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NOTE

Screening for melanogenesis-controlled agents using Sudanese medicinal plants and identification of active compounds in the methanol extract of *Terminalia brownii* bark

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Abstract We isolated and identified compounds in medicinal plant extracts that could control melanogenesis. Sudanese medicinal plants were extracted with methanol (MeOH) and 50 % ethanol (EtOH)/water, yielding 104 extracts that were screened for melanogenic activity using B16 melanoma cells. The MeOH extract of *Terminalia brownii* bark dose-dependently enhanced intracellular and extracellular melanogenesis, with no cytotoxicity. Furthermore, we isolated and identified the components in *T. brownii* MeOH extract. Gallic acid (**1**), α,β-punicalagin (**2**), α,β-terchebulin (**3**), ellagic acid 4-*O*-α-L-rhamnopyranoside (**4**), ellagic acid (**5**), and 3,4,3′-tri-*O*-methylellagic acid (**6**) were isolated by chromatography and identified using nuclear magnetic resonance (NMR), matrix-assisted laser desorption/ionization (MALDI) or ultra-performance liquid chromatography–time-of-flight mass spectrometry (UPLC–TOFMS), and ultraviolet (UV) spectroscopy data. Among the isolated compounds, **2**, **3**, **5**, and **6** enhanced melanogenesis. Furthermore, compound **1** inhibited intracellular and extracellular melanogenesis with no cytotoxicity.

Keywords Sudanese medicinal plants · B16 melanoma cell · *Terminalia brownii* · Tyrosinase

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Introduction

Melanin, a pigment that is synthesized from tyrosine by tyrosinase-mediated enzymatic oxidation, is widely distributed in the body surface, retina, nigra of the brain, and adrenal medullae. The primary function of melanin is to prevent skin cancer by protecting cells from ultraviolet (UV) rays [1, 2]. However, melanin production sometimes causes problems for beauty and health. For instance, decreased melanin production due to aging or stress causes gray hair. Furthermore, excess melanin production causes sunburn and mottle. Thus, control of melanogenesis is strongly desired. Melanin is synthesized in unique cells, called melanocytes, using L-tyrosine as the starting material. The key enzyme in melanin biosynthesis is tyrosinase, which contains copper and catalyzes two reactions [3]. The first step of melanin biosynthesis is the hydroxylation of L-tyrosine to L-DOPA, which is followed by the oxidation of L-DOPA to L-DOPA quinine, which produces the red-orange coloration of pheomelanin and blackish brown coloration of eumelanin [4].

Melanosomes are transported in melanocytes and transferred to keratinocytes. Interestingly, extracellular melanin elicits the skin pigmentation. Nevertheless, most reports evaluating compounds that modulate melanogenesis only determine changes in intracellular melanin. In this paper, we determined both extracellular and intracellular melanin content to identify compounds in Sudanese medicinal plants that regulate melanogenesis.

Sudanese medicinal plants have immunomodulatory [5], anti-bacterial [6, 7], and anti-malarial activity [8]. However, the effect of Sudanese medicinal plant extracts on melanogenesis remains unclear. Here we screened 104 extracts from Sudanese medicinal plants to evaluate their effects on melanogenesis. Furthermore, the active

compounds in the methanol (MeOH) extracts of *Terminalia brownii* Fresen (Combretacae) bark, which is widely used in traditional medicine to treat bacterial, fungal, and viral infections [9], were isolated and identified. We found that these compounds modulate melanogenesis.

Materials and methods

General experimental procedures

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded in methanol-d_4 or dimethyl sulfoxide (DMSO)-d_6 with a JEOL EC600 MHz NMR (Tokyo, Japan). Ultra-performance liquid chromatography-time-of-flight mass spectrometry (UPLC-TOFMS, WatersXevo™ QTof MS, Waters, Milford, MA, USA) was performed using a C_{18} column (2.1 × 100 mm, Waters). The UPLC–TOFMS data were collected in negative ionization mode. The capillary voltage was 3.0 kV. Cone and desolvation gas flow rates were set at 50 and 1000 L/h, respectively. The source and desolvation temperatures were 150 and 500 °C, respectively. Matrix-assisted laser desorption/ionization (MALDI)-TOFMS spectra were measured on an SHIMADZU AXIMA-Resonance spectrometer (Kyoto, Japan) equipped with a nitrogen laser (λ = 337 nm). The samples were mixed with matrices [2,5-dihydroxy benzoic acid (DHB) in 30 % acetonitrile, 10 mg/ml] and loaded onto a 384-well MALDI sample plate. Preparative high-performance liquid chromatography (HPLC, SHIMADZU LC-6AD) was performed using an Inertsil ODS-3 column (20 × 250 mm, GL Sciences Inc., Tokyo, Japan). Analytical HPLC (SHIMAZU SIL-20A) was performed using an Inertsil ODS-3V column (4.6 × 250 mm, GL Sciences Inc., Tokyo, Japan). Commercially available products were purchased from Wako Chemical (Osaka, Japan). UV spectra were recorded on a Shimadzu SPD-M20A diode array detector.

Materials

Plants were collected from Khartoum and Gadarif states in Sudan in March 2011. Voucher specimens are deposited in the Horticultural Laboratory, Department of Horticulture, Faculty of Agriculture, University of Khartoum. A list of voucher numbers and ethnomedical uses of the investigated species are shown in Table 1.

Extraction of plant materials

Extraction of plant materials were performed as previously described [6].

Isolation of compounds from *T. brownii* MeOH extract

T. brownii bark MeOH extract was separated with medium pressure chromatography eluting [ODS-25 (40 × 200 mm) water:MeOH = 95:5 (30 min), 80:20 (60 min), 70:30 (90 min), 40:60 (120 min), 0:100 (150 min), flow rate: 5 ml/min] to obtain fractions (Fr) 1, 2, 3, and 4. Fr. 1 was separated by Sephadex LH-20 gel column chromatography, eluting [LH20 (30 × 430 mm) MeOH 100 % (3 h), acetone:water 70:30 (3 h), flow rate: 0.5 ml/min] to obtain Fr. 1–1, 1–2, 1–3, and 1–4. Compound **1** was isolated from Fr.1–1, **2** was isolated from Fr.1–3, **3** was isolated from Fr.1–4, and **4**, **5**, and **6** were isolated from Fr. 2, 3, and 4, respectively using preparative HPLC [Colum: ODS-3 (20 × 250 mm), flow rate: 9 ml/min, wavelength: 254 nm, gradient program: MeOH:0.05 % TFA aqueous solution = 20:80 (0 min), 20:80 (20 min), 50:50 (50 min), 100/0 (60 min)]. The yields of **1–6** were 8.1, 4.3, 2.6, 4.9, 2.8, and 1.2 mg, respectively, against 291 g of *T. brownii* bark powder. The purity was confirmed using analytical HPLC [Colum: ODS-3V (20 × 250 mm), flow rate: 1 ml/min, wavelength: 256 nm, gradient program: MeOH: 0.05 % TFA aqueous solution = 5:95 (0 min), 10:90 (5–10 min), 20:80 (10–20 min), 50:50 (35 min), 100:0 (45–55 min)].

Identification of isolated compounds from *T. brownii* bark MeOH extracts

The isolated compounds from *T. brownii* bark MeOH extract were identified using the NMR, MALDI or UPLC-TOFMS, and UV spectrometry data. Gallic acid (**1**): white powder; UV λ_{max}: 195, 213, 270 nm; UPLC-TOFMS: *m/z* 169.011 [M-1]$^-$. α,β-Punicalagin (**2**): pale green powder; UV λ_{max}: 212, 253, 372 nm; MALDI-TOFMS: *m/z* 1107.900 [M + Na]$^+$. α,β-Terchebulin (**3**): pale green powder; UV λ_{max}: 200, 253, 379 nm; MALDI-TOFMS: *m/z* 1107.878 [M + Na]$^+$. Ellagic acid 4-*O*-α-L-rhamnopyranoside (**4**): pale yellow powder; UV λ_{max}: 210, 253, 360 nm; UPLC-TOFMS ES$^-$: *m/z* 447.059 [M-H]$^-$. Ellagic acid (**5**): pale yellow powder; UV λ_{max}: 211, 253, 367 nm; UPLC-TOFMS ES$^-$: *m/z* 301.0381 [M-H]$^-$. 3,4,3′-tri-*O*-methylellagic acid (**6**): pale yellow powder; UV λ_{max}: 199, 247, 372 nm; UPLC-TOFMS: *m/z* 343.0429 [M-H]$^-$. NMR data for these compounds were completely the same to previous reports [10–17].

Tyrosinase activity assay

Tyrosinase activity was assayed based on protocols by Yamauchi et al. [18]. The sample (60 µl) was placed in a 96-well plate. Mushroom tyrosinase (30 µl, 333 U/ml in

Table 1 The intracellular melanogenesis activity and cell viability of extracts (100 μg/ml) from Sudanese medicinal plants

No.	Scientific name	Family	Used part	Voucher specimen	Extract	Yield (%)	Intracellular melanogenesis activity (%)	Cell viability (%)
1	*Parkinsonia aculeata* L.	Fabaceae	Leaves	SD-SH-02	M	43.2	116	121
2					E	8.12	89	75
3	*Abrus precatorius* L.		Seeds	SD-OD-22	M	16.8	104	103
4					E	6.80	120	88
5	*Trigonella foenum-graecum* L.		Seeds	SD-OD-44	M	17.9	116	111
6					E	8.55	67	85
7	*Ambrosia maritima* L.	Asteraceae	Arial parts	SD-SH-03	M	16.3	97	109
8					E	4.18	118	91
9	*Vernonia amygdalina* Del.		Leaves	SD-MAPI-19	M	31.1	103	108
10					E	13.4	102	126
11	*Aristolochia bracteolata* Lam.	Aristolochiaceae	Arial parts	SD-SH-04	M	36.8	106	109
12					E	11.8	101	82
13	*Citrullus colocynthis* (L.) Schrad.	Cucurbitaceae	Dry fruit	SD-OD-05	M	34.5	113	95
14					E	12.0	93	77
15	*Ziziphus spina–christi* (L.) Desf.	Rhamnaceae	Fruits	SD-SH-06	M	48.8	97	110
16					E	6.89	117	102
17			Bark		M	36.8	110	89
18					E	5.54	139	95
19			Leaves		M	47.1	100	124
20					E	9.23	124	109
21	*Lawsonia inermis* L.	Lythraceae	Leaves	SD-SH-07	M	39.6	114	109
22					E	6.20	111	95
23	*Moringa oleifera* Lam.	Moringaceae	Leaves	SD-SH-08	M	20.3	91	109
24					E	12.0	81	77
25	*Salvadora persica* L.	Salvadoraceae	Leaves	SD-SH-09	M	36.5	102	122
26					E	5.76	68	77
27			Stems		M	18.4	99	103
28					E	5.17	77	79
29	*Tamarix nilotica* (Ehrenb.) Bunge	Tamaricaceae	Stems	SD-OD-10	M	40.5	105	86
30					E	12.2	89	78
31	*Calotropis procera*	Asclepiadaceae	Leaves	SD-SH-11	M	26.9	109	121
32					E	14.0	93	89
33	*Solenostemma argel* Hayne		Leaves	SD-SH-23	M	37.2	105	104
34					E	13.5	109	104
35	*Xanthium brasilicum* W.	Compositae	Leaves	SD-SH-12	M	23.4	207	7
36					E	9.70	71	61
37	*Lepidium sativum* L.	Cruciferae	Seeds	SD-MAPI-13	M	37.4	87	103
38					E	3.69	79	72
39	*Ammi visnaga* L.	Umbelliferae	Fruits	SD-OD-38	M	20.0	122	85
40					E	13.8	97	97

Table 1 continued

No.	Scientific name	Family	Used part	Voucher specimen	Extract	Yield (%)	Intracellular melanogenesis activity (%)	Cell viability (%)
41	*Khaya senegalensis* (Desr.) A. Juss.	Meliaceae	Bark	SD-SH-14	M	62.1	122	101
42					E	5.75	101	71
43	*Balanites aegyptiaca* (L). Del.	Balanitaceae	Leaves	SD-SH-15	M	25.5	98	110
44					E	19.0	101	124
45			Bark		M	40.7	163	50
46					E	3.61	115	111
47			Fruits		M	74.4	86	91
48					E	6.45	106	74
49			Wood		M	5.64	41	65
50					E	3.15	30	13
51	*Carum carvi* L.	Apiaceae	Fruits	SD-OD-47	M	29.4	108	99
52					E	12.6	92	120
53	*Hibiscus sabdariffa* L.	Malvaceae	Flowers	SD-SH-48	M	58.4	100	94
54					E	27.5	97	117
55	*Abutilon pannosum* (Forst.f.) Schlecht.		Leaves	SD-SH-43	M	16.1	126	113
56					E	9.45	120	101
57	*Terminalia brownii* Fres.	Combretaceae	Bark	SD-GF-02	M	43.0	211	89
58					E	4.46	129	87
59			Wood		M	12.5	102	85
60					E	1.85	103	77
61	*Combretum hartmannianum* (Schweinf.)		Bark	SD-KH-04	M	21.9	129	89
62					E	4.88	105	87
63			Wood		M	4.68	82	77
64					E	1.86	106	100
65	*Terminalia laxiflora* Engl. & Diels		Wood	SD-KH-03	M	14.3	104	10
66					E	2.04	79	63
67	*Guiera senegalensis* J. F. Gmel		Leaves	SD-OD-40	M	26.2	179	9
68					E	12.7	112	94
69	*Acacia seyal* var *seyal* Del.	Mimosaceae	Bark	SD-GF-05	M	32.9	115	90
70					E	6.44	113	86
71			Wood		M	2.52	105	78
72					E	2.17	93	74
73	*Acacia seyal* var *fistula* (Schweinf.)		Bark	SD-GF-06	M	27.1	118	95
74					E	6.88	93	86
75			Wood		M	8.54	115	96
76					E	2.74	92	85
77	*Acacia tortilis* (Forssk.) Hayne		Bark	SD-KH-07	M	5.70	114	85
78					E	2.30	120	111
79			Wood		M	5.70	106	103
80					E	6.53	96	90

Table 1 continued

No.	Scientific name	Family	Used part	Voucher specimen	Extract	Yield (%)	Intracellular melanogenesis activity (%)	Cell viability (%)
81	*Acacia nilotica* (L.)		Pods	SD-OD-01	M	70.5	126	90
82					E	6.15	113	102
83			Bark		M	23.8	138	99
84					E	5.42	98	86
85	*Haplophyllum tuberculatum* Forsk	Rutaceae	Aerial part	SD-WR-32	M	17.2	187	7
86					E	8.75	85	6
87	*Kigelia africana* (Lam.) Benth.	Bignoniaceae	Fruits	SD-SH-21	M	26.2	97	83
88					E	8.65	125	99
89	*Hyphaene thebaica* (L.) Mart.	Palmae	Fruits	SD-OD-41	M	52.9	109	95
90					E	14.4	116	98
91	*Moringa oblongifolia* (Frossk.) A. Rich	Capparidaceae	Stems	SD-SH-45	M	10.7	115	115
92					E	4.20	110	98
93	*Capparis decidua* (Forssk.) Edgew.		Stems	SD-SH-17	M	15.9	109	121
94					E	4.13	94	117
95	*Polygonum glabrum* Willd.	Polygonaceae	Leaves	SD-SH-A-03	M	33.7	115	6
96					E	8.18	105	102
97	*Fagonia cretica* L.	Zygophyllaceae	Aerial part	SD-SH-26	M	14.7	113	113
98					E	8.30	111	97
99	*Nigella sativa* L.	Ranunculaceae	Seeds	SD-OD-16	M	19.3	91	107
100					E	7.00	110	122
101	*Solanum dubium* Fresen	Solanaceae	Fruits	SD-SH-34	M	37.2	94	93
102					E	10.9	96	97
103	*Grewia tenax* (Forsk)	Tiliaceae	Fruits	SD-OD-42	M	69.9	116	99
104					E	8.46	108	95

Intracellular melanogenesis activities and cell viabilities are expressed as means ($n = 2$)

M methanol extract, *E* 50 % ethanol/water extract

50 mM phosphate buffer, pH 6.5) and substrate [110 μl L-tyrosine (2 mM) or L-DOPA (2 mM)] were added. After incubation at 37 °C for 30 min, the absorbance at 510 nm was measured using a microplate reader. Each experiment was repeated twice. The tyrosinase activity was expressed as a percentage of the control treated with the solvent DMSO/water, without samples.

Cell culture

Murine melanoma B16-F0 cells (DS Pharma Biomedical, Osaka, Japan) were grown in Dulbecco's modified Eagle's medium (DMEM) without phenol red, supplemented with 10 % fetal bovine serum and 1 % penicillin/streptomycin. Cells were cultured at 37 °C in a humidified atmosphere containing 5 % CO_2.

Measurement of cellular melanin content

Confluent B16 melanoma cells were rinsed in phosphate-buffered saline (PBS) and removed using 0.25 % trypsin/EDTA. Cells were placed in 24-well plates (0.5×10^5 cells/well) and allowed to adhere at 37 °C for 24 h. After adding samples, cells were incubated for 72 h, and 150 μl

medium was placed in a 96-well plate. The absorbance of the medium was measured at 510 nm with a microplate reader (Biotec. Immuno Mini NJ-2300, Tokyo, Japan). The cells were washed with PBS, lysed in 600 μl NaOH (1 M), and heated for 15 min at 100 °C to solubilize the melanin. An aliquot of the resulting lysate (250 μl) was placed in a 96-well microplate, and the absorbance was measured at 405 nm with a microplate reader. Each experiment was repeated twice. Melanin production was expressed as a percentage of the control cells treated with the solvent DMSO/water without sample.

Cell viability

Cell viability was measured according to a previously reported method [19], using a micro-culture 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) technique. Cells (0.5×10^5 per well) were grown in 24-well plates. After incubation, 50 μl MTT reagent (5 mg/ml) was added to each well. The plates were incubated in a humidified atmosphere at 37 °C for 4 h. After the medium was removed, 1.0 ml isopropyl alcohol containing 0.04 N HCl was added to the plate. An aliquot (150 μl) was placed in a 96-well plate, and the absorbance was measured at 590 nm with a microplate reader. Each experiment was repeated twice. The cell viability was expressed as a percentage of the control cells treated with the solvent DMSO/water without sample.

Statistical analysis

All data in Table 2 were expressed as the mean ± SD. Differences were examined for statistical significance using the Student's *t* test. The data in Table 1 were expressed as the mean.

Result and discussion

Screening of Sudanese medicinal plant extracts

Sudanese medicinal plants were extracted with MeOH and 50 % EtOH. The effect of the 104 obtained extracts on melanogenesis was evaluated in B16 melanoma cells. As shown in Table 1, MeOH extracts of *Xanthium brasilicum* leaves, *Guiera senegalensis* J. F. Gmel, *Balanites aegyptiaca* bark, *T. brownii* bark, and *Haplophyllum tuberculatum* aerial parts enhanced melanogenesis by 207, 179, 163, 211, and 187 %, respectively. Among these, MeOH extracts from *T. brownii* bark had the greatest effect on intracellular melanogenesis, with no observed cytotoxicity. These result indicated that MeOH extracts of *T. brownii* bark should be further investigated.

Isolation and identification *T. brownii* bark MeOH extract compounds

Compounds **1**–**6** were isolated from *T. brownii* by a series of chromatographic experiments, and were identified as gallic acid (**1**), punicalagin (**2**), terchebulin (**3**), ellagic acid 4-*O*-α-L-rhamnopyranoside (**4**), ellagic acid (**5**), and 3,4,3′-tri-*O*-methylellagic acid (**6**) by comparing the NMR, MS, and UV spectrum data (Fig. 1) [10–17]. Among the isolated compounds, **2** and **3** were identified as α/β(3/2)-punicalagin (**2**) and α/β(3/2)-terchebulin (**3**). These compounds existed as an equilibrium mixture of the α- and β-forms.

Effects of isolated compounds on melanogenesis and tyrosinase activity

The effect of the MeOH extract from *T. brownii* bark at 200–50 μg/ml and isolated compounds on intracellular and extracellular melanogenesis and cell viability was evaluated (Table 2). Compound **2** dose-dependently enhanced intracellular melanogenesis; however, it was cytotoxic at 46 and 92 μM. In contrast, compound **3** dose-dependently enhanced extracellular melanogenesis, with less cytotoxicity than compound **2**, even though these compounds had similar chemical structures. The structure differed between the two compounds at the D2 position. In the case of compound **2**, C-D2 was attached to C-5, forming a C–C bond. In contrast, in compound **3**, C-D2 and C-4 were bound via an oxygen, which has higher flexibility than a C–C bond. The difference in flexibility between compounds may cause the difference in cell viability and melanogenesis.

Compounds **4**, **5**, **6** were ellagic acid and its derivatives. Compound **4**, an ellagic acid rhamnoside, had no effect on melanogenesis, whereas **5** and **6** stimulated melanogenesis. Compound **5** dose-dependently increased intracellular melanogenesis with no cytotoxicity. Furthermore, it exhibited higher activity than theophylline, which is known to stimulate melanogenesis. However, its effect on extracellular melanogenesis was less than theophylline. Compound **6**, a methylated form of **5**, induced cell proliferation. Intracellular melanin levels were increased in parallel with the number of melanoma cells.

Among the isolated compounds from *T. brownii* bark MeOH extract, compound **1** inhibited melanogenesis. In particular, extracellular melanogenesis following treatment with 100, 50, or 25 μM compound **1** was 16, 13, and 12 % of the control, respectively. This inhibitor activity was greater than arbutin, a known inhibitor of melanogenesis. Compound **1** had a lesser effect on intracellular melanogenesis, which was inhibited approximately 70 % at the same concentrations. Skin surface pigmentation is

Table 2 Tyrosinase activity, cell viability and intracellular and extracellular melanogenesis activity of *Terminalia brownii* bark MeOH extract and its isolated compounds [gallic acid (**1**), α,β-punicalagin (**2**), α,β-terchebulin (**3**), ellagic acid 4-*O*-α-L-rhamnopyranoside (**4**), ellagic acid (**5**), 3,4,3′-tri-*O*-methylellagic acid (**6**)] using B16 melanoma cells

Cell viability and melanogenesis activity (%)				Substrate	Tyrosinase activity (%)		
Extract concentrations	200 μg/ml	100 μg/ml	50 μg/ml		125 μg/ml	63 μg/ml	32 μg/ml
Cell viability	70 ± 1.6*	96 ± 1.6	97 ± 1.4	L-Tyrosine	37 ± 25.4	80 ± 9.0	84 ± 1.2
Intracellular melanin	299 ± 15.3**	220 ± 33.1*	113 ± 1.8	L-DOPA	55 ± 10.1*	81 ± 10.5	87 ± 6.3
Extracellular melanin	139 ± 3.4	131 ± 4.5	116 ± 1.8				
1 Concentrations	100 μM	50 μM	25 μM		200 μM	100 μM	50 μM
Cell viability	100 ± 7.6	98 ± 1.1	102 ± 1.5	L-Tyrosine	140 ± 5.8	113 ± 2.7	88 ± 0.2
Intracellular melanin	72 ± 0.0*	68 ± 0.8*	73 ± 0.8*	L-DOPA	101 ± 0.8	101 ± 2.3	84 ± 1.8
Extracellular melanin	16 ± 0.5**	13 ± 0.2**	12 ± 0.2**				
2 Concentrations	92 μM	46 μM	23 μM		200 μM	100 μM	50 μM
Cell viability	45 ± 2.8**	48 ± 1.9**	73 ± 8.1*	L-Tyrosine	84 ± 0.1	92 ± 1.1	74 ± 0.9
Intracellular melanin	249 ± 27.4*	106 ± 10.6	94 ± 6.3	L-DOPA	78 ± 0.3	89 ± 0.8	84 ± 0.1
Extracellular melanin	142 ± 12.9	85 ± 0.0	79 ± 0.0				
3 Concentrations	200 μM	100 μM	10 μM		200 μM	100 μM	50 μM
Cell viability	70 ± 0.7*	95 ± 2.8	100 ± 3.3	L-Tyrosine	77 ± 1.9	90 ± 0.8	93 ± 0.6
Intracellular melanin	75 ± 14.2	86 ± 1.7	100 ± 3.3	L-DOPA	80 ± 1.8	86 ± 2.1	91 ± 1.7
Extracellular melanin	146 ± 0.5*	125 ± 0.9	99 ± 1.5				
4 Concentrations	200 μM	100 μM	10 μM		200 μM	100 μM	50 μM
Cell viability	85 ± 3.4	93 ± 0.2	99 ± 6.9	L-Tyrosine	50 ± 0.8*	70 ± 1.1	79 ± 0.7
Intracellular melanin	103 ± 6.9	111 ± 6.4	124 ± 3.7	L-DOPA	70 ± 0.6*	88 ± 0.3	79 ± 1.3
Extracellular melanin	121 ± 0.3	113 ± 2.9	107 ± 2.6				
5 Concentrations	200 μM	100 μM	10 μM		200 μM	100 μM	50 μM
Cell viability	93 ± 3.7	93 ± 7.0	97 ± 1.3	L-Tyrosine	85 ± 1.6	76 ± 0.0	79 ± 1.3
Intracellular melanin	148 ± 5.3*	132 ± 0.0*	106 ± 1.0	L-DOPA	98 ± 2.9	98 ± 1.7	104 ± 2.2
Extracellular melanin	109 ± 2.6	109 ± 0.2	103 ± 2.3				
6 Concentrations	200 μM	100 μM	10 μM		200 μM	100 μM	50 μM
Cell viability	172 ± 5.5**	123 ± 6.1	90 ± 8.9	L-Tyrosine	86 ± 0.4	82 ± 1.2	83 ± 0.8
Intracellular melanin	174 ± 13.6	140 ± 22.4	115 ± 4.4	L-DOPA	94 ± 1.5	95 ± 1.2	98 ± 0.1
Extracellular melanin	132 ± 27.6	115 ± 24.1	112 ± 6.9				
Theophylline	500 μM	250 μM	125 μM				
Cell viability	91.4 ± 1.3	94.4 ± 4.1	95.5 ± 11.0				
Intracellular melanin	166.8 ± 31.7*	131.7 ± 1.9	127.6 ± 6.6				
Extracellular melanin	204 ± 1.6**	183.2 ± 3.2**	170.7 ± 0.7**				
Arbutin	730 μM	365 μM	183 μM				
Cell viability	97.9 ± 1.3	98.4 ± 1.1	88.3 ± 7.5				
Intracellular melanin	81.2 ± 0.6	85.7 ± 0.6	96.0 ± 1.3				
Extracellular melanin	26.1 ± 4.4**	30.3 ± 0.4**	44.5 ± 0.4**				

Cells were treated with the extract or its components for 72 h following melanogenesis activity was measured. Cell viability was determined by the MTT method. Tyrosinase activity of the extract and **1**–**6** were determined using L-tyrosine and L-DOPA as substrate. The activities of melanine production are expressed as percentage to that of control (100 %). Theophylline and arbutin are used as positive control of melanogenic stimulator and inhibitor respectively

The data of theophylline are described in [20]

* $p < 0.05$ and ** $p < 0.01$ compared with respective control values $n = 2$

regulated by extracellular melanin. Thus, compound **1** may be a beneficial lead compound to develop whitening agents.

The effects of the isolated compounds on tyrosinase activity using L-tyrosine and L-DOPA as substrates are shown in Table 2. Compounds **2**, **3**, **5**, and **6** stimulated melanogenesis, but did not stimulate tyrosinase activity at 200, 100, and 50 μM. Furthermore, compound **1** did not inhibit tyrosinase, despite its ability to inhibit extracellular

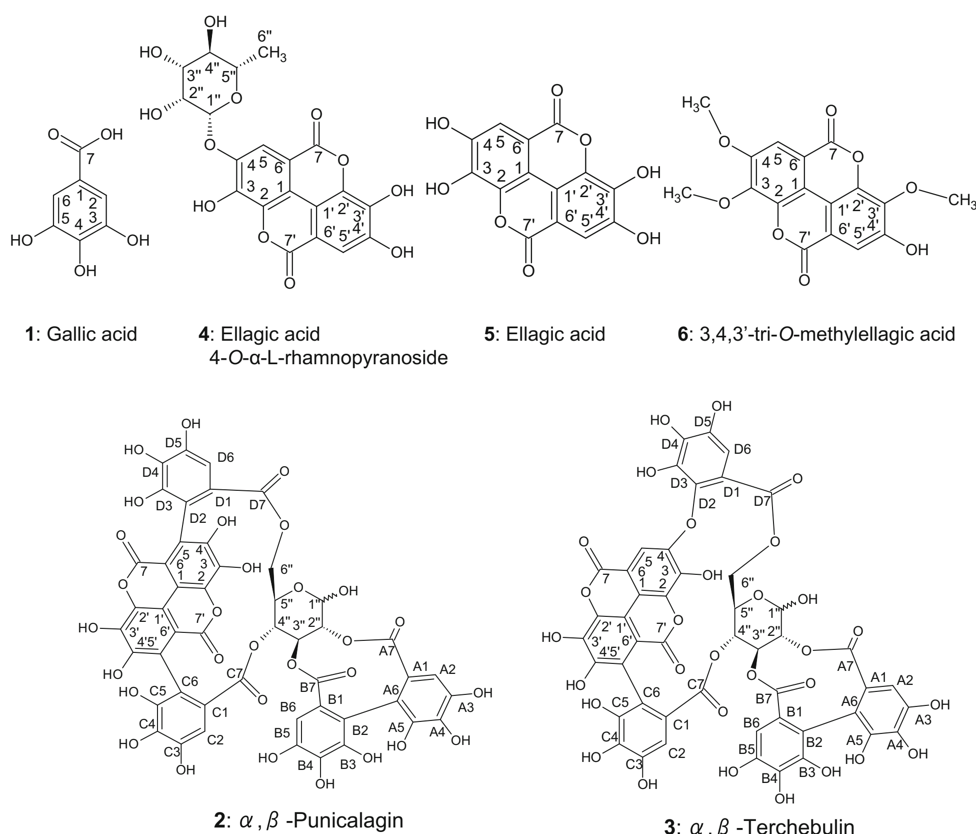

Fig. 1 Structures of isolated compounds from *Terminalia brownii* MeOH extract

melanogenesis in B16 melanoma cells. Thus, the effects of compounds **1**, **2**, **3**, **5**, and **6** on melanogenesis is likely not due to tyrosinase activity. Rather, they may affect tyrosinase expression. Microphthalmia-associated transcription factor (MITF), which upregulates the expression of tyrosinase, is of interest in the study of melanogenesis [21–23]. The effects of compounds **1**, **2**, **3**, **5**, and **6** on melanogenesis may be associated with the expression or activity of MITF. Furthermore, compounds **1** and **3**, which had a strong effect on extracellular melanin content, may alter melanin transport in B16 melanoma cells. Elucidation of the precise mechanisms of these compounds is necessary. Nevertheless, a fundamental investigation of medicinal plant extracts and their components, and their effects on intracellular and extracellular melanin content, was achieved in this study.

Conclusions

Our B16 melanoma cell screen revealed that the MeOH extract of *T. brownii* bark showed the greatest enhancement in melanogenesis among 104 extracts of Sudanese medicinal plants, with no cytotoxicity. We isolated and identified 6 compounds, and determined their effects on intracellular and extracellular melanogenesis and tyrosinase activity. Among the isolated compounds, **2**, **5**, and **6** enhanced intracellular melanogenesis, whereas compound **3** enhanced extracellular melanogenesis. In contrast, compound **1** decreased both extracellular and intracellular melanin content, with a stronger effect on extracellular melanin.

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