

Some Biological Activities and Safety of Mineral Pitch

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Abstract

Mineral pitch, a form of mineral dripping from the cracks of the rocks, has been historically used through topical and oral administrations for health benefits. Its biological activities and safety have not been well characterized. The purpose of this study was to investigate the antioxidative activity, antimicrobial activity, cytotoxicity and heavy metal determination of mineral pitch in comparison to coal tar. The total phenolic content and antioxidative activity of mineral pitch were higher than those of coal tar. Antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* of mineral pitch were less than coal tar. A dichloromethane extract of mineral pitch could inhibit the growth of those three microbes, while the butanol extract showed the growth inhibition on *S. aureus* and *C. albicans*. From the MTT assay, mineral pitch was notably toxic to normal human lung fibroblast (MRC-5), human breast carcinoma cells (MDA-MB-231), human lung carcinoma cells (A549), human cervical carcinoma cells (Hela), human colorectal adenocarcinoma cells (SW-620), human ovarian carcinoma cells (SKOV-3) and human hepatocarcinoma cells (HepG2). Inductively coupled plasma-mass spectrometry (ICP-MS) analysis indicated the high content of heavy metals, especially, As, Hg, and Pb in mineral pitch which might relate to the cytotoxicity.

Key Words: Antimicrobial activity; Antioxidative activity; Cytotoxicity; Mineral pitch

Introduction

Mineral pitch (botanical name: Asphaltum), also known as Shilajit, is a pale-brown to blackish-brown mixture exuding from the rocks in many mountain ranges of the world, especially the Himalayas and Hindukush ranges of the Indian subcontinent (Ghosal et al., 1976). It has been found to consist of a complex mixture of organic humic substances and plant and microbial metabolites occurring in the rock rhizospheres of its natural habitat (Ghosal et al., 1991).

Substances identified in mineral pitch include moisture, gums, albuminoids, calcium, potassium, nitrogen, silica, resin, vegetable matter, magnesium, sulfur, iron, chloride, phosphorous, iodine, glycosides, tannic acid, benzoic acid, and a number of vitamins (Ghosal et al., 2006 a;b). Mineral pitch has been used as a rejuvenator and an adaptogen for thousands of years, in one form or another, as part of traditional systems of medicine in a number of countries (Frotan and Acharya, 1984; Acharya et al., 1988). Mineral

pitch has been attributed with many miraculous healing properties (Agarwal et al., 2007). According to recent reports, mineral pitch was found to have significant anti-inflammatory activity (Goel et al., 1990), free radical elimination functions (Bhattacharya and Sen, 1995), and anxiolytic effects (Jaiswal and Bhattacharya, 1992). Mineral pitch could induce a dose-related increase in superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities in the frontal cortex and striatum of rats (Bhattacharya and Sen, 1995). It was estimated that mineral pitch had both a spermiogenic and ovogenic effect in mature rats (Park et al., 2006). Coal tar is a viscous black liquid that is obtained by the destructive distillation of coal (Azpíroz, 2008). It is used commonly to treat psoriasis, seborrheic dermatitis, and dandruff (DiSepio et al., 1999). The physical appearance of mineral pitch is similar to that of coal tar. Both of them are composed of organic compounds (Richaud et al., 1998; White and Claxton, 2004). Some activities such as antioxidant and antimicrobial actions of mineral pitch have not been well characterized. Typically, heavy metal contamination is one of the problems for a quality control of healthcares (Saper et al., 2004). Currently, mineral pitch has been used for both topical and oral administrations, therefore its safety should be considered. The aim of this investigation was to study the antioxidative property, the antimicrobial activity and the cytotoxicity of mineral pitch in comparison to coal tar. The determination of the heavy metal content was also performed.

Materials and Methods

Materials

Mineral pitch was derived from the National Institute of Traditional Medicinal Service, Timphu, Bhutan in May 2007. Coal tar (lot no. 99-4181-03) was supplied by P.C. Drug Center Co., Ltd., Bangkok, Thailand. Mineral pitch (50 g) was separated by a partition technique using sequential extraction with dichloromethane, butanol and water, respectively,

using 400 mL of each solvent for extraction. The resulting extracts were evaporated until dryness and kept at 4 °C before antimicrobial activity determination. The voucher specimens were deposited in the Department of Pharmacognosy, Silpakorn University, Nakhon Pathom, Thailand.

A. Determination of total phenolic compound and antioxidant activity

Chemicals for determination of total phenolic compound and antioxidant activity

ABTS²⁻, 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate), was obtained as the sulfonic acid from Sigma (St.Louis, USA). Trolox, (+/-)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, 97%, was purchased from Aldrich (Steinheim, Germany). Potassium persulfate was obtained from Asia Pacific Specialty Chemicals Limited (Bangkok, Thailand). Sodium carbonate was purchased from Ajax Finechem (Seven Hills, Australia). Folin-Ciocalteu reagent was purchased from CarLo Erba Reagenti (Milano, Italy). Absolute ethanol and dimethylsulfoxide (DMSO) were purchased from Merck (Darmstadt, Germany). Bidistilled water was produced in this laboratory.

Determination of total polyphenols

The determination of total phenolics was performed using the method described by Kumazawa et al (2002). A 0.5 mL sample of 10 µg/mL coal tar or 10 µg/mL mineral pitch solutions (using 0.1%w/w DMSO solution as solvent) was mixed with 0.5 mL Folin-Ciocalteu reagent and 0.5 mL 10 % Na₂CO₃. The mixture was thoroughly shaken and placed at room temperature for 1 hr (n=3). The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Agilent 8453E UV-Visible Spectroscopy System). The calibration curve for polyphenols was prepared by using gallic acid as a standard in the concentration range of 2-8 mg/L. The total polyphenolic content was calculated in g of gallic acid /100 g of substance (n=6).

Determination of antioxidant

An ABTS.⁺ solution was prepared according to the method described by Re et al., 1999. Briefly, an

equal volume of 7 mM ABTS²⁻ in water was mixed with 4.9 mM potassium persulfate solution. The solution was protected from light and stored at room temperature for 12–16 hrs. ABTS.⁺ formation was checked by its absorbance (A) at 734 nm using a UV-Vis spectrophotometer (Agilent 8453E UV-Visible Spectroscopy System). The absorbance of ABTS.⁺ was equilibrated to 0.7 (± 0.02) by diluting with water at room temperature. Trolox was used as the standard substance. The calibration curve of this substance in the concentration range between 0.00-17.27 x10⁻³ mg/ml was prepared. The experiments were performed in quadruplicate in each concentration. For establishing the calibration curve, a standard solution (50 µL) was mixed with ABTS.⁺ solution (3 mL). Absorbances were measured at 734 nm at 6 min, giving trolox or compound A as presented in equation (1). The absorbance of solvent was determined by mixing absolute ethanol (50 µL) with ABTS.⁺ solution (3 mL) and its absorbance monitored at 6 min, given as solvent A. The % inhibition was calculated as equation (1). The calibration curve was plotted between % inhibition at t = 6 min and concentration of trolox. The regression coefficient (r²) was calculated from the curve.

$$\% \text{ inhibition T} = \frac{(\text{solvent A} - \text{compound A})}{\text{solvent A}} \times 100 \quad (1)$$

% inhibition T = % inhibition of ABTS.⁺ absorbance that was inhibited by trolox (T).

Mineral pitch and coal tar were diluted with absolute ethanol. The final concentrations of the samples were in the range of 0-500 µg/50µL. All experiments were done in quadruplicate. A sample solution (50 µl) was mixed with 3 mL ABTS⁺ solution. Absorbance was measured at the wavelength of 734 nm at 6 min, given as sample A or compound A in equation (1). The absorbance of solvent was deduced by mixing absolute ethanol (50 µL) with 3 mL ABTS.⁺ solution and its absorbance monitored at 6 min, given as solvent A. The curve of each sample was plotted between the % inhibition of absorbance at t = 6 min

and the concentration of each sample.

Calculation for TEAC (trolox equivalent antioxidant capacity)

$$\text{TEAC} = \frac{\% \text{ inhibition of sample}}{\% \text{ inhibition of trolox}} \quad (2)$$

TEAC is the ratio of % inhibition of sample to % inhibition of trolox at the same concentration of sample and trolox.

B. Determination of antimicrobial activity

Chemicals for determination of antimicrobial activity

Tryptic Soy Agar (TSA) (lot No. 3056695, Difco, USA.), Tryptic Soy Broth (TSB) (lot 4259, Difco, USA.), Sabouraud Dextrose Agar (SDA) (lot 6166081, Difco-TM, Becton Dickinson and Company, USA) and Sabouraud Dextrose Broth (SDB) (lot 6345690, Difco-TM, Becton Dickinson and Company, USA) were used as received. Absolute ethanol, methylene chloride and butanol were purchased from Merck (Darmstadt, Germany).

Method for antimicrobial activity determination

The antibacterial activity of coal tar and mineral pitch was assessed using the colony count method. The standard microbes used in this study were *Staphylococcus aureus* (ATCC 6538P), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 17110). The culture media for the antibacterial assay was TSA and TSB for *S. aureus* and *E. coli*. SDA and SDB were used in the case of *C. albicans*. The indicator strains from TSA (for bacteria) or SDA (for fungi) was individually inoculated into liquid of TSB (for bacteria) or SDB (for fungi) and was then incubated at 37 °C for 24 hrs. Once an actively growing broth culture or suspension of microbes was obtained, the turbidity was adjusted to match that of standard 0.5 M MacFarland solution indicating it to contain approximately 10⁷ cells/ml. Coal tar or mineral pitch was separately added to the screw test tube containing the prepared inoculated broth to obtain the concentrations of 5, 20 and 40 mg/mL and

then they were incubated at 37 °C with shaking at 150 rpm. A glass rod was used to spread the incubated broth (0.1 mL) at 0, 4 and 8 hrs onto TSA and SDA in three directions on a whole agar surface area and incubated at 37 °C for 24 hrs. The antimicrobial activity was measured using the colony count method. The tests were carried out in triplicate and the mean total colonies \pm S.D. were calculated.

For the antimicrobial activity testing of the extracts prepared from the partition of mineral pitch, paper disks containing 5 mg of each extract of the mineral pitch were prepared and dried before the testing against *S. aureus*, *E. coli* and *C. albicans* using the agar diffusion technique. Once an actively growing broth culture or suspension of microbes was obtained, the turbidity was adjusted to match that of the standard 0.5 M MacFarland solution. A sterile swab was dipped into the adjusted suspension before rotating and pressing on the inside wall of the tube. The inoculated microbe was spread on TSA for bacteria and SDA for fungi in three directions to ensure the complete spreading of the agar surface. Plates were opened for a few minutes to dry the spread culture. The prepared paper disks containing 5 mg different solvent extracts of mineral pitch were carefully placed on agar and incubated at 37 °C for 24 hrs. The antimicrobial activity was measured as the diameter (mm) of the clear zone of growth inhibition. The tests were carried out in triplicate and the mean clear zone \pm S.D. was calculated. The paper disks filled with the same amount of those solvents were also prepared and tested as the negative control.

C. Determination of cytotoxicity

Chemicals for cytotoxicity determination

Normal human lung fibroblast (MRC-5), human breast carcinoma cells (MDA-MB-231), human lung carcinoma cells (A549), human cervical carcinoma cells (Hela), human colorectal adenocarcinoma cells (SW-620), human ovarian carcinoma cells (SKOV-3), and human hepatocarcinoma cells (HepG2) were obtained from the American Type Culture Collection.

Modified Eagle's medium (MEM), fetal bovine serum, glutamine and trypsin-EDTA were purchased from Gibco (Gibthai, Thailand). 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was from Amersham (Biosciences AB, Sweden). Tissue culture flasks and 96-well plates were purchased from Costar® Corning Inc. (A.N.H., Thailand). Double distilled water was produced in this laboratory. All other chemicals and solvents were of analytical grade.

Method for cytotoxicity determination

Cells were cultured in MEM containing 10% fetal bovine serum and 1% glutamine, and incubated at 37 °C in the 5% CO₂ atmosphere. Cell treatment was initiated from seeding 20,000 cells/100 μ l of serum-free MEM in each well of a 96-well plate. After one night, mineral pitch at concentration of 100 μ g/ml was added in each well and then incubated for 24 hrs. The viability of the cells was determined using the 4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay (Mossman, 1983) with some modifications. Twenty microliters of 5 mg/ml MTT solution were added to each well for 2 hrs, and then the supernatant was removed. The cells were washed once with 100 μ l phosphate buffer saline, pH 7.4. DMSO (100 μ L) was added to each well. The solubilized formazan product was spectrophotometrically quantified with a microplate spectrophotometer (Packard Fusion™) at a wavelength of 550 nm. Solvent for mineral pitch, DMSO, was used as the blank control. One human normal lung fibroblast cells line (MRC-5) and human carcinoma cell lines from 6 various organs, breast (MDA-MB-231), lung (A549), liver (HepG2), colorectal (SW-620), ovary (SKOV-3) and cervix (Hela) were tested. For the determination of 50% effective concentration (ED₅₀), 25-200 μ g/ml of mineral pitch was used in each well and tested as above mentioned and then EC₅₀ value was calculated. Human carcinoma cell lines only from lung (A549), ovary (SKOV-3), breast (MDA-MB-231) and liver (HepG2) were used for ED₅₀ determination.

D. Determination of heavy metals

Chemicals for determination of heavy metals

Nitric acid (Lot C08033, J.T. Baker, U.S.A) was purchased from S.R. Lab, Bangkok, Thailand. Multi-element calibration standard-2A (Lot #6-108VY, Agilent, U.S.A), and single-element standard mercury (Lot #3-12HG, Agilent, U.S.A) were used as received. Deionized water (18 MΩ resistivity) was prepared in an in-house laboratory.

Method for determination of heavy metals

Glass volumetric flasks were cleaned with 20% w/v nitric acid solution and rinsed in deionized water, and dried immediately before use. A stock solution (10,000 ppb) of the standard (multi-element 10 µg/ml calibration standard-2A containing of Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd and Pb, and single-element standard 10 µg/ml Hg) was prepared in 5% w/v nitric acid solution. Working aqueous solutions at 0, 1, 5, 10, 50, 100, 500 and 1,000 ppb were prepared extemporaneously. These samples were analyzed immediately using inductively coupled plasma-mass spectrometry (ICP-MS) (Model 7500ce ICP-MS spectrometer, Agilent Technologies Inc., Palo Alto, CA, USA). Samples (100 mg) of mineral pitch and coal tar were individually accurately weighed and thoroughly mixed with 10.0 mL of 70% w/v nitric acid solution. Acid digestion was performed on a hot plate until the solution was clear. Reagent blanks were also checked in parallel at all stages. After cooling at room temperature, the digested solution was adjusted to 10.0 mL with 5% w/v nitric acid solution. Samples prepared by this method were analyzed immediately using ICP-MS.

Results and Discussion

Mineral pitch is a blackish-brown paste with the unique smell and taste since it is a complex of organic compounds derived from the humification of latex containing plants. Mineral pitch contained higher level of the total phenolic compounds than coal tar (Table 1). Compounds such as dibenzo-*a*-pyrones, along with triterpenes and phenolic lipids have been claimed as

being present in mineral pitch (Phillips, 1997). Fulvic acids and humic acids acting as carrier molecules for the more bioactive smaller compounds of mineral pitch have been reported (Hartman, 2007). In addition, tannic acid has been reported in mineral pitch (Agarwal et al., 2007). The antioxidative activity of coal tar was notably less than that of mineral pitch (Table 2). The potency of the antioxidative activity corresponded to the level of phenolic compounds present. By comparison, the water extract of mineral pitch exhibited antioxidative activity greater than the dichloromethane and butanol extracts, respectively. This result corresponded to the level of total phenolic compounds presented in these extracts (Table 1). The positive correlation between the antioxidant capacity and the total phenolic content has been reported (Andre, 2007). However, the antioxidative activity of mineral pitch was higher prior to partition than after extraction with butanol, and the value of the total phenolic compounds in the first instance was lower.

The results from Table 3 indicate that coal tar could exhibit antibacterial against *S. aureus*. As the time of exposure was increased, the growth inhibition was enhanced. The antibacterial effect of mineral pitch was less than that of coal tar, but was dose and time dependent, except at concentration of 5 mg/mL. This behavior was also found in the case of testing mineral pitch with *E. coli*, except at 20 mg/mL. This latter case was due to the limitation of contact between microbes and test substances during the test and therefore some error was evident. For *C. albicans* inhibition, coal tar was apparently more effective than mineral pitch. However, mineral pitch also exhibited growth inhibition of *C. albicans* compared to the control. Typically, antiseptic property and antifungal activity, especially for the treatment of psoriasis, of coal tar has been mentioned (Thami and Sarkar, 2002).

The percentage yield of the extracts obtained from the partition of mineral pitch with dichloromethane, butanol and water was 1.00, 5.20 and 49.06%, respectively. This signified that most of the substances in mineral pitch were rather hydrophilic. However, the

Table 1 Total polyphenol content in g/100g calculated as gallic acid on substance (n=6).

Substance	Total-phenols (g/100g crude extract calculated as gallic acid)
Coal tar	0.81±0.25
Mineral Pitch (before partition)	4.18±0.07
Mineral Pitch (CH ₂ Cl ₂ extract)	5.23±0.12
Mineral Pitch (BuOH extract)	4.95±0.09
Mineral Pitch (H ₂ O extract)	5.45±0.10

Table 2 The slope, intercept and r² of % inhibition equations, IC₅₀ and TEAC values of the substances and extracts from antioxidative activity test.

Type of substance	Equations			IC ₅₀ µg	TEAC
	slope	intercept	r ²		
Trolox	5.0248	-0.9708	0.9985	10.14	1
Coal tar	0.0114	7.5423	0.1392	3724.36	0.017
Mineral Pitch (before partition)	0.1198	0.7754	0.9963	410.89	0.025
Mineral Pitch (CH ₂ Cl ₂ extract)	0.1187	3.1347	0.9909	394.82	0.03
Mineral Pitch (BuOH extract)	0.0688	-0.4726	0.9978	733.61	0.013
Mineral Pitch (H ₂ O extract)	0.1581	2.8952	0.9887	297.94	0.037

hydrophilic compounds exhibited less antimicrobial activity, as shown in Table 4. The dichloromethane extract could inhibit the growth of the three microbial species and the butanol extract showed the growth inhibition of *S. aureus* and *C. albicans*, but not *E. coli*. The result indicated that the antimicrobial compounds in mineral pitch were rather hydrophobic. However, this was not a high level of hydrophobicity since they could diffuse through the agar to inhibit the growth of the microbes.

Cytotoxicity of mineral pitch was tested in normal lung fibroblast and several human carcinoma cell lines, including breast, lung, liver, colon, ovary and cervix using the MTT technique. It was found that mineral pitch was toxic to all types of cell lines at the concentration of 100 µg/mL including normal cell line, MRC-5 (Fig. 1). This rather high concentration was used in this study for screening the cytotoxicity of

mineral pitch which was not the pure compound but contained many compounds. However, it inhibited growth of MRC-5 about 24.8 %, which was significantly less than other human carcinoma cells, except for the breast carcinoma cells (MDA-MB-231) (Fig. 1). Among the various types of carcinoma cells, mineral pitch highly decreased the viability in cervical carcinoma cells, Hela cells, which exhibited only 31.5 ± 3.85 % (mean ± S.D) compared to the control, same cell with only solvent treatment. However, the rest cells, A549 (lung), HepG2 (liver), SW620 (colon), and SKOV-3 (ovary), were affected quite similarly (44.5-54.9%). By comparison of the same cell type, mineral pitch repressed the growth of lung carcinoma cells (A549) higher than normal lung cells (MRC-5). From ED₅₀ determination, mineral pitch was more toxic to A549 than HepG2, MDA-MB-231, SKOV-3, respectively (Table 5). Although this experiment did

Table 3 Antimicrobial activity of coal tar and mineral pitch from colony count against (A): *S. aureus*; (B): *E. coli* and (C): *C. albicans*

(A)

Conc. (mg/ml)	No. of colony					
	Coal tar			Mineral pitch		
	Time (hr)			Time (hr)		
	0	4	8	0	4	8
5	72±24	23±2	4±2	138±25	829±74	1857±77
20	88±9	31±13	4±2	129±23	66±22	45±19
40	98±54	22±4	2±1	78±13	39±7	6±2

No. colony of control at 0, 4 and 8 hrs was 51±4, 3421±214 and 7872±45, respectively.

(B)

Conc. (mg/ml)	No. of colony					
	Coal tar			Mineral pitch		
	Time (hr)			Time (hr)		
	0	4	8	0	4	8
5	73±22	724±59	32,384±1,886	105±20	27,520±1,430	*
20	71±2	2±2	0±0	69±10	341±48	1,568±418
40	67±7	0±0	0±0	72±6	51±14	40±11

No. colony of control at 0, 4 and 8 hrs was 70±6, 90,514±8778 and more than to be counted, respectively.

* more than to be counted

(C)

Conc. (mg/ml)	No. of colony					
	Coal tar			Mineral pitch		
	Time (hr)			Time (hr)		
	0	4	8	0	4	8
5	3±4	0±0	0±0	2±1	44±58	82±32
20	4±3	0±0	0±0	9±7	8±4	21±2
40	3±2	0±0	0±0	6±3	12±10	17±10

No. colony of control at 0, 4 and 8 hrs was 4±2, 123±25 and 233±28, respectively.

Table 4 Inhibition zone of disk containing 5 mg different solvent extracts of mineral pitch against *S. aureus*, *E. coli* and *C. albicans*

Solvent extract	Diameter (mm)		
	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>
CH ₂ CL ₂	13.0±2.6	12.3±2.5	21.67±3.1
BuOH	8.3±0.6	0.0±0.0	16.0±1.6
Water	0.0±0.0	0.0±0.0	0.0±0.0

There was no inhibition zone for the negative control disk prepared from the solvents.

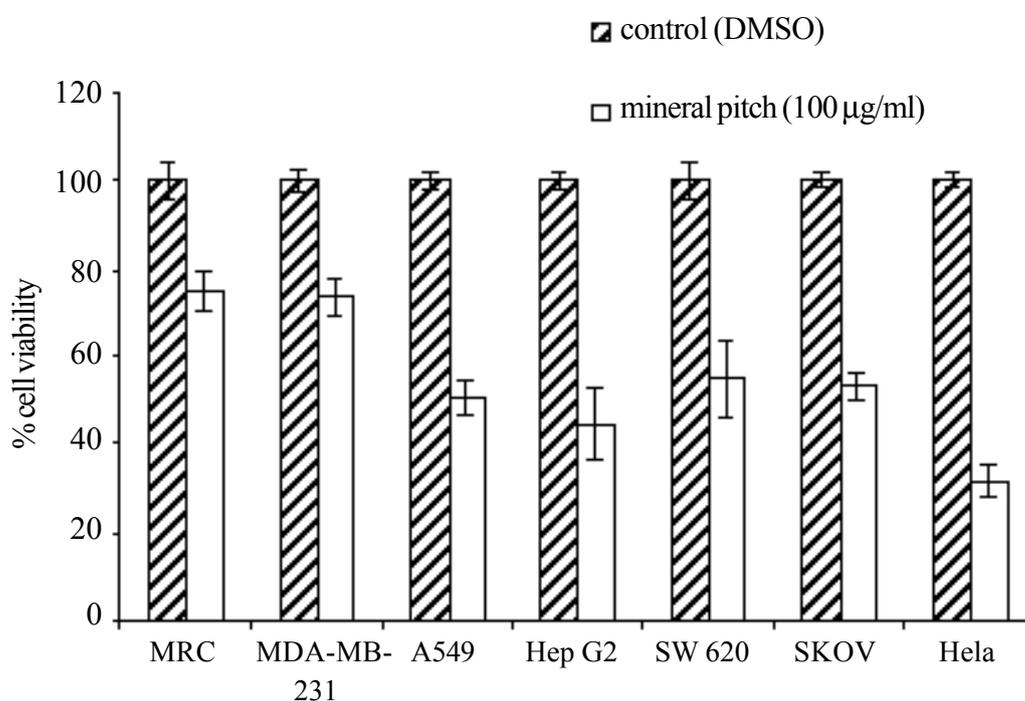


Figure 1 Cytotoxicity of mineral pitch on one human normal lung fibroblast cells line (MRC-5) and human carcinoma cell lines from 6 various organs, breast (MDA-MB-231), lung (A549), liver (HepG2), colorectal (SW-620), ovary (SKOV-3) and cervix (Hela). All cells were treated with 100 µg/ml of the mineral pitch for 24 hrs and followed determined the viability by the MTT technique. Relative cell viability was expressed as % control cells at the same concentration of solvent. All values were mean ± S.E.M; n=3, p < 0.01 (*, **) comparing to the control, only solvent, and p < 0.01 (**) comparing to MRC-5 (normal human cells).

Table 5 Cytotoxic effective dose (ED₅₀) of mineral pitch on human carcinoma cell lines from various organs, lung (A549), liver (HepG2), breast (MDA-MB-231) and ovary (SKOV-3).

Cell line	ED ₅₀
A549	89
HepG2	96
MDA-MB-231	225
SKOV-3	286

Table 6 Heavy metal concentrations in mineral pitch and coal tar.

Metal	Concentration of heavy metal			
	Mineral pitch		Coal tar	
	mg/kg	RSD ^a	mg/kg	RSD ^a
Al	72.395	0.013	18.414	0.074
Cr	1.374	0.006	1.918	0.007
Mn	21.399	0.017	0.000	0.442
Fe	269.921	0.055	16.750	0.140
Ni	260.091	0.036	16.721	0.308
Cu	3.979	0.038	3.422	2.428
Zn	32.519	0.014	25.309	0.073
As ^b	3.648	0.044	6.344	0.004
Cd	1.328	0.001	1.958	0.004
Hg ^b	1.535	0.003	2.280	0.002
Pb ^b	10.937	0.072	15.542	0.079

^aRSD = Relative standard deviation

^bThe recommended maximum levels of As, Hg, and Pb in herbals in the Thai Herbal Pharmacopoeia 2000, are 4, 0.3, and 10 mg/kg, respectively.

not use normal cells which were representative for the topical or oral route of administration, the results clearly indicated the potential toxicity of mineral pitch for human cells. This study did not determine the cytotoxicity of coal tar since this drug has been typically used as the active compound in the topical pharmaceutical products not in the dosage form with oral administration. However, its toxicity should be considered and further studied since some investigation revealed its toxicity for short nose sturgeon (*Acipenser brevirostrum*) embryos and larvae (Kocan et al., 1996).

Historically, some regional people, such as the

Bhutanese, utilized mineral pitch as a power increasing tonic and the medicine taken is orally for peptic ulcer treatment (Acharya et al., 1988; Ghosal et al., 2006b). However, the toxicity data of mineral pitch has not been reported. Since there are presently no state or federal heavy metal guidelines for mineral pitch and coal tar, this study was guided to the heavy metal limitation in the Thai Herbal Pharmacopoeia 2000. The recommended maximum levels of As, Hg, and Pb in herbals in the Thai Herbal Pharmacopoeia 2000, are 4, 0.3, and 10 mg/kg, respectively. This study determined the concentrations of eleven heavy metals such as Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg,

and Pb in mineral pitch and coal tar with ICP-MS. The concentrations of Hg and Pb in mineral pitch and coal tar were found to be higher than the permissible limits as shown in Table 6. The concentration of As in mineral pitch was lower than the safety limit, while that of As in coal tar was higher than the safety limit. The contamination of As, Hg, and Pb in both materials may cause a potential health risk in chronic long term use. Mineral pitch is a compact mass of bituminous substance ejected from crevices of rocks (Ashida et al., 2008) whereas coal tar is a viscous black liquid obtained by the destructive distillation of coal (Azpíroz, 2007). Both are composed of organic compounds (Kipling, 1976; Richaud et al., 1998; White and Claxton, 2004) and are potentially contaminated with toxic heavy metals. Metal contamination is a problem for the safety of both samples. In the last few years, the use of coal tar has been limited to skin diseases, such as psoriasis and chronic dermatitis (Thami and Sarkar, 2002). The French FDA Advisory Panel on over-the-counter drug products determined that coal tar is not safe for use as a topical antifungal agent (IARC, 1984). From the above data, although coal tar and mineral pitch exhibited the interesting biological activities, it is necessary to consider their safety for use by human beings. Additional toxicity tests are needed to understand their effects on animals.

Conclusion

Mineral pitch exhibited both antioxidative and antimicrobial activities, but its cytotoxicity and high heavy metal content should be considered before using this material for human health needs.

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