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Original Article

Phytochemical characterization and mushroom tyrosinase inhibition of different extracts from *Salvia officinalis* L. leaves

[Caracterización fitoquímica e inhibición de la tirosinasa de hongos de diferentes extractos de hojas de *Salvia officinalis* L.]

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Abstract

Context: Sage (*Salvia officinalis*) is an ancient valuable plant used in the treatment of variant health issues.

Aims: To evaluate the depigmentation activity of *S. officinalis* leaf chloroformic (SOCF) and ethanolic (SOMF) extracts via its efficacy to inhibit tyrosinase enzyme using *in vitro* model and bioassay-guided identification and quantification of the main active constituents.

Methods: Plant extracts efficacy as a depigmentation agent has been studied via mushroom tyrosinase inhibition using *in vitro* model at two concentrations (100 and 200 µg/mL). Extracts were analyzed for phenolic compounds that could be responsible for the biological activity using LC-MS/MS analysis.

Results: Significant potency at a high concentration of 200 µg/mL for the methanolic extract were recorded ($p \leq 0.05$). The LC-MS/MS analysis of *S. officinalis* leaf extracts revealed the presence of eight and fourteen analytes of origin of thirty-seven analytes in both SOCF and SOMF, respectively. Analytes' quantification recorded the highest amount for rosmarinic acid (46 016 µg/g) in SOMF and the lowest was hesperidin (0.6 µg/g) in SOCF.

Conclusions: *S. officinalis* extracts recorded significant tyrosinase inhibition potency could control the melanin synthesis process and exhibit beneficiary effect in hyperpigmentation issues.

Keywords: hesperidin; hyperpigmentation; LC-MS/MS spectroscopy; rosmarinic acid; sage; tyrosinase inhibitors.

Resumen

Contexto: La salvia (*Salvia officinalis*) es una planta antigua y valiosa utilizada en el tratamiento de problemas de salud variantes.

Objetivos: Evaluar la actividad despigmentante de los extractos chorofórmico (SOCF) y etanólico (SOMF) de hojas de *S. officinalis* a través de su eficacia para inhibir la enzima tirosinasa utilizando un modelo *in vitro* y la identificación y cuantificación guiada por bioensayos de los principales componentes activos.

Métodos: La eficacia de los extractos de plantas como agente despigmentante se ha estudiado mediante la inhibición de la tirosinasa de hongos utilizando un modelo *in vitro* a dos concentraciones (100 y 200 µg/mL). Los extractos se analizaron en busca de compuestos fenólicos que pudieran ser responsables de la actividad biológica mediante análisis LC-MS/MS.

Resultados: Se registró potencia significativa a alta concentración de 200 µg/mL para el extracto metanólico ($p \leq 0.05$). El análisis LC-MS/MS de extractos de hojas de *S. officinalis* reveló la presencia de ocho y catorce analitos de origen de treinta y siete analitos tanto en SOCF como en SOMF, respectivamente. La cuantificación de los analitos registró la mayor cantidad de ácido rosmarínico (46 016 µg/g) en SOMF y la menor de hesperidina (0,6 µg/g) en SOCF.

Conclusiones: Los extractos de *S. officinalis* registraron una potencia significativa de inhibición de la tirosinasa que podría controlar el proceso de síntesis de melanina y exhibir un efecto beneficioso en los problemas de hiperpigmentación.

Palabras Clave: ácido rosmarínico; espectroscopia LC-MS/MS; hesperidina; hiperpigmentación; inhibidores de tirosinasa; salvia.

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INTRODUCTION

Salvia officinalis L., *Labiatae* (sage) is a perennial, evergreen, aromatic, round shrub, with a woody stem. Sage is a common vegetal species majorly used for food spicing. The plant genus is native to Mediterranean region and Middle East; however, it has cultivated in many regions worldwide (Bisset and Wicht, 2001; Dinç et al, 2009; Walker and Sytsma, 2007). Sage expressed many pharmacological activities such as anticancer (Karmokar et al., 2012; Sharmila and Manoharan, 2012), neuroprotective and antioxidant (De Oliveira et al., 2012; Iuvone et al., 2006), immunomodulatory (Costa et al., 2012; Kang et al., 2003), melanogenic and photoprotection (Psotova et al., 2006). Many of presented biological properties attributed to the polyphenol content of the plant (Bauer et al., 2012; Ben Taarit et al., 2012; Lu and Foo, 2002).

Salvia was derived from the Latin word "salvarem", which means cure or save. Traditionally, the plant has been used to diminish perspiration, to eliminate hot flashes in menopause and to improve regularity of a menstrual cycle, as a gargle for sore throat, in gastroenteritis and other infections, to improve lipid profile and liver functions, to enhance appetite and digestion, and to improve mental abilities (Martina et al., 2019).

Melanin content and distribution in keratinocytes is the major determinant in mammalian skin and hair color, which acts primarily by protecting animals from ultraviolet (UV) radiation. Various dermatological conditions observed due to abnormality in either melanin pigment biosynthesis or accumulation of melanin in melanocytes such as freckles, melasma or age spot, lentigines (Adhikari et al., 2008; Virador et al., 1999).

Melanin is a polymorphous biopolymers produced from a complex and multistep oxidative reaction starting from L-tyrosine amino acid, the key element, which catalyzes the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA), and then to dopaquinone (Adhikari et al., 2008; Chang, 2009; Kim and Uyama, 2005; Lin et al., 2011).

Serious global attempts for treating hyperpigmentation-related problems have been undertaken in producing skin-whitening properties cosmetics preparations, which act via melanin biosynthesis inhibition (Lin et al., 2011). The treatment can be achieved by various mechanisms such as protection of the skin from sun light exposure, removing melanin by corneal ablation, eliminating, or blocking the melanocytes proliferation or tyrosinase activity inhibition (Ha et al., 2005; Wang et al., 2006).

Salvia officinalis leaf is used in different skin issues and incorporated in whitening preparation as traditional herbal remedy in the Kurdistan region of Iraq. The study was aimed to set a scientific proof of plant depigmentation efficacy via the plant extracts activity to inhibit mushroom tyrosinase enzyme using *in vitro* model and marking main active constituents that could be responsible for the corresponding biological activity.

MATERIAL AND METHODS

Plant material

Leaves of *S. officinalis* were collected during April-May 2020 from different areas of the Kurdistan Region, Iraq (36.6085° N, 44.5239° E and 36.4098° N, 44.3202° E). The plant was authenticated by Assistant Professor Alaadin Naqishbandi, the leaves were cleaned, dried in shade for 4-6 days and kept in closed containers at 25°C. The voucher specimen (L-23) was stored at the Pharmacognosy Department, Pharmacy College, Hawler Medical University.

Plant extract preparation

Ultrasonic assisted extraction technique was applied as an extraction method as described by Siriangkhawut and Kaewboo (2013). It was used for extraction of leaves (500 g) powdered plant material by solvents as chloroform and ethanol. The liquid extracts obtained from chloroform and methanol were dried using a rotary vapor machine (Buchi Rotavator, Switzerland) to afford the corresponding chloroformic (SOCF) and ethanolic (SOMF) extracts with the following yields (7.7 g (0.75%, w/w) and 329.6 g (32.99%, w/w), respectively). Extracts were kept at 4°C until further used.

In vitro tyrosinase inhibition assay

Qualitative determination

The assay was conducted according to the protocol described by Macrini et al. (2009), with some modifications. 10 µL aliquots of a solution composed of (125 U/mL) of mushroom tyrosinase (Sigma-Aldrich, Germany) were added to 96-well microplates, and subsequently 70 µL of pH 6.8 phosphate buffer solution and 60 µL of the *S. officinalis* extracts (100 and 200 µg/mL, in 25% dimethyl sulfoxide, DMSO) were also added, separately. Kojic acid used as a positive control, 60 µL (50 µg/mL in 25% DMSO) was used instead of the *S. officinalis* extracts, and 60 µL of DMSO (2.5%) was added used as a negative control. A volume of 70 µL of L-tyrosine as substrate (Sigma-

Aldrich, Germany) was added at a concentration of 0.3 mg/mL in distilled water to the resultant mixture (to make a final volume of 210 µL in each well). The absorbance of the microplate wells was read using ELISA microplate reader (BioTek, American) at 510 nm (T0). Later, the microplates were incubated at 30 ± 1°C for 60 min and the absorbance was measured (T1). A further incubation period of 60 min at 30 ± 1°C was performed, and a new measuring was recorded as (T2) using equation [1]:

$$IA\% = C - S/C \quad [1]$$

Where IA%: inhibitory activity; C: negative control absorbance; S: sample or positive control absorbance (absorbance at time T1 or T2 minus the absorbance at time T0).

Quantitative assay

Quantitative assay was conducted for the extracts that exhibited an IA% greater than 35% (Teixeira et al., 2012). The quantitative evaluation of tyrosinase inhibition activity was determined via a calibration curve and the respective line equation. For this, a serial dilution of the extracts and kojic acid was prepared in the microplate wells of concentrations of 6.25, 12.5, 25, 50, 100, and 200 µg/mL with 25% DMSO. The inhibitory activity at 50% (IA_{50}, in µg/mL) was calculated using the line equation of a tyrosinase inhibition activity percentage and the concentrations curve plot for the extracts and positive control.

Phytochemical study

LC-MS/MS

Shimadzu Nexera model ultra-high performance liquid chromatography (UHPLC) coupled to Shimadzu LCMS 8040 model triple quadrupole mass spectrometer was used according to a method established by Akdeniz to evaluate 37 phenolic compounds qualitatively and quantitatively of Akdeniz (2018). The liquid chromatography composed of LC-30 AD model gradient pump, DGU-20A3R model degasser, CTO-10ASvp model column oven, and SIL-30AC model autosampler. The chromatographic separation was performed on an Inertsil ODS-4 model C18 (100 mm × 2.1 mm, 2 µm) column. The column temperature was kept at 35°C during the analysis. The mobile phase A consisted of water, 10 mM ammonium formate-0.1% formic acid was added to the water phase to facilitate better chromatographic separation and ionization and methanol as mobile phase B. The applied gradient profile was optimized as 5-20% B (0-10 min), 20% B (10-22 min), 20-50% B (22-36 min), 95% B (36-40), 5% B (40-50 min). The flow rate of the mobile phase was 0.25 mL/min, and the injection volume was 4 µL.

The triple quadrupole mass spectrometer was equipped with an ESI (electrospray ionization) source that operates in both negative and positive mode. LC-ESI-MS / MS data were collected and processed by the software registered in LabSolutions (Shimadzu, Kyoto, Japan).

In the MRM (multiple reaction monitoring) mode for the quantitative determination of analytes studied and molecular (parent) ions (product ions) (the first one was used for quantitative purposes and the other was used for qualitative purposes). The peak area of each component in the extracts of *S. officinalis* was obtained from its chromatogram and the abundance of each compound was calculated from its corresponding calibration curve, the concentration of the compounds expressed as µg/g of extract. The other parameters optimized in the spectrometer were: 350°C interface temperature, 250°C desolvation line (DL) temperature, 400°C heat block temperature, 3 L/min and 15 L/min were nebulizer and drying gas (N2) flow rates, respectively.

Validation parameters

The method performance characteristics were established by standard spiked and non-spiked samples. Within the context of method validation, linearity, trueness (recovery), precision (repeatability and reproducibility), limits of detection and quantification (LOD/LOQ) and relative standard uncertainty [U% at 95% confidence level (k = 2)] were expressed in Fig. 1 and Table 1.

Statistical analysis

Data expressed as mean ± SEM (n = 3) were calculated by Microsoft Excel 2010. *Post-hoc* Dunnett's test applied for the comparison of data obtained from plant extracts and the kojic acid (reference drug), using the software Statistical Package for the Social Sciences (SPSS) version 23 for data analysis. P≤0.05 was considered statistically significant.

RESULTS

Tyrosinase inhibition assay

S. officinalis leaf extracts expressed tyrosinase inhibition activity. The methanolic extract (SOMF) (83.06 ± 0.03%) showed greater activity in comparison to the chloroform extract (59.64 ± 1.08%) and a significant potency were recorded in comparison to the positive control (kojic acid), since there was insignificant difference between their efficacy to inhibit tyrosinase enzyme (p≤0.05).

Table 1. Analytical parameters that belong to the LC-MS/MS method.

No	Analytes	RT[a]	Mother ion (m/z)[b]	Fragment ions	Ion. mode	Equation	R^{2c}	RSD%[d]		Linearity Range (µg/L)	LOD/LOQ (µg/L)[e]	Recovery (%)		U[f]
								Interday	Intraday			Interday	Intraday	
1	Quinic acid	1.13	190.95	85.3-93.3	Neg	y=41.06×+10671	0.996	0.00259	0.00274	250-10000	75.8/79.4	1.00288	0.98778	0.0082
2	Malic acid	1.23	133.00	115.2-71.3	Neg	y=316.95×-42041	0.999	0.00477	0.00527	250-10000	55.3/67.5	1.01266	0.99836	0.0113
3	Fumaric acid	1.48	115.00	71.4	Neg	y=64.99×-11592	0.997	0.00536	0.00460	100-5000	28.1/34.5	0.99748	0.99867	0.0124
4	Gallic acid	3.00	168.85	125.2-79.2	Neg	y=226.76×+38152	0.998	0.01601	0.01443	250-10000	95.5/106.9	1.00004	1.00454	0.0282
5	Protocatechuic acid	4.93	152.95	108.3	Neg	y=297.75×+30590	0.995	0.01236	0.01296	100-5000	28.2/31.4	0.99404	1.01070	0.0411
6	Pyrocatechol	6.48	109.00	108.35-91.3	Neg	y=30.61×+14735	0.996	0.01313	0.01339	1000-20000	261.1/278.4	0.99987	0.99936	0.0235
7	Chlorogenic acid	7.13	353.15	191.2	Neg	y=781.36×-18697	0.998	0.00058	0.00076	25-1000	6.2/8.1	1.00806	0.99965	0.0069
8	4-OH-benzoic acid	7.39	136.95	93.3-65.3	Neg	y=409.03×+112079	0.998	0.01284	0.01538	250-10000	33.2/38.1	0.99662	1.00058	0.0289
9	Vanillic acid	8.57	166.90	152.3-108.3	Neg	y=35.84×-12097	0.999	0.00528	0.00619	1000-20000	122.2/139.7	1.00093	1.04095	0.0508
10	Caffeic acid	8.80	178.95	135.2-134.3	Neg	y=3963.32×+178156	0.998	0.01454	0.01469	25-1000	18.4/22.4	1.00917	0.98826	0.0354
11	Syringic acid	9.02	196.95	182.2-167.3	Neg	y=42.33×-52547	0.996	0.01049	0.01345	1000-20000	212.5/233.3	0.99922	0.99977	0.0238
12	Vanillin	10.87	151.00	136.3-92.2	Neg	y=446.10×+70934	0.998	0.00696	0.00793	250-10000	44.3/53.1	0.99679	0.99611	0.0280
13	Salicylic acid	11.16	136.95	93.3-65.3	Neg	y=5286.26×+309192	0.989	0.01016	0.01242	25-1000	5.0/6.5	1.00989	0.99013	0.0329
14	p-Coumaric acid	11.53	162.95	119.3-93.3	Neg	y=3199.20×+13002	0.992	0.01820	0.01727	25-1000	7.3/9.1	1.00617	1.01224	0.0516
15	Rutin	12.61	609.05	300.1-271.1	Neg	y=561.91×-16879	0.997	0.00473	0.00624	25-1000	5.5/6.5	1.00994	0.98017	0.0159
16	Ferulic acid	12.62	192.95	178.3	Neg	y=80.45×-31782	0.997	0.00708	0.00619	250-10000	36.6/42.0	0.99987	1.00289	0.0494
17	Sinapinic acid	12.66	222.95	208.3-149.2	Neg	y=141.96×-73294	0.992	0.01446	0.01517	250-10000	78.7/86.1	1.00164	0.99962	0.0281
18	Hesperidin	12.67	610.90	303.1-465.1	Poz	y=1340.27×-43769	0.998	0.00945	0.01126	25-1000	3.4/4.2	1.01733	1.01263	0.0262
19	Isoquercitrin	13.42	463.00	300.1-271.1	Neg	y=803.23×+4981	0,999	0.00682	0.00515	25-1000	5.4/6.3	1.00594	1.00722	0.0133
20	Rosmarinic acid	14.54	359.00	161.2-197.2	Neg	y=909.67×-201692	0.994	0.02014	0.01751	100-5000	6.6/8.8	0.99206	1.03431	0.0713
21	Nicotiflorin	14.68	593.05	285.1-255.2	Neg	y=498.38×+79274	0,991	0.00737	0.00875	100-5000	22.4/25.5	1.02558	1.00970	0.0276
22	o-Coumaric acid	15.45	162.95	119.4-93.3	Neg	y=1219.34×-10915	0.999	0.02730	0.02566	25-1000	24.4/31.1	0.98344	0.99061	0.0513
23	Rhoifolin	16.11	577.05	269.2-211.1	Neg	y=237.15×+11887	0,999	0.00747	0.01528	100-5000	23.1/27.9	1.01046	1.01739	0.0941

Table 1. Analytical parameters that belong to the LC-MS/MS method (continued…)

No	Analytes	RT[a]	Mother ion (m/z)[b]	Fragment ions	Ion. mode	Equation	R^{2c}	RSD%[d]		Linearity Range (µg/L)	LOD/LOQ (µg/L)[e]	Recovery (%)		U[f]
								Interday	Intraday			Interday	Intraday	
24	Quercitrin	16.41	447.15	301.1-255.1	Neg	y=339.39×+38910	0.999	0.01528	0.02320	100-5000	22.0/25.2	0.99726	1.00620	2.0079
25	Apigetrin	16.59	431.00	268.2-239.2	Neg	y=1775.55×+91121	0,993	0.01797	0.01607	25-1000	5.4/6.1	1.01394	1.00419	0.0597
26	Coumarin	17.40	147.05	91.0-103.2	Poz	y=33.64×-89700	0.994	0.01306	0.01239	1000-20000	208.4/228.4	0.99947	1.00081	0.0237
27	Myricetin	18.72	317.00	179.2-151.3	Neg	y=583.55×+205727	0,999	0.00652	0.00711	250-10000	53.2/57.2	0.99982	1.00042	0.0126
28	Fisetin	19.30	284.95	135.2-121.3	Neg	y=547.46×+274791	0,991	0.00557	0.00820	250-10000	54.4/61.4	0.99877	1.00031	0.0148
29	Cinnamic acid	25.61	147.00	103.15-77.3	Neg	y=9.06×-12403	0.996	0.00648	0.00816	5000-20000	821.8/859.7	1.00051	0.99927	0.0143
30	Liquiritigenin	25.62	254.95	119.3-135.1	Neg	y=2384.96×+59141	0,996	0.01849	0.01738	25-1000	5.5/6.6	1.00333	0.99957	0.0341
31	Quercetin	28.17	300.90	151.2-179.2	Neg	y=1198.48×+480562	0.990	0.01589	0.01360	100-5000	23.3/28.9	0.98470	1.00103	0.0543
32	Luteolin	28.27	284.75	133.2-151.2	Neg	y=3272.65×+150557	0,997	0.00575	0.00696	25-1000	5.4/6.5	1.00772	0.99524	0.0174
33	Naringenin	30.68	270.95	151.2-119.3	Neg	y=4315.1×+178410	0.995	0.02054	0.02019	25-1000	5.4/6.4	0.99883	1.01002	0.0521
34	Apigenin	31.43	268.95	117.3-151.2	Neg	y=4548.36×+295252	0.990	0.02304	0.02204	25-1000	5.4/6.3	1.01444	1.01331	0.0650
35	Hesperetin	31.76	300.95	164.2-136.2	Neg	y=876.67×+48916	0.997	0.03209	0.02605	25-1000	5.6/6.9	0.98850	0.99435	0.0562
36	Kaempferol	31.88	284.75	255.1-117.3	Neg	y=26.29×+87558	0,992	0.01436	0.01070	1000-20000	206.6/214.3	0.99971	0.99851	0.0209
37	Chrysin	36.65	252.95	143.3-119.4	Neg	y=2032.13×+95593	0,993	0.00490	0.00630	25-1000	5.4/6.2	1.00338	1.00437	2.0083

[a]RT: Retention time, [b]Mother ion(*m/z):* Molecular ions of the standard compounds (m/z ratio), [c]R^2: Coefficient of determination, [d]RSD: Relative standard deviation, [e]LOD/LOQ (µg/L): Limit of detection/quantification, f U (%): percent relative uncertainty at 95% confidence level (k = 2).

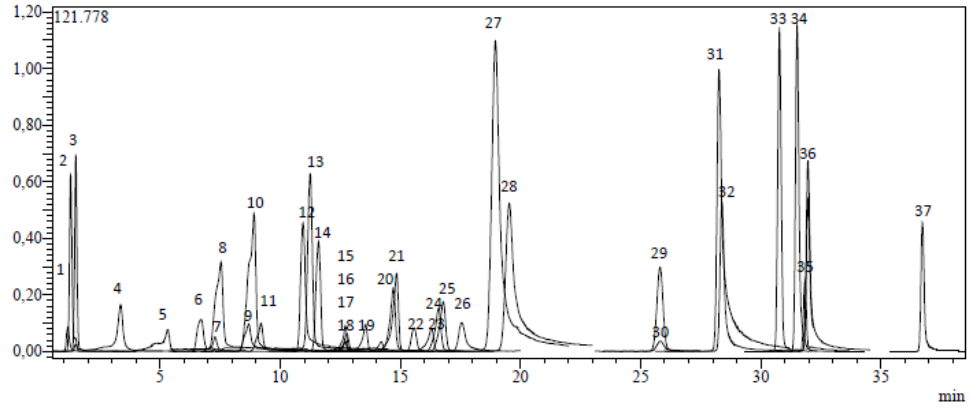

Figure 1. Standard chromatogram for 37 phenolic compounds.

Table 2. Tyrosinase inhibitory activity of *S. officinalis* extracts.

Sample[1]	Concentration (µg/mL)	$IA_{60\ min}$ (%)[2] Mean ± SEM	IA_{120min} (%) Mean ± SEM	IA_{50} (µg/mL)
SOCF	100	31.33±0.98	18.20±1.36	53.41
SOCF	200	59.64±1.08	37.56±1.33	
SOMF	100	54.24±1.18	19.97±2.01	32.33
SOMF	200	83.06±0.03*	44.08±0.95*	
Kojic acid	50	90.17±1.02	45.88±1.39	6.73*

[1]$SOCF_1$ **stands for *S. officinalis* chloroform extract, SOEF stands for *S. officinalis* methanol extract,** [2]**IA stands for percentage of inhibitory effect, *p≤0.05 *versus* positive control.**

The IA50 of the methanolic extract (32.33 µg/mL) confirms the potency of the extract in comparison to the IA50 chloroform extract (53.41 µg/mL) (p≤0.05) while in comparison to the IA50 of the standard kojic acid (6.73 µg/mL) both extract showed less potency (p≥0.05). Continuous effect has been detected for the extracts after 120 min evaluation approximately approaching the positive control effectiveness. Dose dependent manner were detected for both extracts in their exhibited activity (Table 2).

Phytochemical study

The LC-MS/MS analysis for the phenolic compounds in the *S. officinalis* leaf extracts revealed the presence of eight and fourteen analytes in both SOCF and SOMF, respectively (Figs. 2-3). The quantification of the analytes showed the highest amount were recorded for rosmarinic acid (46 016.0 µg/g) in SOMF and lowest was for hesperidin (0.6 µg/g) in SOCF (Table 3).

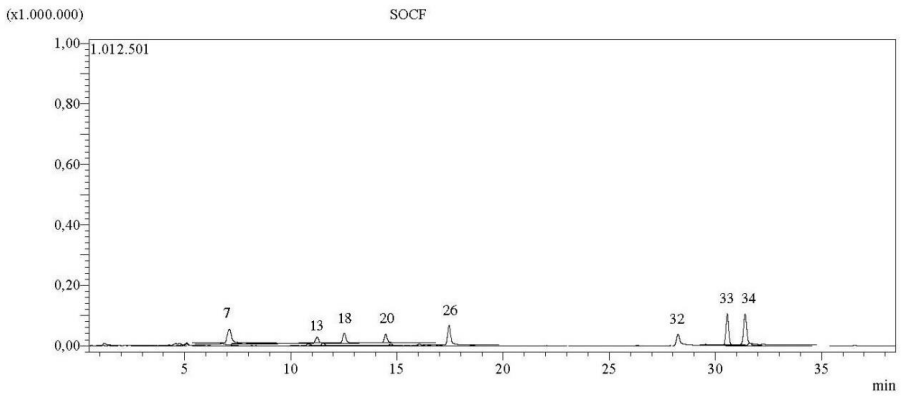

Figure 2. LC-MS/MS chromatogram for *Salvia officinalis* chloroform extract (SOCF).

7: Chlorogenic acid; **13:** Salicylic acid; **18:** Hesperidin; **20:** Rosmarinic acid; **26:** Coumarin; **32:** Luteolin; **33:** Naringenin; **34:** Apigenin.

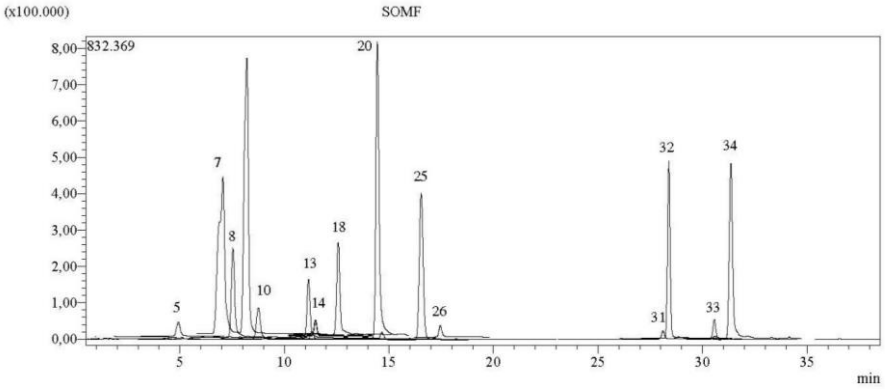

Figure 3. LC-MS/MS chromatogram for *Salvia officinalis* methanol extract (SOMF), 5: Protocatechuic acid.
7: Chlorogenic acid; **8:** 4-OH-benzoic acid; **10:** Caffeic acid; **13:** Salicylic acid; **14:** p-Coumaric acid; **18:** Hesperidin; **20:** Rosmarinic acid; **25:** Apigetrin; **26:** Coumarin; **31:** Quercetin; **32:** Luteolin; **33:** Naringenin; **34:** Apigenin.

Table 3. Quantity of the evaluated analytes in *S. officinalis* leaf extracts using LC-MS/MS technique.

Analyte		Quantity of analyte (µg/g of extract)[1]	
		SOCF	**SOMF**
1	Quinic acid	ND	1880.00
2	Malic acid	ND	ND
3	Fumaric acid	ND	331.00
4	Gallic acid	ND	ND
5	Protocatechuic acid	ND	3.40
6	Pyrocatechol	ND	N.D.
7	Chlorogenic acid	0.67	301.00
8	4-OH-benzoic acid	ND	626.00
9	Vanillic acid	ND	ND
10	Caffeic acid	ND	795.00
11	Syringic acid	ND	ND
12	Vanillin	ND	ND
13	Salicylic acid	2.00	92.00
14	*p*-Coumaric acid	ND	174.00
15	Rutin	ND	ND
16	Ferulic acid	ND	ND
17	Sinapinic acid	ND	ND
18	Hesperidin	0.60	23.80
19	Isoquercitrin	ND	57.00
20	Rosmarinic acid	90.00	46 016.00
21	Nicotiflorin	ND	ND
22	o-Coumaric acid	ND	ND
23	Rhoifolin	ND	ND
24	Quercitrin	ND	ND
25	Apigetrin	ND	69.20

Table 3. Quantity of the evaluated analytes in *S. officinalis* leaf extracts using LC-MS/MS technique (continued...)

Analyte		Quantity of analyte (µg/g of extract)[1]	
		SOCF	SOMF
26	Coumarin	12.00	7.00
27	Myricetin	ND	ND
28	Fisetin	ND	ND
29	Cinnamic acid	ND	ND
30	Liquiritigenin	ND	ND
31	Quercetin	ND	4.35
32	Luteolin	9.52	597.60
33	Naringenin	30.00	14.00
34	Apigenin	40.04	391.24
35	Hesperetin	ND	ND
36	Kaempferol	ND	ND
37	Chrysin	ND	ND

[1]**ND stands for not detected in the extract.**

DISCUSSION

Tyrosinase has a curial role in melanogenesis. Controlling of the tyrosinase enzyme activity is one of the most strategies explored by scientists, hence it is the key enzyme involved in melanin biosynthesis, the pigment responsible for hyperpigmentation in humans and mammals. Most of the skin care agents specifically lightening products are containing tyrosinase inhibitors. Since tyrosinase inhibitors may specifically inhibit the melanogenesis in cells without other side effects because tyrosinase enzyme produced only by melanocytes (Gunia-Krzy˙zak et al, 2016; Lee et al., 2016; Kumari et al., 2018; Pillaiyar et al., 2018; Thanigaimalai et al., 2017; Zolghadri et al., 2019). There is a growing intertest for developing skin-lightening agents from nature with lower backwards, which is consequently used in medicine, cosmetics (Biswas et al., 2015). A standard compound kojic acid is a fungal metabolite exhibited tyrosinase enzyme inhibitory activity, it is effect mediated via copper chelating mechanism to the active site of the enzyme, because of it is efficacy it is used as positive control in tyrosinase inhibition assay and in cosmetic and food industry. But it is applications have been limited due to many recorded side effects (Burnett et al., 2010).

Sage is a well-known plant has a long history of documented benefits in various health condition (Jakovljević et al., 2019). *S. officinalis* leaf extracts assessed for it is efficacy as natural skin whitening agent, the plant extracts showed an appreciable tyrosinase inhibition activity in comparison to the standard drug kojic acid ($p \leq 0.05$). The methanolic extract (SOMF) expressed greater efficacy in comparison to the chloroform extract (SOCF). The dose-response assay showed a positive relationship, the greater response for greater doses were recorded for both extracts. The potency of the extracts in inhibition of tyrosinase enzyme were determined by calculating the IA_{50} (the concentration of the extract capable of inhibition of 50% of the enzyme), the SOMF extract expressed greater potency in comparison to the SOCF since it has lower IA_{50} value.

A diversity group of compounds in nature acting as tyrosinase inhibitors interestingly most of them are phenolic compounds (Panzella and Napolitano, 2019). *S. officinalis* extracts introduced to phytochemical studies using LC-MS/MS for detecting and quantifying phenolic compound that could be responsible for the corresponding biological activity of the extracts. The extracts were evaluated for the presence of 37 phenolic compounds, the SOCF showed the presence of eight phenolic compounds (chlorogenic acid, salicylic acid, hesperidin, rosmarinic acid, coumarin, luteolin, naringenin, and apigenin) and the SOMF showed the presence of fourteen phenolic compounds (protocatechuic acid, chlorogenic acid, 4-OH-benzoic acid, caffeic acid, salicylic acid, p-coumaric acid, hesperidin, rosmarinic acid, apigetrin, coumarin, quercetin, luteolin, naringenin, and apigenin). Sharing constituents with variable quantities were detected in both extract as indication for the activity of both extracts. The concentration of the most detected phenolic compounds is higher in methanolic extract in comparison to the chloroform extract due to solvent polar-

ity effect could rationalize the variation in the extract's efficacies for inhibition of the tyrosinase enzyme.

In general, it believed that there are four main mechanisms mediated tyrosinase enzyme inhibition, copper chelating at the active site of the enzyme leading to irreversible tyrosinase deactivation (Sarikurkcu et al., 2015; Souza et al., 2012), oxygen activation prevention by tyrosinase via introduction of powerful antioxidant (Boonsiripiphat and Theerakulkait, 2009; Fiocco et al., 2011), melanin formation prevention by a free radical scavenging agent (Fatiha et al., 2015), and tyrosinase competitive inhibition mediated by antioxidants and radical scavenging activities (Nagarani et al., 2014). Among phenolic compounds the flavonoid class represent the most potent inhibitors of tyrosinase enzyme such luteolin, quercetin, and apigenin recording low IA_{50} value (20.8 µM, 10.73 µM, and 17.3 µM, respectively) (Kim and Lee, 2019; Omar et al., 2018). Phenolic acids caffeic acid, p-coumaric acid, and rosmarinic acid potency for inhibition of tyrosinase enzyme were recorded by Crespo et al. (2019) and Kim and Lee (2019). *S. officinalis* plant efficacy for controlling pigmentation were recorded by Karina et al. (2013).

S. officinalis plant extracts might have competitive tyrosinase inhibition activity, competing melanin substrates such as L-dopa for the same active site on the enzyme. Consequently, the substrate and the inhibitor could be correspondent and could be non-metabolizable substrate, copper chelator, or substrate derivatives. Copper chelating compounds can inhibit tyrosinase enzyme activity since copper is an essential element in the enzymatic activity (Chang, 2009). *S. officinalis* plant extract showed phenolic flavonoid constituents from the phytochemical analysis. The phenolic and flavonoid compounds could inhibit tyrosinase enzyme activity via competition of the substrate on the enzyme active site since they have L-tyrosine structural analogue properties (Chang, 2009).

Results of the present study confirm the potency of the methanolic *S. officinalis* plant extract have persisting tyrosinase inhibitory effect that could be used safely as natural depigmentation remedy for approximately 2 h in comparison to the standard compound kojic acid.

CONCLUSION

An appreciable tyrosinase inhibition efficacy was recorded for *S. officinalis* extracts that could control the melanin synthesis process and exhibiting beneficiary effect in hyperpigmentation issues. The high phenolic content of the plant could contribute majorly to the plant tyrosinase inhibition efficacy. *S. officinalis* could be considered as a valuable natural depigmentation remedy.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Juee LYM
Concepts or ideas	x
Design	x
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