/z: 308.28) with a similarity greater than 95%. Finally traces of fatty acids were observed from the 15.71st min such as linoleic acid (m/z: 279.20), palmitic acid (m/z: 255.203) (Yang et al. 2015) and oleic acid (m/z: 281.22, 99.9% MoNA).

The results of this study showed that all the plant extracts used have a powerful anti-glycation activity greater than 60% for the 2 mg/mL concentration. This effect translates into very remarkable IC^{50} values between 70 and 360 µg/mL. Suggest that these extracts inhibit the formation of AGEs and have therapeutic potential in patients with diabetes or aging. A recent study has shown that the methanolic extract of *S. officinalis* induces a decrease in the formation of AGEs from concentrations of 0.75 and 1 mg/mL; this inhibitionwas observed after the second week of incubation of the plant extracts with glucose and BSA (BenKhedher et al. 2020). Similarly, the methanolic extract of *S. hydrangea* has anti-glycation activity with an IC^{50} greater than 1.6 mg/mL. This activity may be related to the antioxidant power of this plant (Safari et al. 2018). The use of methanolic extracts of *S. macilenta*, *S. lachnocalyx*, *S. reuterana* and *S. sahendica* at different concentrations reduces the formation of protein carbonyl groups with fructose by protecting albumin and its thiol groups from glycation (Esmaeili et al. 2010; Tusi and Khodagholi 2014). The inhibition of the formation of AGEs by the different extracts under study can come down to the phenolic compounds present such as rosmarinic acid and carnosic acid (Govindaraj and Sorimuthu Pillai 2015; Jean et al. 2015; Ou et al. 2018; Sheng et al. 2018). These latter constituents were known to inhibit the glycolization of albumin by glucose by blocking the formation of glyoxal and methylglyoxal and/or their binding with BSA (Ou et al. 2017).

To measure the degree of ROS impacted by plant extracts, luminol was utilized as a probe. As luminol has a low molecular weight, it may enter cells and subsequently interact with intracellular ROS to determine how much ROS are produced (Jantan et al. 2011). The results showed that the two types of extract prepared from the plants of the genus *Salvia* under study exhibit an inhibitory activity in the formation of the chemiluminescent signal greater than that obtained with the aqueous extract of *S. viridis* L., *S. multicaulis Vahl*, *Stachys byzantina C. Koch* and *Eromotachys laciniata* (L.) Bunge (Erdemoglu et al. 2006). The presence of flavonoids in the produced extracts may be the cause of this action. In a prior research, it was shown that *S. Mirzayanii'*s ethyl acetate and aqueous fractions were both enriched in methoxyflavones and effectively inhibited the generation of ROS in phagocyte cells (Ayatollahi et al. 2015). One of the main components of plants in the Lamiacea family, rosmarinic acid, was present in substantial amounts in the methanolic extract of *Rosmarinus officinalis* and has an inhibitory effect on neutrophils' respiratory outbreaks (Rocha et al. 2015). As compared to other biological compounds like ascorbic acid and hydroxyquercetin, this effect was greater (Popov et al. 2013). Additional investigations have shown that other forms of flavonoids including luteolin and salvianolic acid B, extracted from the roots of *S. miltiorrhiza*, reduce ROS generation by immune cells, notably neutrophils (Ribeiro et al. 2013; Tao et al. 2018; Yang et al. 2018). These substances act by preventing NADPH oxidase, myeloperoxidase, and nitric oxide synthase from producing reactive oxygen species (ROS) within the body (Amira et al. 2012; Jiang et al. 2015; Revoltella et al. 2018; Tao et al. 2018; Yang et al. 2018; Zhou et al. 2017).

A study by Firuzi et al. (2013) demonstrated that methanolic extracts (80%) of several species of *Salvia* such as *S. aegyptiaca*, *S. aethiopis*, *S. atropatana*, *S. hypoleuca*, *S. limbata*, *S. nemorosa*, *S. sclarea*, *S. syriaca*, and *S. xanthocheila* have no effect on HL60, K562 and MCF-7 cell lines. Other studies have confirmed the non-toxicity of methanolic, ethanolic, chloroform or hexane extracts of several plants of the genus *Salvia* including *S. argentea*, *S. pratensis* and *S. officinalis* on cell lines 3T3, HeLa and MCF-7 (Janicsák et al. 2011; Keshavarz et al. 2010). However, the ethanolic extract of *S. Libanotica* showed toxicity against 3T3 cells and not HeLa cells (Soomro et al. 2019). Recently, *S. verbenaca* plant extracts have been shown to have a moderate effect on RD and vero cell lines (rhabdomyosarcoma-derived cells and kidney tumor cells) (Guaouguaou et al. 2018). On the other hand, other research has shown that the methanolic extracts of *S. aegyptiaca* and *S. verbenaca* have toxicity on several cell lines such as colorectal adenocarcinoma (HT-29 Caco-2 and DLD-1), human lymphoma cells (U- 937 GTB) and breast cancer (MCF-7, T47D, ZR-75-1 and BT 474) (Abdallah et al. 2018; Abu-Dahab et al. 2012;

El-Seedi et al. 2013; Kamatou et al. 2008). The cytotoxic effect of *S. aegyptiaca* on Ehrlich ascites tumor cellswas due to the activation of apoptosis (Tohamy et al. 2016). Similarly, the cytotoxic effect of *S. argentea* on erythromyeloblastoid leukemia (K562) and fibrosarcoma (HT1080) cellswas probably due to the active triterpenoids isolated from this plant (Bechkri et al. 2019; Lehbili et al. 2018). Rosmarinic acid, one of the major compounds of *S. officinalis*, inhibits the proliferation of colon cancer cell lines (HCT 15) by blocking the MAPK/ERK pathway via KRAS inhibition (Xavier et al. 2008). One of the mechanisms involved in the anti-cancer activity of medicinal plants of the Lamiaceae familywas the inhibition of NO synthesis in cancer cells (which will activate apoptosis) and the activation of secretion of TNF-α of immune cells. These effects wereobserved when using the hexane and ethyl acetate extracts of *Rosmarinus officinalis* and *S. officinalis* (Kontogianni et al. 2013). The slight proliferation of cells can be explained by the fact that these plants wereendowed with protective activity against cytotoxicity like the aqueous extract of *S. officinalis*. Al-Barazanjy et al. (2013) demonstrated that *S. officinalis* extract induces proliferation of normal murine fibroblast cells (L20B). This effectwas explained by the presence of the most abundant phenolic compounds such as rosmarinic acid and luteolin-7-glucoside which have significant protective potential against hepatic cell carcinoma (HepG2) cell death (Lima et al. 2007). Similarly, incubation of human colon carcinoma cells (HCT15) with ursolic acid or olealonic acid for 36 to 72 hours induces cell proliferation with repression of apoptosis, contrary to the results obtained in the first 24 hours, showing a variety of cell response to the active compounds of medicinal plants (Li et al. 2002).

Conclusion

Four common compounds were detected in the methanolic extracts. These extracts have been identified as, caffeic acid glycoside, apigenin, rosmarinic acid and p-coumarylquinnic acid. The methanolic extract of *S. aegyptiaca*was characterized by the presence of Salvianolic acid B and these derivatives, while luteolin and luteolin glucoside have been identified in the other extracts.

All the extracts studied showed potent antiglycation activity, suggesting their usefulness in the prevention of diabetic complications. The different extracts prepared have anti-inflammatory activity by inhibiting immune cells. This activitywas probably due to the phenolic compounds present such as rosmarinic acid and these derivatives. The origin of the biological activities of these medicinal plants could be due to the phenolic compounds (rosmarinic acid and Salvianolic acid) and can be attributed to the radical-scavenging phenolic compounds and their derivatives.

The absence of toxic effect on mice and cytotoxicity on 3T3 and HeLa cell lines confirms the safety of use of these plants either in traditional or modern medicine.

Additional information

Conflict of interest

All authors declare that they have no conflicts of interest.

Ethics Statements

All studies on cells from human blood were carried out after approval from an independent ethics committee, ICCBS, UoK, No: ICCBS/IEC-008-BC-2015/Protocol/1.0.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Supplementary material

Total ion chromatogram of the methanolic extract. SAE, SVR, SBA and SAR

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Data type: pdf

Link: https://rrpharmacology.ru/index.php/journal/article/view/534/588