Black Salve Composition: An evaluation of the potential for Normal Tissue Toxicity and Treatment Failure from Black Salve Products

Andrew Croaker[a,b], Graham J. King[a], John H. Pyne[c], Shailendra Anoopkumar-Dukie[d], and Lei Liu[a]*.

[a]Southern Cross Plant Science, Southern Cross University, Lismore, NSW, Australia;
[b]Wesley Medical Research Institute, Wesley Hospital, Auchenflower, QLD, Australia;
[c]School of Medicine, University of Queensland, St Lucia, QLD, Australia;
[d]School of Pharmacy, Griffith University, Gold Coast Campus, Gold Coast, QLD, Australia;

* Address Correspondence to:

Dr. Lei Liu Southern Cross Plant Science, Southern Cross University.
Military Rd East Lismore NSW 2480 Australia
ben.liu@scu.edu.au T: +61 2 6620 3293

Abstract

Black salve, used as an alternative skin cancer therapy, contains both herbal and chemical constituents, including extracts of the rhizomes of *Sanguinaria canadensis* and zinc chloride. Black salves may be ordered online and have been associated with cases of extensive tissue necrosis and treatment failures that have resulted in patient fatalities. Despite these adverse outcomes and continued use by patients, black salve products have not been quantitatively assessed to determine their constituent concentrations.

Thirteen different black salve products from eight manufacturers were analysed using validated HPLC-MS and ICP-MS methods to assess *S. canadensis* alkaloid and zinc chloride concentrations. This analysis revealed a dramatic variation in constituent concentrations

between manufacturers of black salve products. The alkaloid sanguinarine was found at concentrations significantly exceeding the cytotoxic IC_{50} of normal human epidermal keratinocytes in the majority of black salve products, with one black salve examined having a concentration 900 times the IC_{50} level. The majority of products contained zinc chloride at concentrations known to kill normal human tissue, while one black salve product was found to contain an elevated lead level of 258 ppm, which exceeds the 10 ppm FDA determined lead limit for cosmetic products. Whilst some of the black salve products were found to contain high concentrations of constituents cytotoxic to normal tissue, others were found to contain insufficient cytotoxic constituents to be reasonably expected to exert any anti-cancer activity.

In their current formulations, the majority of black salves analysed pose a significant risk of harm to patients and should not be available for unregulated clinical use. Further black salve toxicity testing is urgently required.

Keywords: skin cancer, black salve, sanguinarine, topical, escharotic, zinc chloride

Abbreviations: HPLC-MS - High Performance Liquid Chromatography–Mass Spectrometry; ICP-MS - Inductively Coupled Plasma–Mass Spectrometry; IC50 - Half Maximal Inhibitory Concentration; CAM - Complementary and Alternative Medicine; QBA - Quaternary Benzophenanthridine Alkaloid

1. Introduction

Originally developed in the 19th century, cytotoxic escharotics containing *Sanguinaria canadensis*, commonly known as bloodroot and zinc chloride continue to be used today in the form of black salve. Despite multiple case studies associating black salve use with concerning

toxicities and treatment failures (Affleck and Varma, 2007; Bickle and Bennett, 2008; Brown et al., 2001; Eastman et al., 2014; Hou and Brewer, 2015; Jellinek and Maloney, 2005; Leecy et al., 2013; Ma et al., 2012; Osswald et al., 2005; Saltzberg et al., 2009; Schlichte et al., 2014) that in two cases resulted in patient death from metastatic basal cell carcinoma (Laub DR 2008) and metastatic melanoma (Sivyer and Rosendahl, 2014), this controversial, complementary and alternative medicine (CAM) continues to be poorly regulated by government agencies.

Black salve is not registered as a good therapeutic in any jurisdiction, with each manufacturer producing their own unique formulation. This lack of standardization or regulatory imposed quality control, combined with the natural phytochemical variability of botanical constituents, suggests black salves may be unpredictable therapeutic products (Croaker et al., 2016).

Little is known about the quantitative composition of black salve preparations. Only one paper found in the peer reviewed medical literature has performed an analysis of black salve. The manuscript analyzed a single black salve preparation from an unknown manufacturer, with gas chromatography-mass spectrometry (GC-MS) and scanning electron microscopy (SEM) with energy dispersive X-ray analysis (EDXA). Numerous compounds were identified, but were not quantified (Osswald et al., 2005).

As some black salve constituents have concentration dependent normal cell cytotoxicity (Croaker et al., 2017a) and may possess genotoxic and carcinogenic potential (Das et al., 2005), a quantitative analysis of black salve constituents is of significant clinical relevance. For this reason, we conducted a HPLC-MS and ICP-MS analysis of a broad selection of black salve products with a particular focus on the alkaloids sanguinarine, chelerythrine, protopine and allocryptopine, as well as zinc chloride concentrations.

2. Materials & methods

2.1 Black salve products

Twelve different black salve products were purchased from eight online retailers. An additional black salve was sourced from a local user in Northern New South Wales, Australia. Black salve products were kept at room temperature, unopened and stored in a lightproof container until their time of analysis.

2.2 Bloodroot rhizome

Wild harvested *S. canadensis* rhizomes were purchased from Pacific Botanicals (Oregon, United States) and confirmed as *S. canadensis* by Mr Peter Mouatt after comparison with herbarium sample voucher number: PHARN-12-0480 in the Medicinal Plant Herbarium, Southern Cross University, Australia. A voucher specimen of the rhizomes used in the present study has also been retained in the herbarium, voucher number: PHARM-18-0078. Bloodroot rhizome fragments were homogenized to a powder using a Retsch MM301 Tissue Lyzer, then stored in sealed amber vials at room temperature.

2.3 Alkaloid standard solutions

Sanguinarine and chelerythrine standards were purchased from Sigma-Aldrich Co. LLC. Protopine and allocryptopine standards were kindly supplied by Dr. Eve Taborska, Masaryk University, Czech Republic. A mixed standard solution containing the four alkaloids (250 μg/mL each) was prepared in methanol. A serial dilution (1 in 5) of this stock was made at 50, 10, 2, 0.4, 0.08, 0.016 and 0.0032 μg/mL.

2.4 Methanol extraction

Black salve products were stirred and homogenized with a measuring spatula in a low light environment for 30 seconds at room temperature (22^{o}C). The homogenized samples (20 mg each) were accurately weighed and dissolved in 1500 μl methanol in 2 ml amber glass vials

with the aid of sonication (40 minutes, 35^{o}C). The contents were then centrifuged at 3000 rpm for 5 minutes (1308g) and supernatant was removed by pipette and transferred to 2 ml HPLC-MS glass wells for analysis. The extractions were carried out in duplicate for each sample. Dried *S. canadensis* rhizomes were ground to powder using a ball mill (Mixer Mill MM301, Retsch) and then underwent the same methanol extraction procedure as the black salve samples.

2.5 HPLC-MS analysis

The analysis was undertaken using Agilent High Performance Liquid Chromatography (HPLC, series 1290) equipped with a vacuum degasser, binary pump and autoinjector, diode array detector (DAD, 1260) and quadrupole mass detector (MSD, 6120). The quantification method was developed in a similar manner to those reported by Liu et al. (2014). A Phenomenex Kinetex 1.7 μm F5 (100 A, 150x2.1 mm) column was used and column temperature was set at 40 ^{o}C. The injection volume was 3 μL per injection. The HPLC-MS system was controlled using ChemStation software.

A linear gradient elution program containing Milli-Q water with 0.05% trifluoroacetic acid (TFA) and acetonitrile with 0.05% TFA was used. The solvent gradient was programmed from 10 to 95% acetonitrile in 20 min with a flow rate of 0.3 mL/min and held at 95% acetonitrile for 3 min. The UV absorbance was monitored between 190 and 600 nm, and chromatograms were recorded at 288 nm for protopine, 284 nm for allocryptopine, 274 nm for sanguinarine and 269 nm for chelerythrine. The MSD was carried out in electrospray ionization (ESI) mode using the following parameters: drying gas flow, 12.0 L/min; nebulizer pressure, 35 psig; drying gas temperature, 350^{o}C; capillary voltage, 3000 V (positive). Single ion monitoring (SIM) mode was used for signal 1: *m/z* 354 [M+H^{+}] for protopine (5.0 - 6.5 min), *m/z* 332 [M+H^{+}] for sanguinarine (6.5 - 11.0 min) and signal 2: *m/z* 370 [M+H^{+}] for

allocryptopine (5.0 - 7.25 min), *m/z* 348 [$M+H^+$] for chelerythrine (7.25-11.0 min). MSD was also set up to scan mass range 100 – 1200 at signal 3.

The extract of *S. canadensis* was used as a positive control, while pure methanol was used as a negative control. The method was validated for linearity, precision, limit of detection, limit of quantification and working concentration range prior to quantification. The alkaloid concentration was calculated based on the UV response when at ppm level and calculated based on the response in MSD SIM when at ppb level.

2.6 Black salve ICP-MS metal and chloride analysis

Black salve samples underwent acid digestion metal analysis on a Perkin Elmer ELAN DRCe ICPMS. The instrument was calibrated before analysis using certified standards with calibration coefficients 0.9999 or greater. In duplicate, black salve samples (50 mg each) were accurately weighed and placed in digestion tubes, to which 5.0 ml of nitric acid was added. These were placed in a hot block digestor preheated to 120 ^oC, digested for 1 hour, cooled and allowed to settle for 2 hours prior to ICP-MS analysis. Chloride analysis was performed on a Perkin Elmer NexION 300D ICP-MS. Black salve samples (100 mg each) were accurately weighed and added to Falcon tubes, and dissolved in Milli-Q water (10 ml). The Falcon tubes were weighed using an analytical balance (0.1 mg) before and after adding black salve sample and water to acquire the accurate sample/water ratios (w/w). The Black salve Zn^{2+} and Cl^- concentrations were determined in duplicate for each black salve and used to calculate the zinc chloride concentration of each black salve product.

3. Results

3.1 Alkaloids in black salves

The HPLC-MS and ICP-MS analysis of 13 black salve products from 8 online retailers and one local user showed significant alkaloid compositional variation. *S. canadensis* alkaloids

had a striking concentration range, sanguinarine having a four order of magnitude variation with a 12500 fold difference between the black salves with the highest and lowest concentrations (Figure 1). Nine of the thirteen salves had sanguinarine levels exceeding the IC_{50} for epidermal keratinocytes (Ahmad et al., 2000). Product 2 and Product 13 had sanguinarine concentrations of 1.7 μg/g and 1.3 μg/g respectively, below the IC_{50} for epidermal keratinocytes but above that for skin fibroblasts. The concentration of Product 7 (0.27 μg/g) was at a level unlikely to confer normal or tumour cell sanguinarine induced cytotoxicity (Figure 1).

Chelerythrine, the other major quaternary benzophenanthridine alkaloid (QBA) present in bloodroot rhizomes also has *in vitro* normal cell cytotoxicity, with skin fibroblasts (KF-II) having an IC_{50} of 0.6 μg/ml (Malikova et al., 2006). As can be seen from Figure 2, a number of the tested black salves significantly exceeded this concentration level and would be expected to confer chelerythrine induced normal cell toxicity (Figure 2).

3.2 Metal and chloride in black salves

Zinc chloride is a major component of black salve formulations and our analysis shows the majority of black salve products tested to contain high concentrations of $ZnCl_2$. The majority of black salves were found to contain 20-45% $ZnCl_2$ by weight. The zinc concentration and water soluble chloride concentration were determined separately and then compared, allowing us to determine that the majority of zinc and chloride present in black salve formulations was in the form of zinc chloride (Table 1).

On testing the black salves for the presence of heavy metals by ICP-MS, Product 2 was found to contain lead (Pb) at a concentration of 258 ppm, which exceeds the 10 ppm lead level established by the FDA for externally applied cosmetics (FDA, December 2016).

4. Discussion

Sanguinaria canadensis rhizomes are a key ingredient in black salve formulations and contain a number of alkaloids that individually target multiple molecular pathways (Croaker et al., 2016). The most prevalent alkaloids in *S. canadensis* from the benzophenanthridine family are sanguinarine and chelerythrine, while those from the protopin family are protopine and allocryptopine (Slavik, 1960). Sanguinarine has previously been reported to represent 37% of the alkaloid pool found in *S. canadensis* rhizomes (Slavik, 1960); other studies confirm sanguinarine as the most prevalent bloodroot alkaloid (Graf et al., 2007; Salmore and Hunter, 2001). Sanguinarine confers concentration-dependent normal and malignant cell cytotoxicity, with A431 squamous carcinoma cells (SCC) having an IC_{50} of 0.7 μg/ml and epidermal keratinocytes having an IC_{50} of 3.7 μg/ml *in vitro* (Ahmad et al., 2000). A small increase in sanguinarine concentration may therefore result in bloodroot products having general indiscriminate cytotoxicity and the ability to disrupt the dermo-epidermal junction, thus permitting circulatory access of these topical agents through dermal capillaries.

While the topical pharmacokinetics of *S. canadensis* alkaloids have not been studied, patients have developed epidemic dropsy following the topical application of sanguinarine contaminated massage oil suggesting sanguinarine can be absorbed systemically through the skin (Sood et al., 1985). This condition, characterised by altered capillary permeability has a mortality rate of 3-7% (Wadia et al., 1971). The topical application of 20-40 ml of contaminated oil containing 0.0015% sanguinarine for 30 to 60 minutes before bathing has resulted in the development of mild cases of epidemic dropsy (Sood et al., 1985). While there have been no reported cases of epidemic dropsy developing as a result of black salve application (Croaker et al., 2018), the highest black salve sanguinarine concentration detected in our current study was 0.34%, which is over 200 times the sanguinarine concentration of

the adulterated massage oil. As a result, there is a theoretical risk of black salve, with its longer 24 hour contact period, causing epidemic dropsy, especially if used at multiple sites.

Several topical skin cancer therapies are currently available, each having a different pharmacokinetic profile. The maximum flux (Jmax), or amount of a compound that can penetrate the skin in a given time, is an important determinant of the local, systemic or toxicological effects of a topically applied drug (Roberts and Walters, 1998). The Jmax of *S. canadensis* alkaloids cannot be gauged by the compound concentration of a particular preparation; specific pharmacokinetic studies need to be performed in order to characterize the absorption profile. For example, an assessment of 5-fluouracil (5-FU) 5% cream in human cadaver skin, showed that only 2.1% of a 5-FU dose penetrates the skin over a 24 hour period (Levy et al., 2001). In contrast, ingenol mebutate 0.05% in an Epiderm ™ skin model, showed an initial rapid penetration with 25% of the compound present in the epidermal compartment after 2 hours, reducing to 6% by 24 hours (Stahlhut et al., 2012).

The topical pharmacokinetics of bloodroot alkaloids has not been studied as it has in the pharmaceutical agents mentioned above. Further studies should be undertaken to determine the association between alkaloid concentration and penetration. Despite the penetration kinetics of black salve alkaloids being currently unknown, our study demonstrates that a number of black salve products contain alkaloid concentrations that exceed normal cell cytotoxicity levels by several orders of magnitude.

A compound's molecular weight is the primary predictor of its skin penetration and absorption potential, with low molecular weight compounds (<500 Da) typically having greater Jmax pharmacokinetic function (Magnusson et al., 2004). The alkaloids present in black salve all have molecular weights below 400 g/mol, suggesting these small molecules pose a considerable risk of penetrating the skin barrier, and subsequently causing normal cell

toxicity. The extent of this risk will need to be determined by future alkaloid topical pharmacokinetic analysis.

Methanol extraction of black salve products marketed as 'therapeutics' may also release more constituents than would normally be available for stratum corneum penetration. Despite this consideration, we established that the highest sanguinarine concentration, of 3.4 mg/g in Product 1, was 900 times more concentrated than the IC_{50} of epidermal keratinocytes. This particular black salve, along with products 8 to 12, may cause significant normal tissue toxicity and pose a considerable risk to patients.

Individually, the alkaloids present in black salve are unselective allelochemicals that target multiple molecular processes, and although similar in structure, they appear to have differing affinity profiles, which have been reviewed and discussed in our previous papers (Croaker et al., 2017a; Croaker et al., 2017b). This amplifies the potential for neoplastic and normal cell cytotoxicity. In addition to differentially targeting molecular pathways, the alkaloids present in black salve appear to induce different modes of cell death. Sanguinarine induces cell death in keratinocytes by necrosis but in SCC cells by apoptosis (Ahmad et al., 2000), while sanguilutine has been found to induce cell death by autophagy and necroptosis (Hammerova et al., 2012).

Most studies to date on *S. canadensis* alkaloids have examined their effects in isolation. As black salve contains a number of cytotoxic alkaloids, black salve constituents may possess additive or synergistic cytotoxicity. A synergistic action between *S. canadensis* alkaloids has been suggested in microbiological experiments using *Helicobacter pylori* (Mahady et al., 2003), where sanguinarine (a major alkaloid in bloodroot) had a minimum inhibitory concentration (MIC) of 50 μg/ml and chelerythrine (the other major alkaloid in bloodroot) 100 μg/ml, whereas *S. canadensis* extract, which would contain both of these two

major bloodroot alkaloids and others, had a lower MIC of 12.5 μg/ml. The additive or synergistic cytotoxic effects between each of the constituents in black salve against normal or cancer cells have not been assessed, and so the individual constituent concentrations reported in the current study may underestimate the normal tissue toxicity risk posed by black salve.

Despite often being promoted as a natural skin cancer therapy, zinc chloride, which represents the main constituent by weight of some of the black salves tested, also has cytotoxic effects, causing 40% HLE B-3 human epithelial cell apoptosis and necrosis at a concentration of 80 μg/ml *in vitro* (Du et al., 2014). Human *in vivo* studies have shown zinc chloride to have an escharotic effect, with its depth of application determining the depth of tissue fixation and death (Mohs, 1941). $ZnCl_2$ concentrations of 20 to 45% kill and histologically fix both benign and malignant tissue (Mohs and Guyer, 1941), facilitating the staged complete removal of skin tumours. The majority of black salves we tested had $ZnCl_2$ concentrations that fell within this range. They would therefore be expected to lack cancer specificity as is often claimed. The zinc chloride used in Moh's fixed tissue technique allowed the surgical removal of skin cancers; the fixative was never used nor recommended as a standalone skin cancer treatment by Mohs.

Of added concern, Product 2 contained lead (Pb) levels 25 times higher than the safety limit recommended by the FDA. Thus some black salves, in addition to their other risks, may place patients at risk of heavy metal toxicity.

In addition to the normal cell cytotoxicity risk of some black salves (Brown et al., 2001) and the treatment failure risk of others (McDaniel and Goldman, 2002), the major alkaloid present in black salve formulations, sanguinarine, may have carcinogenic potential. Evidence from genotoxicity (Matkar et al., 2008; Das et al., 2004), murine carcinogenesis tests (Ansari and Das, 2010), and the association of sanguinarine-containing mouthwashes

with oral leukoplakia development (Damm et al., 1999), all suggest black salve should not be used by patients until its mutagenic and carcinogenic potential has been conclusively established (Croaker et al., 2017b).

These previous tests have generally used low *in vitro* sanguinarine concentrations, below 10 μM, due to compound cytotoxicity (Kevekordes et al., 2001) and low murine *in vivo* intraperitoneal doses at 10 mg/kg body weight or below (Das et al., 2004). These concentration ranges do not reflect the sanguinarine exposure that would ensue from using some of the black salve products tested. The systemic absorption and carcinogenic potential of high sanguinarine topical exposures should, therefore, be assessed as should the mutagenic potential of black salve therapies in human skin equivalent or human skin explant models, especially in adjacent skin tissue that remains viable and does not undergo necrosis.

5. Conclusion

The authors targeted quantitative analysis of black salve composition and its variability between sources are to our knowledge the first study to report on this important issue. The majority of black salves assessed contain high concentrations of cytotoxic alkaloids and $ZnCl_2$ that are expected to result in significant tissue necrosis. Moreover, a proportion of the black salves tested lacked sufficient constituent concentrations to confer tumour cytotoxicity, potentially placing patients at risk of cancer persistence and subsequent metastasis. Black salve manufacturers should be subjected to adequate and regulated quality control processes. The finding of heavy metal lead contamination in one black salve product also raises questions about manufacturer quality control processes. Black salve and other topical CAM therapies should be subjected to the same rigorous scientific and clinical evaluations required of pharmaceutical products. Not doing so, as our analysis suggests, places patients at considerable risk of toxicity and therapy failure. It is our contention that black salve has a

poor therapeutic index and should not be used in any circumstance until further efficacy and toxicity studies are undertaken.

Conflict of Interest

The authors declare they have no conflicts of interest.

Acknowledgements

We wish to thank Professor Eva Taborska of Masaryk University, Czech Republic for the provision of protopine and allocryptopine standards.

References

Affleck, A.G., Varma, S., 2007. A case of do-it-yourself Mohs' surgery using bloodroot obtained from the internet. The British journal of dermatology 157(5), 1078-1079.

Ahmad, N., Gupta, S., Husain, M.M., Heiskanen, K.M., Mukhtar, H., 2000. Differential antiproliferative and apoptotic response of sanguinarine for cancer cells versus normal cells. Clin. Cancer Res. 6(4), 1524-1528.

Ansari, K.M., Das, M., 2010. Potentiation of tumour promotion by topical application of argemone oil/isolated sanguinarine alkaloid in a model of mouse skin carcinogenesis. Chem.-Biol. Interact. 188(3), 591-597.

Bickle, K., Bennett, R.G., 2008. Combined hinge flap full-thickness skin graft for a through-and-through nasal defect. Dermatologic surgery: official publication for American Society for Dermatologic Surgery [et al.] 34(3), 389-392.

Brown, C.W., Goldstein, G.D., Birkby, C.S., 2001. Auto-Mohs.com. Dermatol. Surg. 27(11), 975-978.

Croaker, A., King, G.J., Pyne, J.H., Anoopkumar-Dukie, S., Liu, L., 2016. Sanguinaria canadensis: Traditional Medicine, Phytochemical Composition, Biological Activities and Current Uses. International Journal of Molecular Sciences 17(9), 1414.

Croaker, A., King, G.J., Pyne, J.H., Anoopkumar-Dukie, S., Liu, L., 2017a. A Review of Black Salve: Cancer Specificity, Cure, and Cosmesis. Evidence-Based Complementary and Alternative Medicine 2017, 11.

Croaker, A., King, G.J., Pyne, J.H., Anoopkumar-Dukie, S., Liu, L., 2018. Assessing the risk of epidemic dropsy from black salve use. Journal of Applied Toxicology doi:10.1002/jat.3619.

Croaker, A., King, G.J., Pyne, J.H., Anoopkumar-Dukie, S., Simanek, V., Liu, L., 2017b. Carcinogenic potential of sanguinarine, a phytochemical used in 'therapeutic' black salve and mouthwash. Mutation Research/Reviews in Mutation Research 774, 46-56.

Damm, D.D., Curran, A., White, D.K., Drummond, J.E., 1999. Leukoplakia of the maxillary vestibule - an association with Viadent? Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 87(1), 61-66.

Das, A., Mukherjee, A., Chakrabarti, J., 2004. Sanguinarine: an evaluation of in *vivo* cytogenetic activity. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 563(1), 81-87.

Das, M., Ansari, K.M., Dhawan, A., Shukla, Y., Khanna, S.K., 2005. Correlation of DNA damage in epidemic dropsy patients to carcinogenic potential of argemone oil and isolated sanguinarine alkaloid in mice. Int. J. Cancer 117(5), 709-717.

Du, Y., Guo, D., Wu, Q., Liu, D., Bi, H., 2014. Zinc Chloride Inhibits Human Lens Epithelial Cell Migration and Proliferation Involved in TGF-β1 and TNF-α Signaling Pathways in HLE B-3 Cells. Biological trace element research 159(1), 425-433.

Eastman, K.L., McFarland, L.V., Raugi, G.J., 2014. A Review of Topical Corrosive Black Salve. J. Altern. Complement Med. 20(4), 284-289.

FDA, December 2016. Limiting Lead in Lipstick and Other Cosmetics. http://www.fda.gov/cosmetics/productsingredients/products/ucm137224.htm. (Accessed 07-01-2017).

Graf, T.N., Levine, K.E., Andrews, M.E., Perlmutter, J.M., Nielsen, S.J., Davis, J.M., Wani, M.C., Oberlies, N.H., 2007. Variability in the yield of benzophenanthridine alkaloids in wildcrafted vs cultivated bloodroot (Sanguinaria canadensis L.). J Agric Food Chem 55(4), 1205-1211.

Hammerova, J., Uldrijan, S., Taborska, E., Vaculova, A.H., Slaninova, I., 2012. Necroptosis modulated by autophagy is a predominant form of melanoma cell death induced by sanguilutine. Biological chemistry 393(7), 647-658.

Hou, J.L., Brewer, J.D., 2015. Black salve and bloodroot extract in dermatologic conditions. Cutis 95(6), 309-311.

Jellinek, N., Maloney, M.E., 2005. Escharotic and other botanical agents for the treatment of skin cancer: a review. J Am Acad Dermatol 53(3), 487-495.

Kevekordes, S., Spielberger, J., Burghaus, C.M., Birkenkamp, P., Zietz, B., Paufler, P., Diez, M., Bolten, C., Dunkelberg, H., 2001. Micronucleus formation in human lymphocytes and in the metabolically competent human hepatoma cell line hep-G2: Results with 15 naturally occurring substances. Anticancer Res. 21(1A), 461-469.

Laub, D.R., 2008. Death from metastatic basal cell carcinoma: herbal remedy or just unlucky? Journal of Plastic, Reconstructive & Aesthetic Surgery, 61(7), pp.846-848.

Leecy, T.N., Beer, T.W., Harvey, N.T., Kumarasinghe, S.P., McCallum, D., Yu, L.L., Wood, B.A., 2013. Histopathological features associated with application of black salve to cutaneous lesions: a series of 16 cases and review of the literature. Pathology 45(7), 670-674.

Levy, S., Furst, K., Chern, W., 2001. A comparison of the skin permeation of three topical 0.5% fluorouracil formulations with that of a 5% formulation. Clinical therapeutics 23(6), 901-907.

Liu, L., Tong, C., Bao, J., Waters, D.L., Rose, T.J., King, G.J., 2014. Determination of starch lysophospholipids in rice using liquid chromatography–mass spectrometry (LC-MS). Journal of agricultural and food chemistry 62(28), 6600-6607.

Ma, L., Dharamsi, J.W., Vandergriff, T., 2012. Black salve as self-treatment for cutaneous squamous cell carcinoma. Dermatitis : contact, atopic, occupational, drug 23(5), 239-240.

Magnusson, B.M., Anissimov, Y.G., Cross, S.E., Roberts, M.S., 2004. Molecular size as the main determinant of solute maximum flux across the skin. Journal of Investigative Dermatology 122(4), 993-999.

Mahady, G.B., Pendland, S.L., Stoia, A., Chadwick, L.R., 2003. In vitro susceptibility of Helicobacter pylori to isoquinoline alkaloids from Sanguinaria canadensis and Hydrastis canadensis. Phytotherapy research : PTR 17(3), 217-221.

Malikova, J., Zdarilova, A., Hlobilkova, A., Ulrichova, J., 2006. The effect of chelerythrine on cell growth, apoptosis, and cell cycle in human normal and cancer cells in comparison with sanguinarine. Cell Biol. Toxicol. 22(6), 439-453.

Matkar, S.S., Wrischnik, L.A., Hellmann-Blumberg, U., 2008. Sanguinarine causes DNA damage and p53-independent cell death in human colon cancer cell lines. Chem.-Biol. Interact. 172(1), 63-71.

McDaniel, S., Goldman, G.D., 2002. Consequences of using escharotic agents as primary treatment for nonmelanoma skin cancer. Arch. Dermatol. 138(12), 1593-1596.

Mohs, F., Guyer, M., 1941. Pre-excisional fixation of tissues in the treatment of cancer in rats. Cancer Research 1(1), 49-51.

Mohs, F.E., 1941. Chemosurgery: A microscopically controlled method of cancer excision. Archives of Surgery 42(2), 279-295.

Osswald, S.S., Elston, D.M., Farley, M.F., Alberti, J.G., Cordero, S.C., Kalasinsky, V.F., 2005. Self-treatment of a basal cell carcinoma with "black and yellow salve". J. Am. Acad. Dermatol. 53(3), 509-511.

Roberts, M.S., Walters, K.A., 1998. The relationship between structure and barrier function of skin. Drugs and the pharmaceutical sciences 91, 1-42.

Salmore, A.K., Hunter, M.D., 2001. Elevational trends in defense chemistry, vegetation, and reproduction in Sanguinaria canadensis. Journal of chemical ecology 27(9), 1713-1727.

Saltzberg, F., Barron, G., Fenske, N., 2009. Deforming self-treatment with herbal "black salve". Dermatol. Surg. 35(7), 1152-1154.

Schlichte, M.J., Downing, C.P., Ramirez-Fort, M., Gordon, R., Tyring, S., 2014. Bloodroot associated eschar. Dermatology online journal 20(7).

Sivyer, G.W., Rosendahl, C., 2014. Application of black salve to a thin melanoma that subsequently progressed to metastatic melanoma: a case study. Dermatology Practical & Conceptual 4(3), 77-80.

Slaninova, I., Slunska, Z., Sinkora, J., Vlkova, M., Taborska, E., 2007. Screening of minor benzo(c)phenanthridine alkaloids for antiproliferative and apoptotic activities. Pharm. Biol. 45(2), 131-139.

Slavik, J., Slavikova, L, 1960. Alkaloide der mohngewächse (Papaveraceae) XVII. Über neue alkaloide aus Sanguinaria canadensis L. Collect. Czech. Chem. Commun. 25(6), 1667-1675.

Sood, N.N., Sachdev, M.S., Mohan, M., Gupta, S.K., Sachdev, H.P., 1985. Epidemic dropsy following transcutaneous absorption of Argemone mexicana oil. Transactions of the Royal Society of Tropical Medicine and Hygiene 79(4), 510-512.

Stahlhut, M., Bertelsen, M., Hoyer-Hansen, M., Svendsen, N., Eriksson, A.H., Lord, J.M., Scheel-Toellner, D., Young, S.P., Zibert, J.R., 2012. Ingenol mebutate: induced cell death patterns in normal and cancer epithelial cells. Journal of drugs in dermatology : JDD 11(10), 1181-1192.

Wadia, R., Relwani, G., Batra, R., Ichaporia, R., Grant, K., 1971. Epidemic dropsy in Poona 1969 (clinical features and 1 year follow up). The Journal of the Association of Physicians of India 19(9), 641.

Figure 1

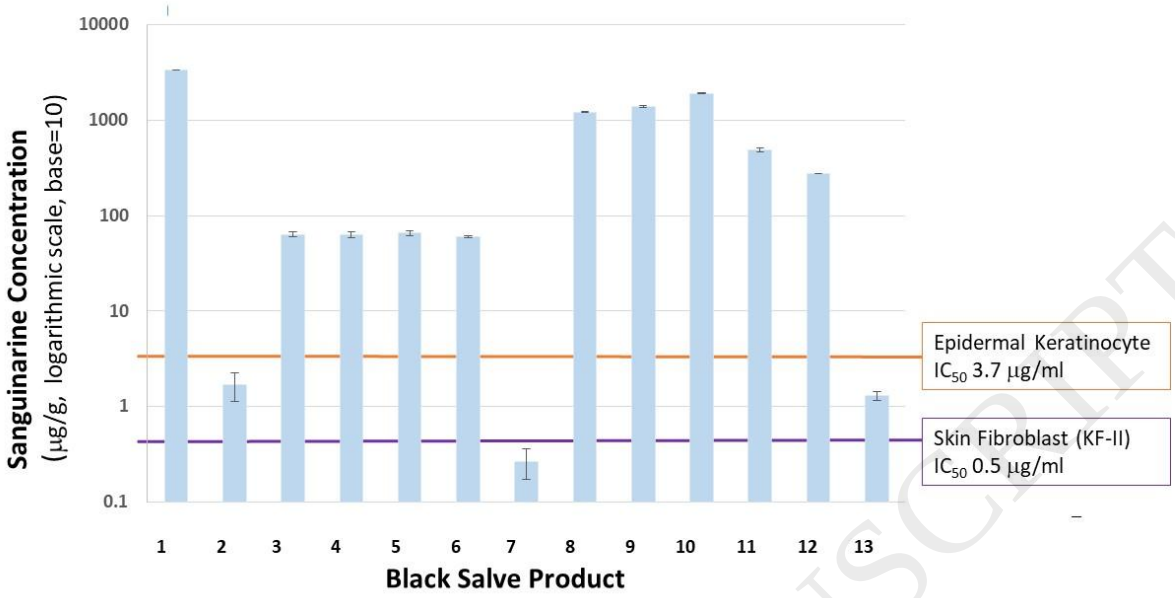

Figure 1. Sanguinarine concentrations of black salve products (µg/g) and their relationship to the historical IC_{50} of normal skin cells stated in the literature (Ahmad et al., 2000; Slaninova et al., 2007). Error bars represent standard deviation (n=2).

Figure 2

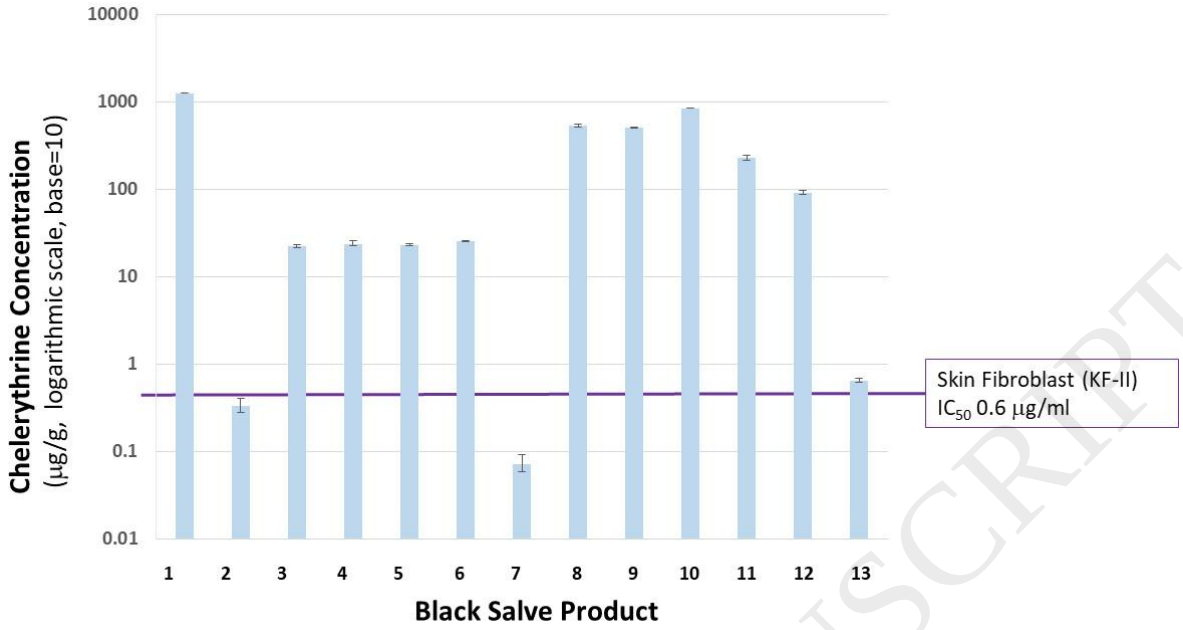

Figure 2: Chelerythrine concentrations of black salve products (µg/g) and their relationship to the historical IC_{50} of normal skin cells stated in the literature (Slaninova et al., 2007). Error bars represent standard deviation (n=2).

Tables

Table 1: Zinc Chloride Content in Black Salve Products (Mean ± Standard Deviation, n=2)

Product	$ZnCl_2$ Content (%) calculated from Zinc concentration			$ZnCl_2$ Content (%) calculated from Chloride concentration			$ZnCl_2$ Content (%) average from both calculations		
1	28.5	±	0.1	27.1	±	0.4	27.8	±	0.9
2	54.5	±	21.9	37.0	±	0.9	45.7	±	16.2
3	42.3	±	0.4	40.1	±	0.4	41.2	±	1.4
4	42.7	±	0.5	42.0	±	0.3	42.4	±	0.5
5	37.9	±	0.7	36.6	±	0.8	37.2	±	1.0
6	40.5	±	1.1	40.1	±	0.1	40.3	±	0.7
7	25.2	±	0.5	22.7	±	0.1	23.9	±	1.5
8	46.5	±	0.3	42.1	±	0.1	44.3	±	2.5
9	32.4	±	1.3	30.2	±	1.3	31.3	±	1.7
10	25.0	±	0.4	22.7	±	0.3	23.8	±	1.4
11	0.2	±	0.2	0.2	±	0.1	0.2	±	0.1
12	0.003	±	0.0001	0.3	±	0.1	0.1	±	0.1
13	12.2	±	0.1	11.9	±	0.8	12.1	±	0.5

Title: Black Salve Composition: An evaluation of the potential for Normal Tissue Toxicity and Treatment Failure from Black Salve Products

Authors: Andrew Croaker, Graham J. King, John H. Pyne, Shailendra Anoopkumar-Dukie, Lei Liu

PII: S2210-8033(18)30057-5
DOI: https://doi.org/10.1016/j.hermed.2018.11.002
Reference: HERMED 246

To appear in:

Received date: 7 June 2017
Revised date: 20 September 2018
Accepted date: 16 November 2018

Please cite this article as: Croaker A, King GJ, Pyne JH, Anoopkumar-Dukie S, Liu L, Black Salve Composition: An evaluation of the potential for Normal Tissue Toxicity and Treatment Failure from Black Salve Products, *Journal of Herbal Medicine* (2018), https://doi.org/10.1016/j.hermed.2018.11.002