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# Triterpene glycosides and other polar constituents of shea (*Vitellaria paradoxa*) kernels and their bioactivities

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ABSTRACT

The MeOH extract of defatted shea (*Vitellaria paradoxa*; Sapotaceae) kernels was investigated for its constituents, and fifteen oleanane-type triterpene acids and glycosides, two steroid glucosides, two pentane-2,4-diol glucosides, seven phenolic compounds, and three sugars, were isolated. The structures of five triterpene glycosides were elucidated on the basis of spectroscopic and chemical methods. Upon evaluation of the bioactivity of the isolated compounds, it was found that some or most of the compounds have potent or moderate inhibitory activities against the following: melanogenesis in B16 melanoma cells induced by α-melanocyte-stimulating hormone (α-MSH); generation of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, against Epstein–Barr virus early antigen (EBV-EA) activation induced by 12-*O*-teradecanoylphorbol 13-acetate (TPA) in Raji cells; t TPA-induced inflammation in mice, and proliferation of one or more of HL-60, A549, AZ521, and SK-BR-3 human cancer cell lines, respectively. Western blot analysis established that paradoxoside E inhibits melanogenesis by regulation of expression of microphthalmia-associated transcription factor (MITF), tyrosinase, and tyrosinase-related protein-1 (TRP-1) and TRP-2. In addition, tieghemelin A was demonstrated to exhibit cytotoxic activity against A549 cells ($IC_{50}$ 13.5 μM) mainly due to induction of apoptosis by flow cytometry. The extract of defatted shea kernels and its constituents may be, therefore, valuable as potential antioxidant, anti-inflammatory, skin-whitening, chemopreventive, and anticancer 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 the Sapotaceae family] 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, 2004b; 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. 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 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, 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 36 shea kernel samples from seven sub-Saharan countries were recently determined (Akihisa et al., 2010c, 2011). In addition, it was

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demonstrated that cinnamyl and acetyl triterpene esters isolated from the kernel fat could be valuable as anti-inflammatory agents and chemopreventive agents in chemical carcinogenesis (Akihisa et al., 2010b). From such perspectives, the evaluation of pharmacological and cosmeceutical potentials of the constituents of defatted shea kernel were of interest, since there seems to be little industrial utilization of defatted shea kernel (residue), other than as fuel. Herein, the isolation of oleanane-type triterpene acids and their glycosides, phenolic compounds, and other polar constituents from the defatted shea kernel, and the evaluation of bioactivity of the isolated compounds, are described.

## 2. Results and discussion

### 2.1. Melanogenesis-inhibitory, antioxidant, EBV-EA-induction-inhibitory, anti-inflammatory, and cytotoxic activities of defatted shea kernel extracts

Dried and pulverized shea kernels were treated with hexane to remove the lipid fraction (Akihisa et al., 2010c), and the defatted kernels were then treated with MeOH in order to isolate the soluble hydrophilic components. The MeOH extract was fractionated into EtOAc-, *n*-BuOH-, and $H_2O$-soluble fractions. Extracted fractions were evaluated for melanogenesis-inhibitory and cytotoxic activities in α-melanocyte-stimulated hormone (α-MSH)-stimulated B16 melanoma cells, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity, inhibitory effects on Epstein–Barr virus early antigen (EBV-EA) induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in Raji cells, anti-inflammatory activity against TPA-induced inflammation in mice, and cytotoxic activity against four human cancer cell lines by means of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay, respectively. As compiled in Table 1, the MeOH extract, and the EtOAc- and BuOH-soluble fractions exhibited potent melanogenesis-inhibitory activities (28.2–58.0% melanin content) at 100 μg ml$^{-1}$ concentration, which were more potent than that of the reference arbutin (4-hydroxyphenyl β-D-glucopyranoside; 87.1% melanin content at 100 μg ml$^{-1}$), but with some cytotoxicities (37.9–63.4% cell viability). The MeOH extract and the three fractions exhibited potent DPPH free radical-scavenging activities ($IC_{50}$ 6.8–24.3 μg ml$^{-1}$) similar to, though slightly less inhibitory than, that of the reference α-tocopherol ($IC_{50}$ 5.6 μg ml$^{-1}$; Table 1). Upon evaluation of the inhibitory effects against TPA (20 ng)-induced EBV-EA activation in Raji cells, the MeOH extract and the EtOAc-soluble fraction exhibited potent inhibitory effects (6.9% and 5.3% induction of EBV-EA at 100 μg ml$^{-1}$ concentration, respectively), while both the MeOH extract and the $H_2O$-soluble fraction showed inhibitory activity (70% and 81% inhibition at 1.0 mg ml$^{-1}$ concentration, respectively) against TPA (1.0 μg)-induced inflammation in mice (Table 2). On the other hand, the MeOH extract and the EtOAc-soluble fraction exhibited moderate cytotoxic activity against HL-60 (leukemia) cell line ($IC_{50}$ 76.6 and 69.5 μg ml$^{-1}$, respectively), and the BuOH-soluble fraction exhibited moderate cytotoxicity against all of the HL-60, A549 (lung), AZ521 (stomach), and SK-BR-3 (breast) cell lines tested ($IC_{50}$ 43.2–88.0 μg ml$^{-1}$).

### 2.2. Isolation, identification, and structure elucidation

All three fractions from the MeOH extract were subjected to successive column chromatography (CC) on Diaion HP-20, silica gel ($SiO_2$), octadecyl silica gel (ODS), and Sephadex LH-20 columns, and to reversed-phase HPLC which led to the isolation of five compounds, **17** and **18** (as the tetraacetate derivatives; **17a** and **18a**, respectively), **23**, **26**, and **27**, from the EtOAc-soluble fraction, 20 compounds, **1**–**3**, **6**–**12**, **14**–**16**, **19**–**22**, **24**, **25**, and **28**, from the BuOH-soluble fraction, and four compounds, **4**, **5**, **29**, and **30**, from the $H_2O$-soluble fraction. Among these, five compounds, **1**, **2**, **8**, **9**, and **14**, were new, and the 21 known compounds were identified as tieghemelin A (**3**), arginine C (**5**), and 3-*O*-β-D-glucopyranosyl 16α-hydroxyprotobassic acid (**7**) (Gosse et al., 2002), butyroside D (**4**) and 3-*O*-β-D-glucuronopyranosyl protobassic acid (**10**) (Li et al., 1994), 3-*O*-β-D-glucuronopyranosyl 16α-hydroxyprotobassic acid (**6**) (Gosse et al., 2002; Li et al., 1994), Mi-glycoside I (**11**) and 3-*O*-β-D-glucopyranosyl bassic acid (**15**) (Nigam et al., 1992), protobassic acid (**12**) (Nigam et al., 1992; Sahu, 1996; Toyota et al., 1990), bassic acid (**16**) (Sahu, 1996; Toyota et al., 1990), spinasterol 3-*O*-β-D-glucopyranoside (**17**) and 22-dihydrospinasterol 3-*O*-β-D-glucopyranoside (**18**) (as the tetraacetate derivatives, **17a** and **18a**, respectively) (Furuya et al., 1990; Kojima et al., 1990), (2*S*,4*S*)-2-*O*-(β-D-glucopyranosyl)pentane-2,4-diol (**19**) and (2*R*,4*S*)-2-*O*-(β-D-glucopyranosyl)pentane-2,4-diol (**20**) (Hybelbauerová et al., 2009; Kaneko et al., 1998), isotachioside (**22**) (Inoshiri et al., 1987), gallic acid (**23**) (Dini, 2011), (+)-catechin (**24**) and (−)-epicatechin (**25**) (Seto et al., 1997), quercetin (**26**) (Atta et al., 2011), rutin (**27**) (Savage et al., 2011), and *proto*-quercitol (**28**) (Machado and Lopes, 2005; Wacharasindhu et al., 2009), also known as quercitol, by comparison of MS, $^1$H NMR, and $^{13}$C NMR spectroscopic and optical rotation data with corresponding literature data (Fig. 1). On the other hand, three known compounds, arbutin (**21**), sucrose (**29**), and maltose (**30**), were identified by comparison of their spectroscopic signatures against those of reference standards.

The structures of the five new compounds were elucidated on the basis of spectroscopic data by comparison with literature as described below, and their proposed structures were supported by analysis of the DEPT, $^1$H–$^1$H COSY, HMQC, HMBC, and NOSEY data.

**Table 1**
Melanogenesis-inhibitory activities and cytotoxicities in B16 mouse melanoma cell line, and DPPH free-radical- scavenging activities of defatted shea kernel extract.

| Extract or fraction | Melanogenesis-inhibitory activity and cytotoxicity[a] | | | | DPPH Free-radical-scavenging activity, $IC_{50}$ (μg ml$^{-1}$)[b] |
|---|---|---|---|---|---|
| | Melanin content (%) | | Cell viability (%) | | |
| | 10 μg ml$^{-1}$ | 100 μg ml$^{-1}$ | 10 μg ml$^{-1}$ | 100 μg ml$^{-1}$ | |
| Control (100% DMSO) | 100.0 ± 4.2 | 100.0 ± 4.2 | 100.0 ± 3.1 | 100.0 ± 3.1 | |
| MeOH extract | 92.0 ± 6.7 | 28.2 ± 5.9 | 101.2 ± 3.0 | 51.8 ± 8.2 | 6.8 ± 0.8 |
| EtOAc-soluble fraction | 99.2 ± 1.4 | 58.0 ± 3.9 | 105.0 ± 3.7 | 63.4 ± 3.5 | 24.3 ± 0.7 |
| BuOH-soluble fraction | 100.5 ± 4.6 | 48.4 ± 8.7 | 101.8 ± 4.6 | 37.9 ± 2.3 | 13.2 ± 1.4 |
| $H_2O$-soluble fraction | 101.6 ± 4.6 | 114.9 ± 6.0 | 108.9 ± 6.5 | 90.4 ± 4.6 | 6.8 ± 1.5 |
| Arbutin[c] | 98.7 ± 9.7 | 68.9 ± 2.3 | 96.5 ± 2.9 | 87.1 ± 2.8 | |
| α-Tocopherol[c] | | | | | 5.6 ± 0.1 |

[a] Melanin content and cell viability were determined based on the absorbances at 405 , and 570 (test wavelength) – 630 (reference wavelength) nm, respectively, by comparison with those for DMSO (100%). Each value represents the mean ± S.D. ($n$ = 3). Concentration of DMSO in the sample solution was 2 μl ml$^{-1}$.
[b] Values of fourfold experiments. Concentration of DMSO in the sample solution was 5 μl/ml.
[c] Reference compounds.