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Compositions and melanogenesis-inhibitory activities of the extracts of defatted shea (*Vitellaria paradoxa*) kernels from seven African countries

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ABSTRACT

Constituents of shea (*Vitellaria paradoxa*; Sapotaceae) kernels are commonly used as functional food and for skin care by indigenous peoples in Africa. Twenty-six MeOH extract samples of defatted shea kernels from seven sub-Saharan countries, *i.e.*, Côte d'Ivoire, Ghana, Nigeria, Cameroon, Chad, Sudan, and Uganda, were investigated in this study. HPLC coupled to an evaporative light-scattering detector and LC-QTOF (time-of-flight)-MS revealed that the extracts contained sugars (25.6–80.2%) as the most predominant constituents, followed by oleanane-type triterpene glycosides (4.9–35.2%), accompanied by minor amounts of cucurbates (1.0–21.0%) and phenolic compounds (0.3–16.0%). Principal component analysis and hierarchical clustering analysis classified the twenty-six samples into three groups. Upon evaluation of the melanogenesis-inhibitory activities of all samples in B16 melanoma cells induced by α-melanocyte-stimulating hormone, it was found that samples S2, S3, and S21 were lower-risk melanogenesis inhibitors (39.4–42.5% melanin content, 78.6–91.6% cell viability) with a small activity-to-cytotoxicity ratio (0.18–0.94), and were superior to reference arbutin at a concentration of 100 μg/mL. These results suggest that the extracts of defatted shea kernels and their constituents can be regarded as skin-whitening agents.

1. Introduction

The shea tree (*Vitellaria paradoxa* C.F. Gaertner; synonyms *Butyrospermum paradoxum* (C. F. Gaertn.) Hepper, *Butyrospermum parkii* (G. Don) Kotschy; belonging to family Sapotaceae) is indigenous to the savanna belt extending across sub-Saharan Africa north of the equator, ranging from Mali in the west to Ethiopia and Uganda in the east (Di Vincenzo et al., 2005; Maranz et al., 2004a,b; Masters et al., 2004). The fruit of the tree is edible and nutritious, while the most widely valued product of shea tree is shea butter, the edible fat extracted from the seed kernel, consisting of an olein fraction and a stearin fraction along with non-saponifiable (non-lipid) compounds (Bail et al., 2009). Fractionated shea stearin is used primarily as a cocoa butter substitute or extender in chocolate manufacture (Masters et al., 2004). These applications are due to properties imparted by the structures of its component triacylglycerols. In addition, shea butter is increasingly popular as a component of skin care products and cosmetic product formulations, in part due to the unusually high level of non-saponifiable lipid (NSL) constituents in the fat (Alander, 2004).

In order to characterize and quantify the constituents of shea butter among widely dispersed *V. paradoxa* populations, we have determined the contents and compositions of triterpene alcohol fractions from the NSL, and fatty acid, triacylglycerol, and triterpene ester compositions of the kernel lipids (hexane extracts) from twenty-six shea kernel samples from seven sub-Saharan countries (Akihisa et al., 2010a, 2011), and have demonstrated that acetyl and cinnamyl triterpene esters isolated from the kernel fat could be valuable as anti-inflammatory agents and chemopreventive agents in chemical carcinogenesis (Akihisa et al., 2010b). On the other hand, since there seems to be little industrial utilization of defatted shea kernel, other than as fuel, we were interested in the evaluation of pharmacological and cosmeceutical potentials of the constituents of defatted shea kernel. In this context, we have

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investigated the oleanane-type triterpene glycosides, phenolic compounds, and sugar constituents (Zhang et al., 2014), as well as cucurbates (3-hydroxy-2-(2*Z*-pentenyl)-cyclopentane-1-acetic acid derivatives), of the defatted shea kernel, and have evaluated their bioactivities (Zhang et al., 2015).

We now report, in this paper, the contents and compositions, similarity evaluation, and melanogenesis-inhibitory activities of the MeOH extracts of defatted shea kernels for twenty-six samples from seven sub-Saharan countries. All extracts were further evaluated for their inhibitory effects against Epstein–Barr virus early antigen (EBV–EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells. The profile analysis of the MeOH extracts was performed using HPLC–evaporative light-scattering detector (ELSD) and LC-QTOF (time-of-flight)-MS systems.

2. Materials and methods

2.1. Plant materials

The shea nut samples used in this study were collected and identified by one of the authors (Eliot T. Masters) on behalf of the World Agroforestry Centre (ICRAF), an international research institute constituted under the Consultative Group for International Agricultural Research (GGIAR), in parallel to project CFC/FIGOOF/23 'Improving Product Quality and Market Access for Shea Butter Originating from sub-Saharan Africa' (Pro*Karité*). Near the geographic center of a regional sampling mission undertaken from Senegal to South Sudan, the specific samples described in this study were collected as fresh fruits from fallow ground beneath the crowns of healthy mature trees at twenty-six sites of seven countries in Africa (Table 1 and Fig. 1) (Akihisa et al., 2010a). These included one site in Côte d'Ivoire (sample S1), one in Ghana (S2), eleven in Nigeria (S3–S13), two in Cameroon (S14 and S15), eight in Chad (S16–S23), two in Sudan (S24 and S25), and one in Uganda (S26). The nut samples were numbered sequentially based on the longitude of the collection site, beginning with the westernmost site.

2.2. Chemicals and reagents

The chemicals and reagents were purchased as follows: fetal bovine serum (FBS), RPMI-1640 medium, antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin), Dulbecco's modified Eagle's medium (D-MEM), Eagle's minimal essential medium (MEM), DL-α-tocopherol, α-melanocyte-stimulated hormone (α-MSH), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) from Sigma-Aldrich Japan Co. (Tokyo, Japan). All other chemicals and reagents were of analytical grade. Paradoxoside A (**1**), paradoxoside B (**2**), tieghemelin A (**3**), butyroside D (**4**), arganine C (**5**), glucosylcucurbic acid (**6**), methyl glucosylcucurbate (**7**), arbutin (**8**), gallic acid (**9**), (+)-catechin (**10**), rutin (**11**), quercetin (**12**), *proto*-quercitol (**13**), sucrose (**14**), and maltose (**15**), isolated from the defatted residue of shea kernel (sample S9 collected in Nigeria) (Zhang et al., 2014, 2015), were used as the reference compounds.

2.3. Extraction and preparation of sample solution

The defatted shea kernel (5.0 g) was extracted 3 times with 100 mL of MeOH at 60 °C for 3 h. The extract was centrifuged at 5000 rpm for 10 min with a CAX-370 centrifuge (Tomy Seiko Co., Ltd., Tokyo, Japan). The supernatant was evaporated under reduced pressure at 45 °C using a rotary vacuum evaporator. The yield of the extract was determined gravimetrically by dividing the extracted material by the defatted shea kernel weight. The extract was then dissolved in 100% MeOH to obtain a final volume of 2.0 mL. About 0.2 mL of the solution was filtered through a 0.45-μm syringe filter unit (GL Science Inc., Tokyo, Japan), and then 10.0 μL of this solution was injected into the HPLC–ELSD system for analysis.

Table 1
Geographical positions of the sites of shea nut collection, and the yields of the twenty-six shea kernel extracts.

shea nut sample		site of shea nut collection			yields of extracts (%)	
		longitude	latitude	elevation (m)	hexane extract	MeOH extract[a]
Côte d'Ivoire	S1	W 7° 12′ 58”	N 10° 1′ 35”	453	46.1	23.5
Ghana	S2	W 0° 29′ 27”	N 6° 47′ 11”	145	36.8	22.0
Nigeria	S3	E 4° 28′ 48”	N 11° 35′ 3”	280	45.7	13.7
	S4	E 5° 18′ 7”	N 10° 58′ 9”	337	41.8	16.4
	S5	E 6° 13′ 52”	N 10° 10′ 22”	347	36.6	15.5
	S6	E 6° 44′ 43”	N 9° 5′ 50”	242	30.6	10.8
	S7	E 6° 54′ 45”	N 8° 35′ 4”	173	51.2	12.3
	S8	E 6° 56′ 35”	N 10° 38′ 44”	683	37.7	21.1
	S9	E 7° 27′ 9”	N 9° 40′ 53”	365	53.7	12.3
	S10	E 8° 58′ 54”	N 8° 38′ 34”	160	38.5	12.5
	S11	E 11° 34′ 36”	N 9° 9′ 16”	313	39.1	4.8
	S12	E 12° 29′ 6”	N 9° 19′ 15”	245	43.8	13.8
	S13	E 12° 52′ 13”	N 9° 33′ 36”	267	39.1	6.3
Cameroon	S14	E 10° 28′ 49”	N 5° 12′ 55”	1421	29.7	8.2
	S15	E 11° 2′ 56”	N 5° 50′ 12”	988	30.4	15.0
Chad	S16	E 14° 19′ 34”	N 9° 36′ 30”	314	39.1	10.3
	S17	E 15° 29′ 30”	N 9° 35′ 54”	363	39.0	7.2
	S18	E 15° 38′ 17”	N 9° 19′ 41”	400	47.5	10.4
	S19	E 16° 9′ 17”	N 8° 31′ 25”	468	46.4	15.7
	S20	E 16° 21′ 12”	N 9° 22′ 45”	366	46.4	12.9
	S21	E 17° 4′ 44”	N 8° 38′ 16”	390	36.6	9.6
	S22	E 17° 26′ 45”	N 9° 1′ 12”	387	48.0	13.7
	S23	E 18° 2′ 19”	N 9° 3′ 56”	418	48.0	15.9
Sudan	S24	E 28° 26′ 55”	N 7° 17′ 19”	473	45.0	23.4
	S25	E 30° 31′ 27”	N 6° 34′ 57”	471	48.4	16.2
Uganda	S26	E 33° 40′ 59”	N 2° 21′ 0”	1203	46.7	20.1
	means				42.0	13.8

[a] MeOH extract of the defatted residue.