I Fanghi Termali del Bagnaccio: Una Fonte Inesauribile di Sostanze Naturali Bioattive Per il Benessere e la Salute

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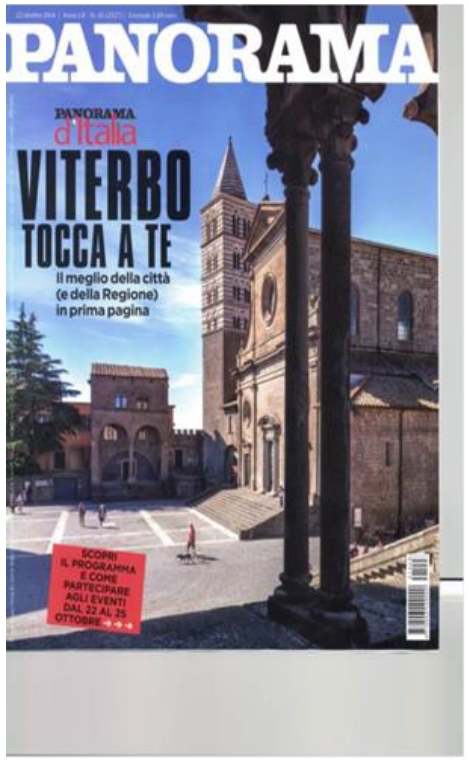
Spin-Off GENTOXCHEM Citogenotossicologia e Chimica Farmaceutica e Industriale

Vincitori del premio Start Cup Lazio Come migliore impresa innovativa



Queste le schede dei progetti vincitori di Start Cup Lazio:

GENTOXchem dell'Università degli studi della Tuscia - Viterbo intende realizzare e commercializzare una crema e un olio solare, con l'innovativa formula dei "filtri solari naturali", protettiva della pelle e con proprietà antiossidanti e anti-invecchiamento. Verrà utilizzata lignina, uno dei principali componenti del legno. L'innovazione di prodotto e di processo consiste proprio nella sostituzione di componenti chimici di sintesi, normalmente presenti nei prodotti di make-up e nei filtri solari, con sostanze organiche naturali, prive di tossicità e in grado di garantire un'uguale o superiore efficacia. Infatti, nonostante l'attenzione rivolta negli ultimi ani alle problematiche della salute umana e più in generale a quelle ambientali, numerosi prodotti cosmetici contengono ancora sostanze classificate dalle UE come "estremamente preoccupanti". L'idea di GENTOXCHEM è "trasferire l'ombra degli alberi in un filtro solare per proteggere la pelle nel modo più naturale possibile". La lignina è, insieme con la cellulosa e la emi-cellulosa, il principale componente del legno ed è uno dei biopolimeri rinnovabili più abbondanti presenti sul nostro pianeta.

FC | FAMIGLIA CRISTIANA.it

DI COSA PARLIAMO | CHIESA E FEDE | VOLONTARIATO E VALORI | SPETTACOLO E CULTURA | BLOG | M

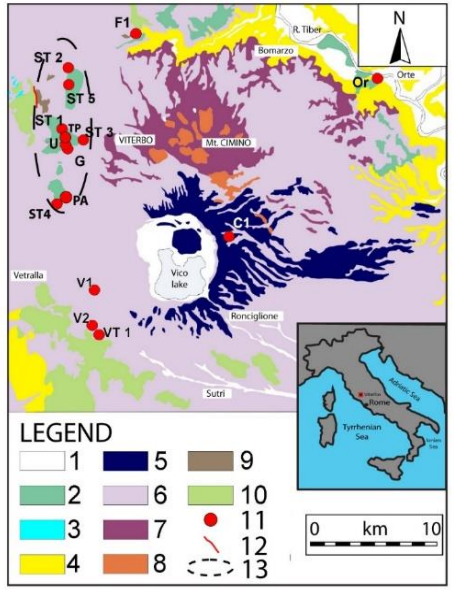
I RICERCATORI LAZIALI IN GARA

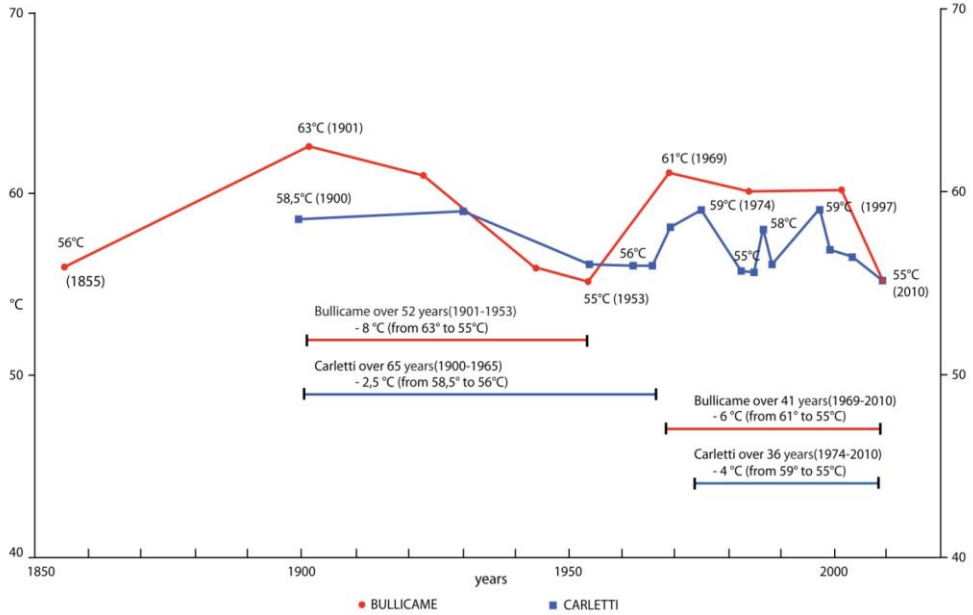
Ha preso parte al:

WORKING CAPITAL
materia per le idee
PNI CUBE
Associazione Italiana degli Incubatori Universitari delle Business Plan Competition
TELECOM ITALIA

COFI
GENTOXCHEM

Localizzazione e caratteristiche generali del sito di raccolta della località Bagnaccio

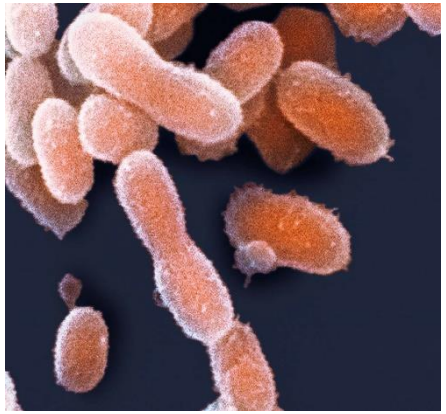


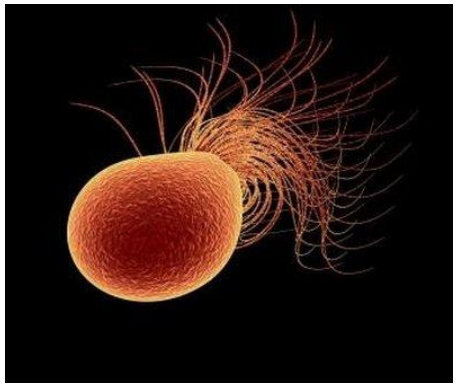


XIV canto dell'Inferno

"QUALE DEL BULICAME ESCE IL RUSCELLO
CHE PARTON POI TRA LOR LE PECCATRICI,
TAL PER LA RENA GIÙ SEN GIVA QUELLO.
LO FONDO SUO ED AMBO LE PENDICI
FATTE ERAN PIETRA, E I MARGINI DA LATO"

Extremophiles





Specificità del territorio

Fitocomplesso Adsorbito

Argilla, sabbia, limo
Sali e ioni inorganici

Tempo
Temperatura
pH

Extremophiles

Rilascio

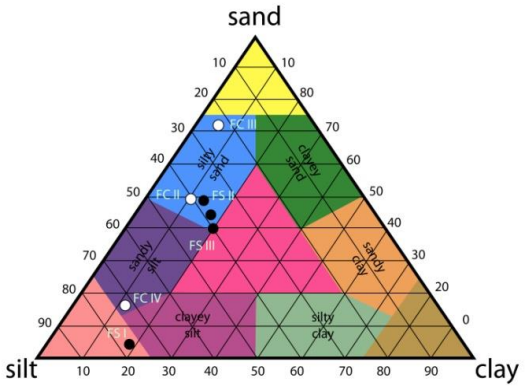
Matrice Inorganica

Specificità della Popolazione microbica

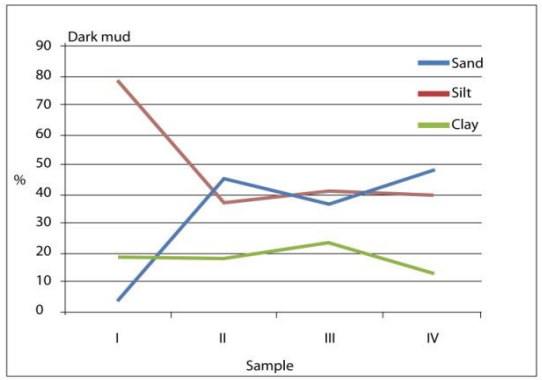
Attività Biologica

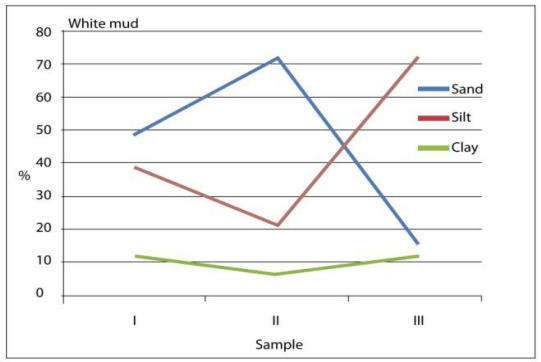
Fitocomplesso Libero

Tipologia e proprietà della componente inorganica dei fanghi termali



Texture diagram of Shepard.
FC, white mud; FS, dark mud.





Trend of the percentages of sand, silt and clay in the samples of DM and WM.

The inorganic particles Trans-Epidermal Water Loss (TEWL) occlusion process induces the active transport of organic substances through the epidermal barrier.

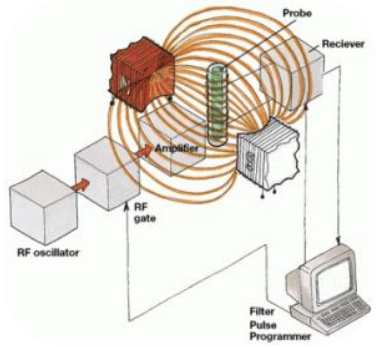
	I	II	III	IV	Mean	St. dev.
T °C	23	31.5	35	28.5	29.5	5.1
pH	6.3	6.3	6.4	6.3	6.3	01
Conductivity	2100	2500	2550	2200	2337.5	221.3
Ca	458.1	453.5	433.6	450.6	449.0	10.7
Mg	115.2	110.8	118.8	121.4	116.6	4.6
Na	13.5	12.1	14.3	12.3	13.1	1.0
K	28.7	26.7	25.8	28.8	27.5	1.5
Cl	15.4	16.8	18.5	20.5	17.8	2.2
SO_4	1300	1350	1380	1420	1362.5	50.6
HCO_3	335	348	360	320	340,8	17,2
F	1.9	2.1	2.2	2	2.1	0.1
NO_3	9,9	10,4	9,6	10,3	10,1	0,4
TDS	2277	2320	2363	2386	2336.5	48.2
Li	13,13	11,1	12	11	11,8	1,0
V	0.2	1.2	1.7	2.2	1.3	0.9
Mn	29,6	26,2	24,4	25,1	26,3	2,3
Fe	0.3	0.1	0.4	0.8	0.4	0.3
As	15	25	22,2	25,1	21,8	4,7
Rb	24.5	33.4	31.8	33.1	30.7	4.2
Sr	806.8	950	988	970	928.7	82,7
Cs	17.6	15.1	12.4	11.2	14.1	2.9
Ba	6.9	8.3	9.3	9.8	8.6	1.3
U	0,1	0,1	0,2	0,4	0,2	0,1
Al	1.6	1.4	2.1	3.1	2.1	0.8
$^{87}Sr/^{86}Sr$	0.70822	0.70835	0.70830	0.70850	0.70834	0.0
$\delta^{18}O$	-6.22	-6.35	-6.45	-6.15	-6.3	0.1
$\delta^{2}H$	-39.45	-40.12	-41.22	-40.88	-40.4	0.8

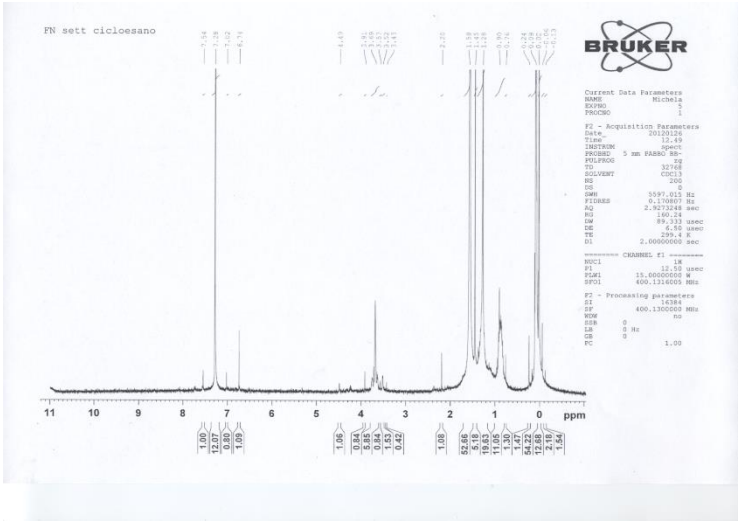
Results of the chemical-physic analyses and values of the isotopic ratio $^{87}Sr/^{86}Sr$ and isotopes $\delta^{18}O$ and $\delta^{2}H$ of thermal water of the spring pool Bagnaccio. The major elements are reported in mg/L, the trace and minor elements in mg/kg.

Estrazione e separazione della componente organica: metodologia sperimentale in scala di laboratorio.
Campionamenti con cadenza mensile per un anno.

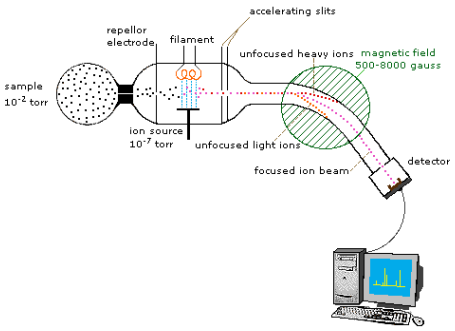
Estrazione Eluotropica

Analisi qualitativa e quantitativa della componente organica- Centro Grandi Apparecchiature (CGA)

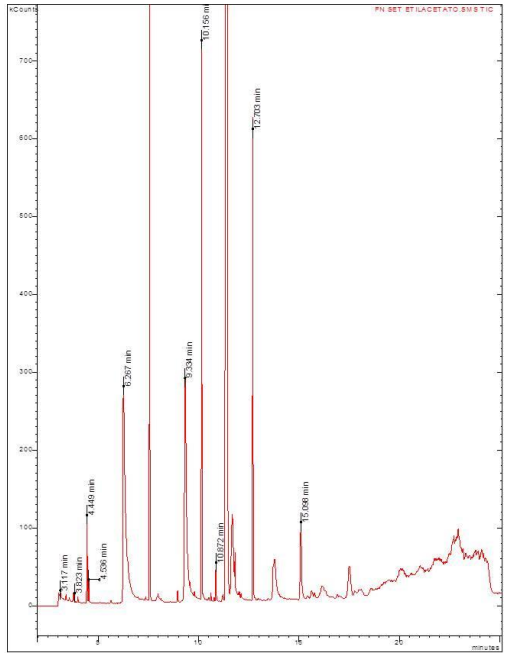


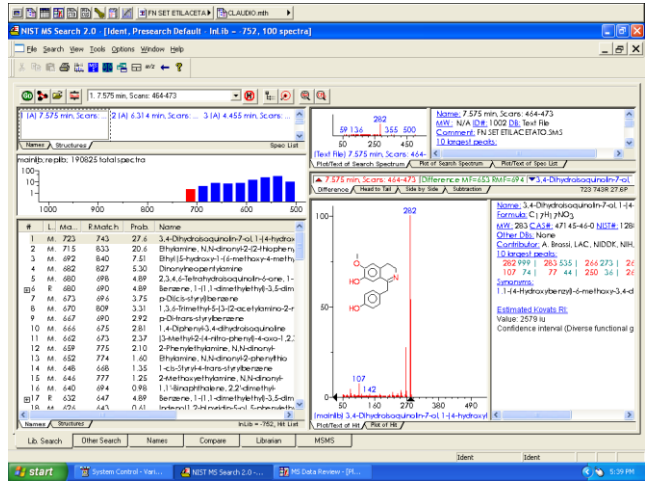


FT-NMR Superconduttore AVANCE III 400 BRUKER BIOSPIN

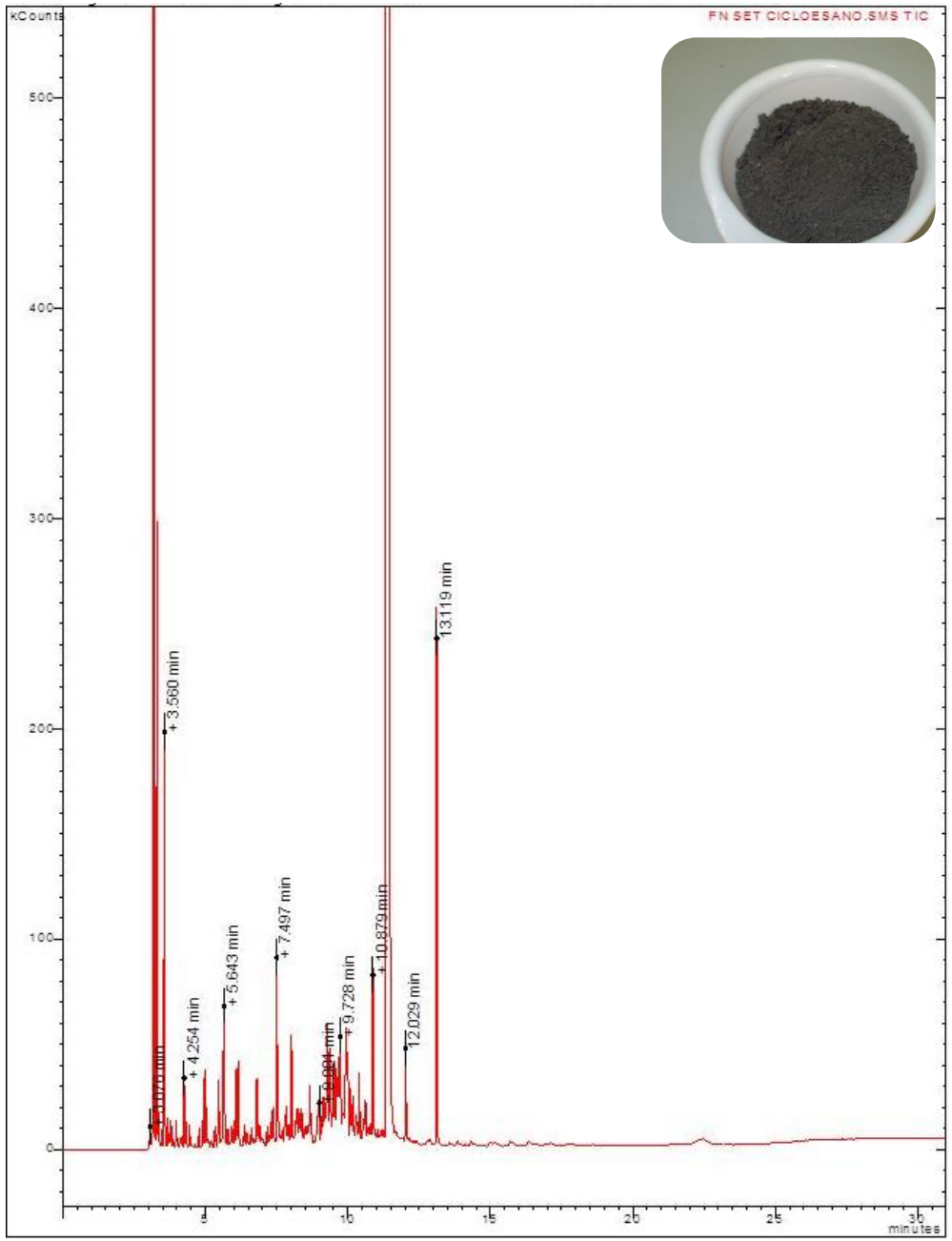


Combo GC-LC MS Varian





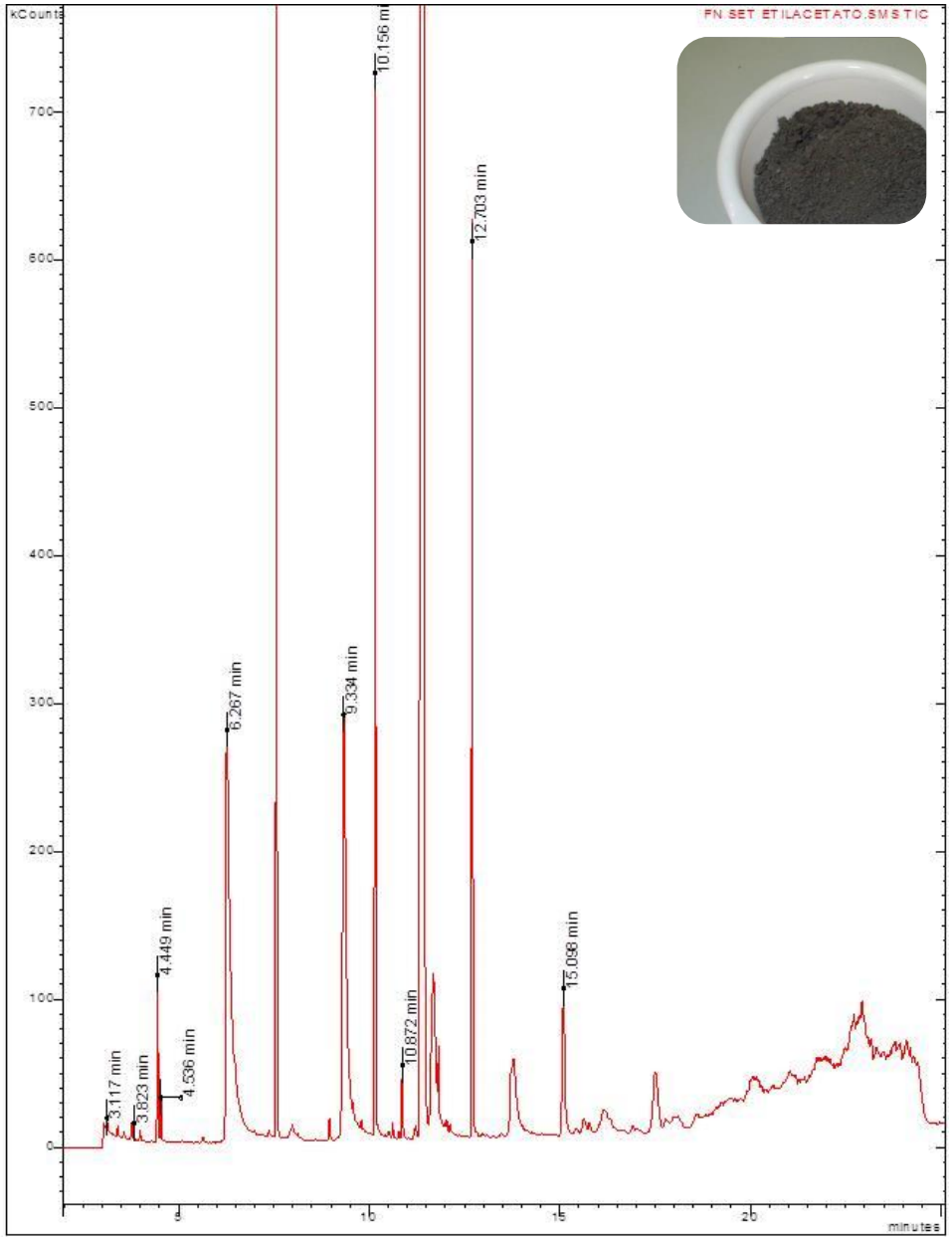
Risultati Fango Nero frazione non polare: sono state identificate 34 sostanze organiche naturali bioattive



Entry	Fraction	Class of compounds	Compound(s)	Yield[a]
1	n-Heptane	Alkanes	Pentadecane	0.03
			Dodecane	0.15
		Alkenes	Tridec-1-ene	$2.5\ 10^{-3}$
			Tetradec-1-ene	$4.6\ 10^{-3}$
		Aromatic hydrocarbons	Ethylbenzene	$8.2\ 10^{-4}$
			Ortho-xylene	$1.7\ 10^{-3}$
			1,4-Diethylbenzene	$4.0\ 10^{-3}$
			1,2,4-trimethylbenzene	$1.7\ 10^{-3}$
2	Cyclohexane	Alkanes	Decane	$3.5\ 10^{-3}$
			Undecane	0.18
			Dodecane	0.02
			Tridecane	0.06
			Pentadecane	0.02
			2,4-dimethylhexane	0.01
		Alkenes	Dodec-1-ene	0.05
			Tridec-1-ene	0.05
			Hexa-2,4-diene	0.09
		Aromatic hydrocarbons	Ethylbenzene	0.02
			Ortho-xylene	0.02
			Meta-xylene	0.04
		Alkanols	Undecan-1-ol	$8.7\ 10^{-3}$
			Tretradecan-1-ol	0.02
			2-Methyldecan-1-ol	0.01
			2-Butyloctan-1-ol	0.02
			2,7-dimethyloctan-1-ol	$4.5\ 10^{-3}$
			Oleyl alcohol	0.02
3	Di-isopropyl ether	Alkanes	Decane	$3.7\ 10^{-3}$
			Dodecane	0.17
			Hexadecane	0.03
		Alkenes	Hexa-2,4-diene	0.01
			Tridec-1-ene	$4.8\ 10^{-3}$
		Aromatic hydrocarbons	Ethylbenzene	0.01
			Ortho-xylene	0.05
			Para-xylene	0.02
			1,2,3-trimethylbenzene	$8.9\ 10^{-3}$
		Alkanols	Tetradecan-1-ol	$3.5\ 10^{-3}$
			2-Butyl-octan-1-ol	$1.7\ 10^{-3}$
			2-Methyl-undecan-1-ol	$1.5\ 10^{-3}$

[a]Yield is defined as mg of compounds per gram of DM sample.

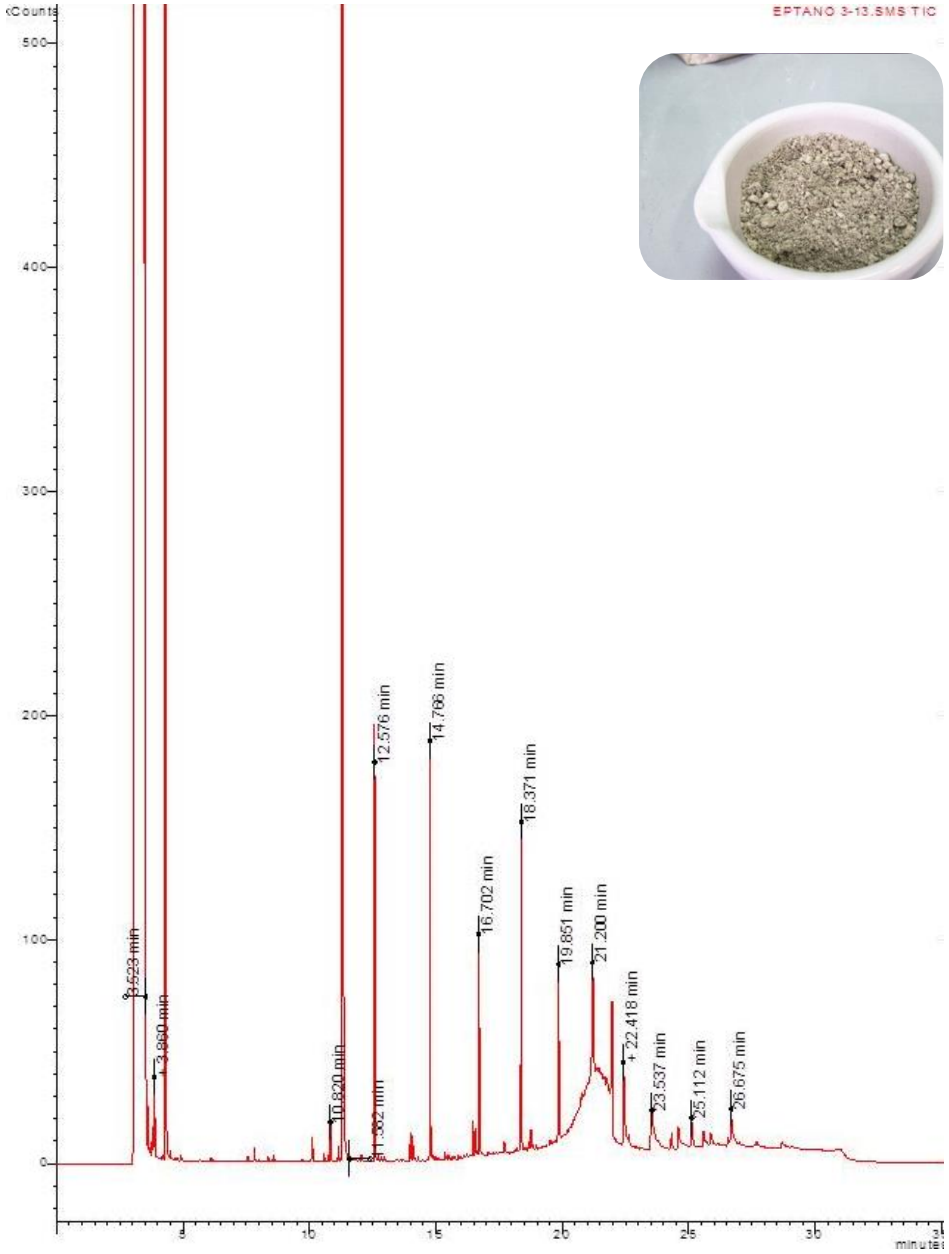
Risultati Fango Nero frazione polare: sono state identificate 34 sostanze organiche naturali bioattive



Entry	Fraction	Class of compounds	Compound(s)	yield[a]
1	EtOAc	Alkanols	n.d.	n.d.
		Acids and derivatives	Myristic ac.	0.78
			Palmitic ac.	0.90
			Stearic ac.	0.62
			Palmitoleic ac.	0.57
			Oleic ac.	0.60
			Methyl stearate	1.68
			Methyl palmitate	2.23
			Methyl oleate	0.38
		Terpenoids	Retinoic acid	0.46
			Retinol (Vitamin A)	0.40
			Retinol acetate	0.14
		Phenols	2-hydroxybenzaldhyde	0.02
			Salicylic acid	0.04
			Thymol	0.02
		Alkaloids	cholchicine	0.02
2	Acetone	Alkanols	Tetradecan-1-ol	0.01
			Tridecan-1-ol	0.02
			Hexadec-9-en-1-ol	0.06
		Acids and derivatives	Palmitoleic ac.	0.37
		Terpenoids	Retinoic acid	0.06
		Phenols	2,4-dimethylphenol	0.02
		Alkaloids	colchicine	$6\ 10^{-3}$
		Inorganic compounds	Octhathiocane	0.01
3	MeOH	Alkanols	Tridecan-1-ol	0.05
			Pentadecan-1-ol	0.25
			Tetradecan-1-ol	0.03
		Acids and derivatives	Ricinoleic ac.	0.02
		Terpenoids	Gibbelleric ac.	0.05
			Methyl retinoate	0.04
			Retinol (Vitamin A)	0.21
		Phenols	Hydroquinone	0.01
			Gallaldehyde	0.20
			Gallic acid	0.27
		Alkaloids	Colchicine	0.01

[a]Yield is defined as mg of compounds per gram of DM sample.

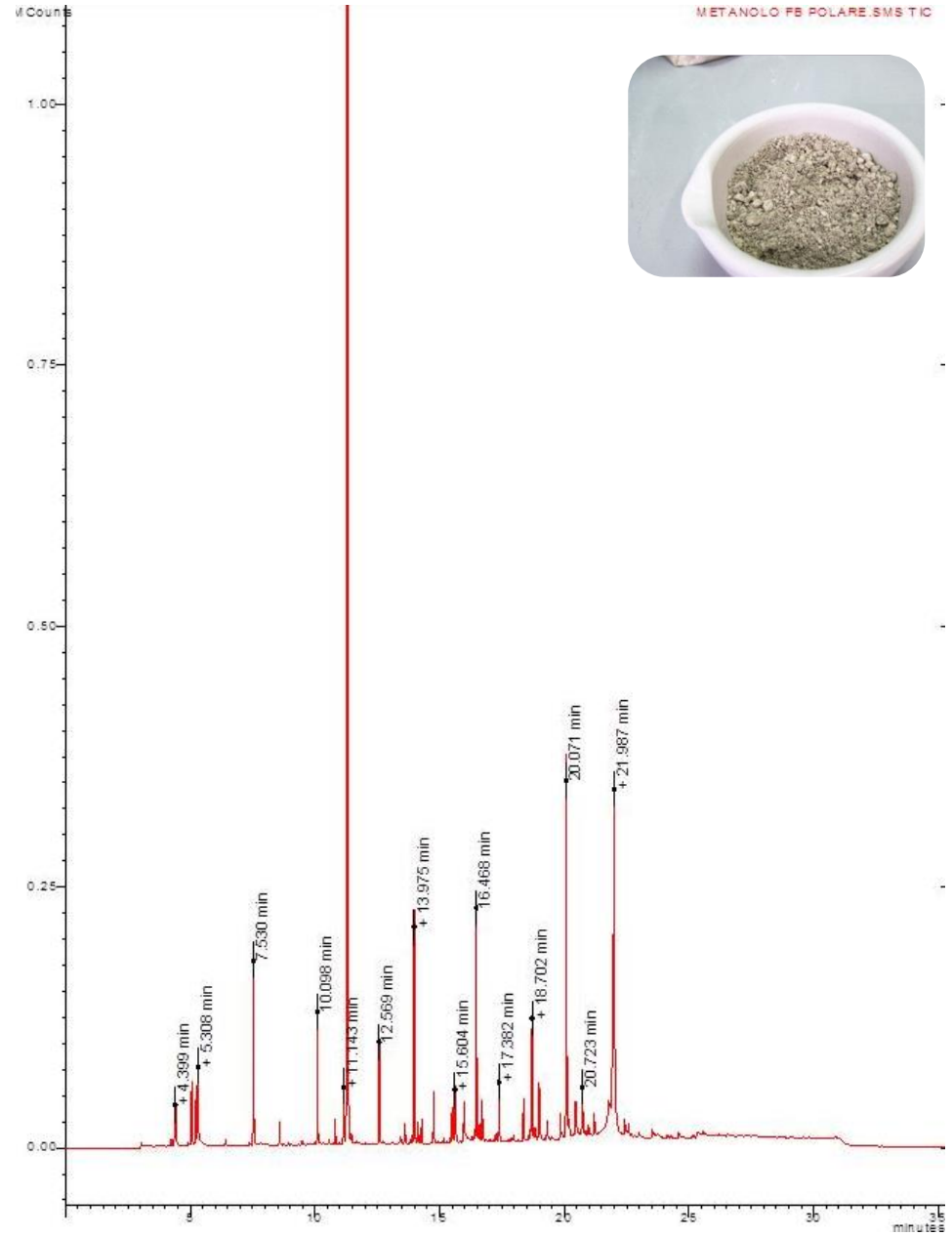
Risultati Fango Bianco frazione non polare: sono state identificate 31 sostanze organiche naturali bioattive



Entry	Fraction	Class of compounds	Compound(s)	Extraction yield
1	n-Heptane	Alkanes	Nonane	$6.2\ 10^{-3}$
			Decane	$2.8\ 10^{-3}$
			Dodadecane	0.01
			Pentadecane	0.01
			Hexadecane	0.01
			Octadecane	$2.0\ 10^{-3}$
			Icosane	$1.0\ 10^{-3}$
			3,5-Dimethyloctane	$8.8\ 10^{-3}$
		Alkenes	Oct-1-ene	$1.8\ 10^{-3}$
			Dodec-1-ene	$2.2\ 10^{-3}$
			Tridec-1-ene	$4.6\ 10^{-3}$
			Pentadec-1-ene	0.02
			Tetradec-1-ene	$2.2\ 10^{-3}$
			Nonadec-1-ene	$2.1\ 10^{-3}$
		Alkanols	Undecan-1-ol	$7.0\ 10^{-3}$
			Tetradecan-1-ol	$2.2\ 10^{-3}$
			Pentadecan-1-ol	$2.3\ 10^{-3}$
			Octadecan-1-ol	$2.2\ 10^{-3}$
			Tridecan-1-ol	0.01
2	Cyclohexane	Alkanes	Decane	0.06
			Undecane	0.08
			Tridecane	0.01
			Pentadecane	0.04
			Hexadecane	0.02
			2,4-Dimethylhexane	0.01
			3,5-Dimethyloctane	0.01
		Alkenes	Tridec-1-ene	0.06
			Pentadec-1-ene	0.11
		Alkanols	Tridecan-1-ol	0.01
			2-propenyl-pentan-1-ol	0.10
			Octadecan-1-ol	0.02
		Miscellanea	Dodecanal	0.05
3	Di-isopropylether	Alkanes	Dodecane	0.01
			Hexadecane	0.02
			Octadecane	0.09
			Icosane	0.05
			Tetracosane	0.13
			Octacosane	0.12
		Alkanols	Tetradecan-1-ol	0.06
			Octadecan-1-ol	0.04
		Acids and derivatives	Enoic ac.	0.02
			Dodecanoic ac.	0.05
			Methyl oleate	0.01
			Isopropyl palmitate	0.20
			Tetradecyltetradecanoate	0.09

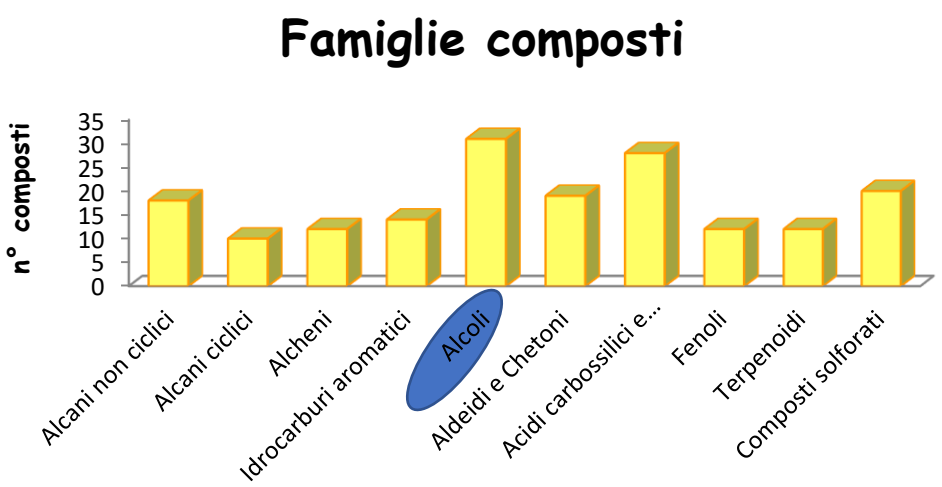
[a]Yield is defined as mg of compounds per gram of WM sample.

Risultati Fango Bianco frazione non polare: sono state identificate 25 sostanze organiche naturali bioattive



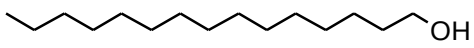
Entry	Fraction	Class of compounds	Compound(s)	Extraction yield
1	EtOAc	Alkanes	Dodecane	0.01
			Nonadecane	$1.9\ 10^{-3}$
			2,3-Dimethylundecane	0.03
			3,7,11-Trimethyldecane	$4.3\ 10^{-3}$
		Alkanols	Dodecan-1-ol	$4.8\ 10^{-3}$
			2-Methylhexadecan-1-ol	0.02
			2-Butyl-ocatan-1-ol	$5.7\ 10^{-3}$
		Aromatics hydrocarbons	Para-Xylene	$2.3\ 10^{-3}$
		Phenols	Phenol	0.01
			2-Hydroxybenzaldehyde	0.01
			Salicylic acid	0.03
		Alkaloids	Colchicine	0.04
		Terpenoids	Limonene	$3.2\ 10^{-3}$
		Acids and derivatives	Butyl acetate	0.02
			2-amino-4-mercaptobutyric ac.	$3.4\ 10^{-3}$
2	Acetone	Alkanes	Dodecane	0.05
		Alkenes	isoprene	0.02
			Heicos-10-ene	0.01
			Hexadec-9-en-1-ol	0.02
		Alkanols	Octan-1,2-diol	0.01
			Icosan-1-ol	0.22
			5-Methylicosan-1-ol	0.16
			Nonadecan-1-ol	0.05
		Alkaloids	colchicine	0.01
		Terpenoids	b-Carotene	0.14
			a-Tocopherol	0.50
		Acids and derivatives	Oleic acid	0.04
			Dioctyl adipate	0.30
			Hex-2-enylhexanoate	0.10
3	MeOH	Alkanes	Dodecane	0.01
		Alkenes	Nonadec-1-ene	0.23
			Henicos-10-ene	0.21
		Alkanols	Icosan-1-ol	0.12
		Alkaloids	Colchicine	0.01
		Terpenoids	a-Tocopherol (vitamin E)	0.05
			d-Tocopherol (vitamin E)	0.06
		Miscellanea	Isonicotinic acid	0.03
		Inorganic compounds	Hexathione	n.d
			Octathione	n.d

[a]Yield is defined as mg of compounds per gram of WM sample.

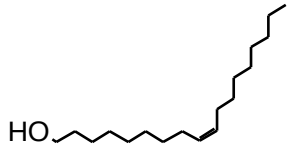


Alcoli a lunga catena di atomi di carbonio. Alcoli grassi saturi ed insaturi.

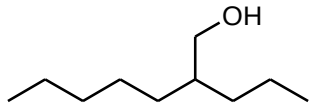
I fanghi FN e FB presentano numerosi alcoli a lunga catena (cioè contenenti un numero di atomi di carbonio compreso tra 8 e 22 atomi), indicati nella nomenclatura comune come alcoli grassi. Gli alcoli grassi sono largamente impiegati in cosmetica come composti gelificanti, come agenti condizionanti della pelle, come umettanti ed emollienti. Inoltre gli alcoli grassi sono anche noti per la loro attività antiossidante soprattutto nella protezione delle lipoproteine a bassa densità, inibendo i processi di invecchiamento della pelle dovuti alla generazione di radicali liberi.



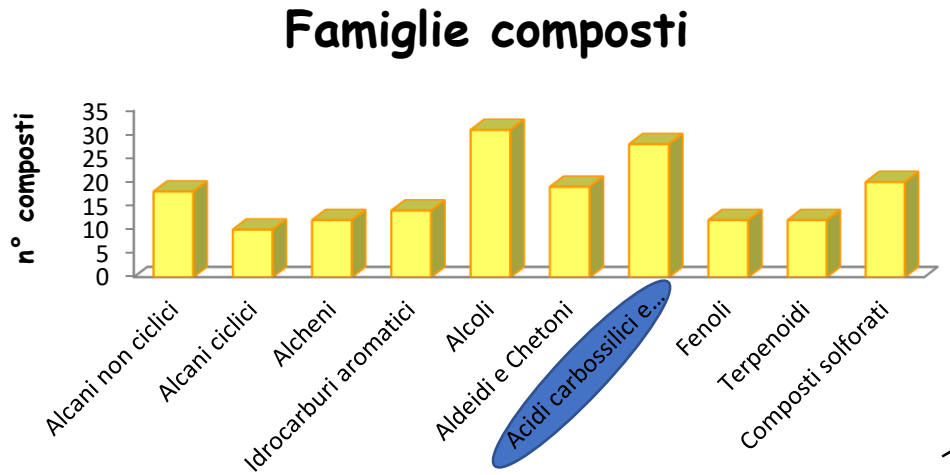
pentadecan-1-ol
Chemical Formula: $C_{15}H_{32}O$
Molecular Weight: 228,41



Oleyl Alcohol
Chemical Formula: $C_{18}H_{36}O$
Molecular Weight: 268,48

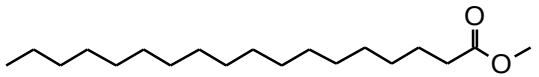


2-propylheptan-1-ol
Chemical Formula: $C_{10}H_{22}O$
Molecular Weight: 158,28

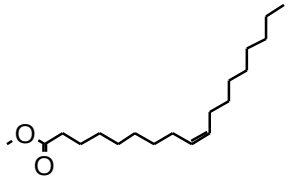


Acidi carbossilici grassi e Esteri degli acidi carbossilici grassi.

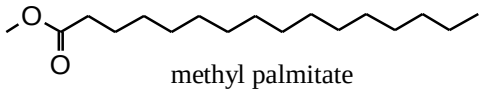
Gli acidi carbossilici a lunga catena insaturi (cioè contenenti uno o più doppi legami C=C), sono sostanze naturali largamente presenti in natura con rilevanti proprietà biologiche, tra le quali una marcata attività antiossidante che si esplica principalmente tramite la loro capacità di inibire l'azione dei radicali liberi. Per questa ragione gli acidi grassi sono largamente impiegati per la cura e la salute della pelle, ed utilizzati in formulazioni cosmetiche, cosmeceutiche ed in presidi sanitari per combattere l'invecchiamento della pelle e i fenomeni da stress-ossidativo. Un comportamento del tutto analogo, ed a volte anche superiore nelle prestazioni salutistiche, è associato agli esteri degli acidi grassi. Infatti, gli esteri degli acidi grassi presentano attività antiossidante ed antiinfiammatoria.



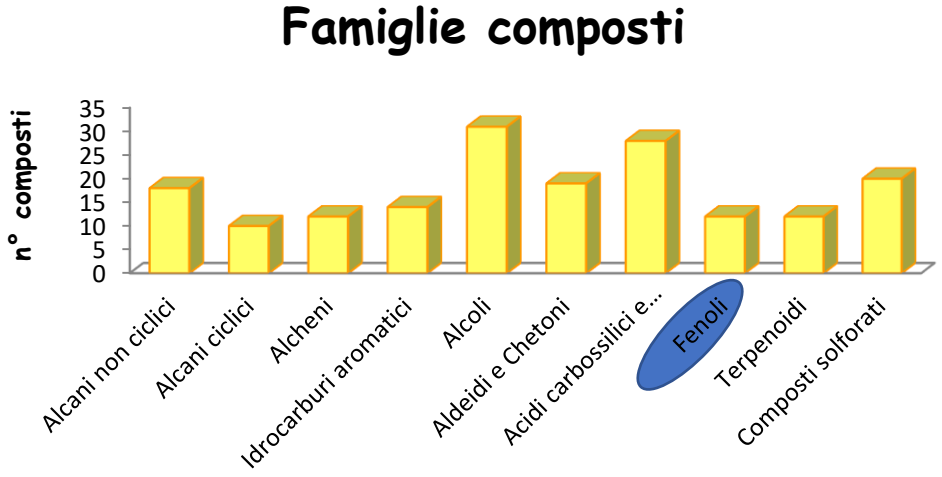
methyl stearate
Chemical Formula: $C_{19}H_{38}O_2$
Molecular Weight: 298,50



methyl oleate
Chemical Formula: $C_{19}H_{36}O_2$
Molecular Weight: 296,49

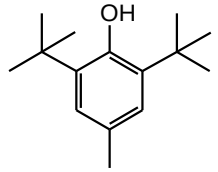


methyl palmitate
Chemical Formula: $C_{17}H_{34}O_2$
Molecular Weight: 270,45

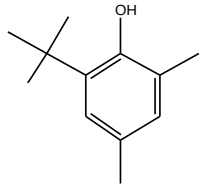


Composti fenolici e polifenolici

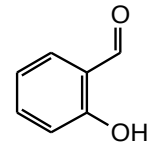
I composti fenolici e polifenolici sono sostanze aromatiche naturali, prodotte come metaboliti secondari nella cellula vegetale ed animale tramite la via biosintetica dell'acido scichimico o, in alternativa, dei polichetidi. I composti fenolici ingombrati sono dei potenti antiossidanti in grado di catturare i radicali liberi all'ossigeno prodotti dai fenomeni di stressossidativo. Tra i fenoli identificati nei campioni FN e FB risultano di particolare interesse il timolo, presente normalmente nell'olio essenziale del *Thymus vulgaris*. E' importante sottolineare che il numero maggiore di composti fenolici è risultato essere contenuto in FN.



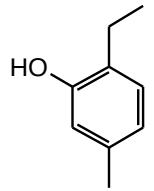
2,6-di-*tert*-butyl-4-methylphenol
Chemical Formula: $C_{15}H_{24}O$
Molecular Weight: 220,35



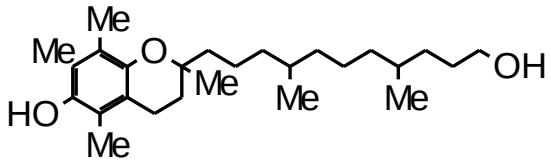
2-*tert*-butyl-4,6-dimethylphenol
Chemical Formula: $C_{12}H_{18}O$
Molecular Weight: 178,27



2-hydroxybenzaldehyde
Chemical Formula: $C_7H_6O_2$
Molecular Weight: 122,12



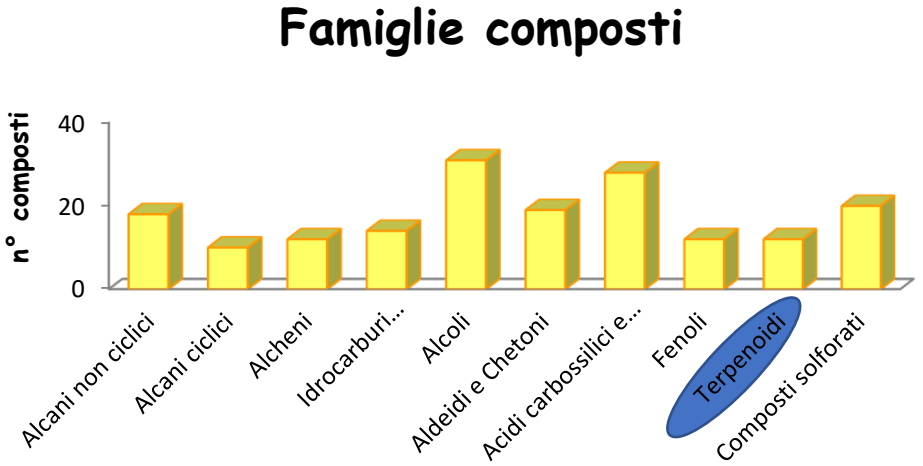
thymol
Chemical Formula: $C_9H_{12}O$
Molecular Weight: 136,19



a-Tocopherol vitamin E

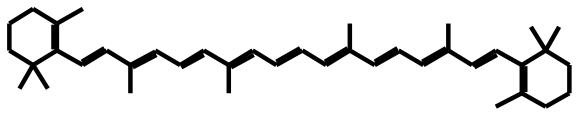
Chemical Formula: $C_{28}H_{50}O_3$

Molecular Weight: 434,69

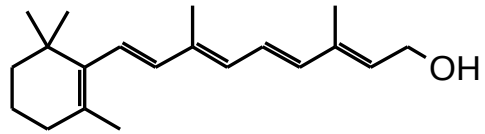


Terpeni e Terpenoidi

Di maggiore rilevanza è la presenza della **vitamina E**, di suoi derivati quali la vitamina E estere dell'acido succinico e il beta-carotene. Infatti la vitamina E ed i suoi esteri sono caratterizzati da una elevata attività antiossidante per la pelle. In modo analogo l'attività elevata antiossidante e anti-radicali liberi del beta-carotene è estremamente elevata, proteggendo la pelle dai danni associati dalla esposizione alla radiazione solare e ad agenti tumorali, soprattutto nel caso di forme di tumore della pelle (melanoma). Il fango FB risulta più ricco in queste sostanze, contenendo vitamina E, l'estere succinico della vitamina E ed il beta-carotene.



beta Carotene

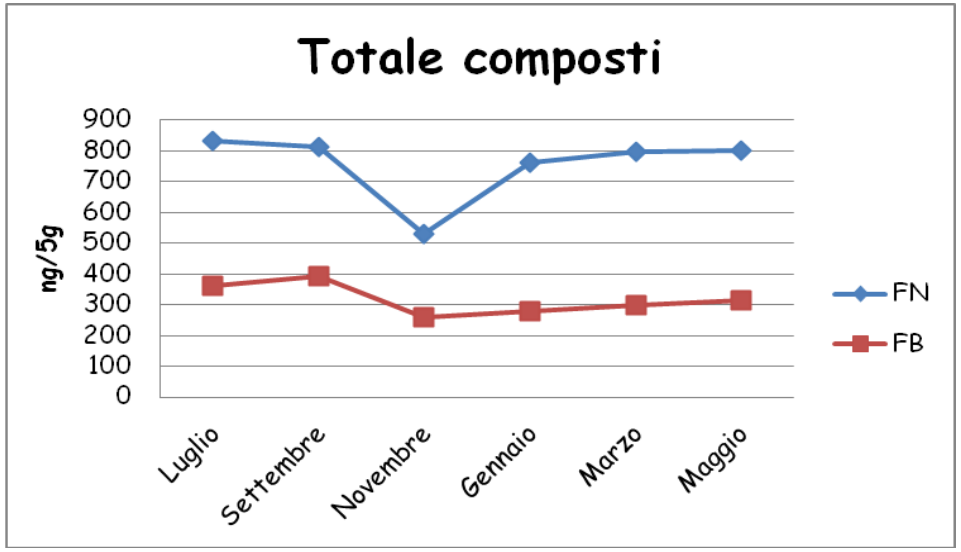


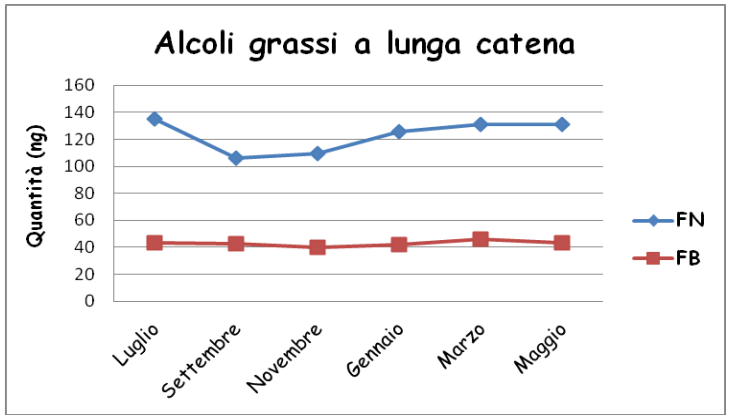
Retinol

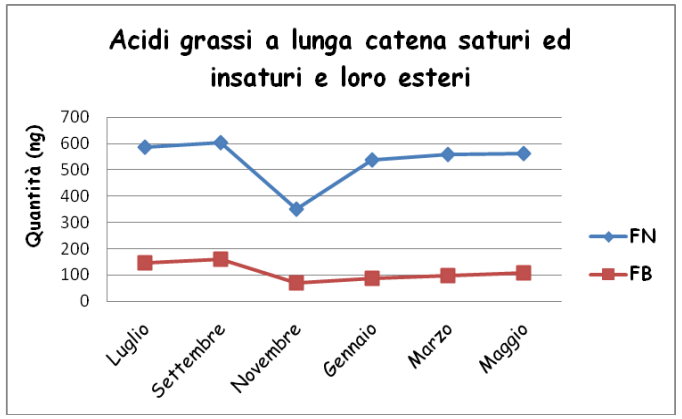
Chemical Formula: $C_{20}H_{30}O$

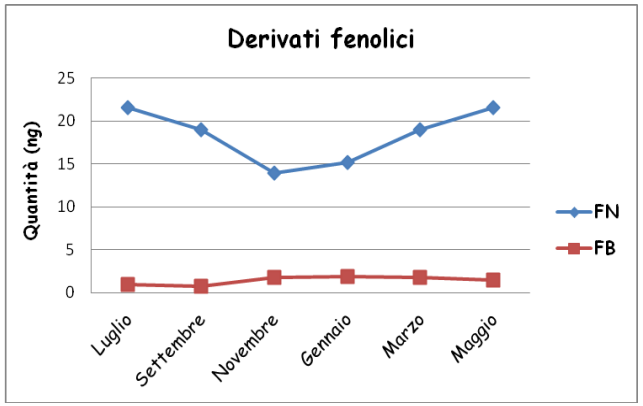
Molecular Weight: 286,45

il Retinolo, identificato nel solo fango FN, noto anche con il nome di **vitamina A**, è un composto con importanti proprietà salutistiche e cosmetiche, ed impiegato in particolare in formulazioni cosmetiche per la rigenerazione della pelle e per il trattamento di fenomeni di infiammazione cutanea, per il trattamento delle rughe, per la protezione della pelle dalla radiazione Ultra-violetta (UV), e per l'attività antiossidante.

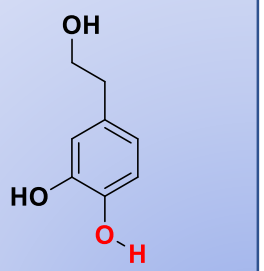

Totale composti
ng/5g
900
800
700
600
500
400
300
200
100
0
Luglio
Settembre
Novembre
Gennaio
Marzo
Maggio
FN
FB


Alcoli grassi a lunga catena
Quantità (ng)
160
140
120
100
80
60
40
20
0
Luglio
Settembre
Novembre
Gennaio
Marzo
Maggio
FN
FB

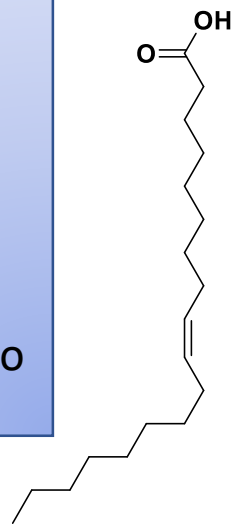

Acidi grassi a lunga catena saturi ed insaturi e loro esteri
Quantità (ng)
700
600
500
400
300
200
100
0
Luglio
Settembre
Novembre
Gennaio
Marzo
Maggio
FN
FB


Derivati fenolici
Quantità (ng)
25
20
15
10
5
0
Luglio
Settembre
Novembre
Gennaio
Marzo
Maggio
FN
FB

Idrofilo

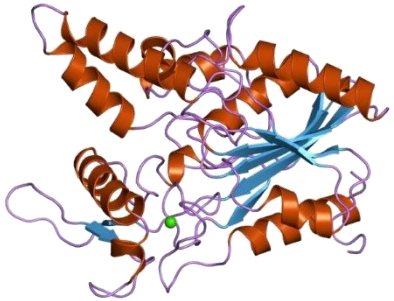


Idrossitirosolo

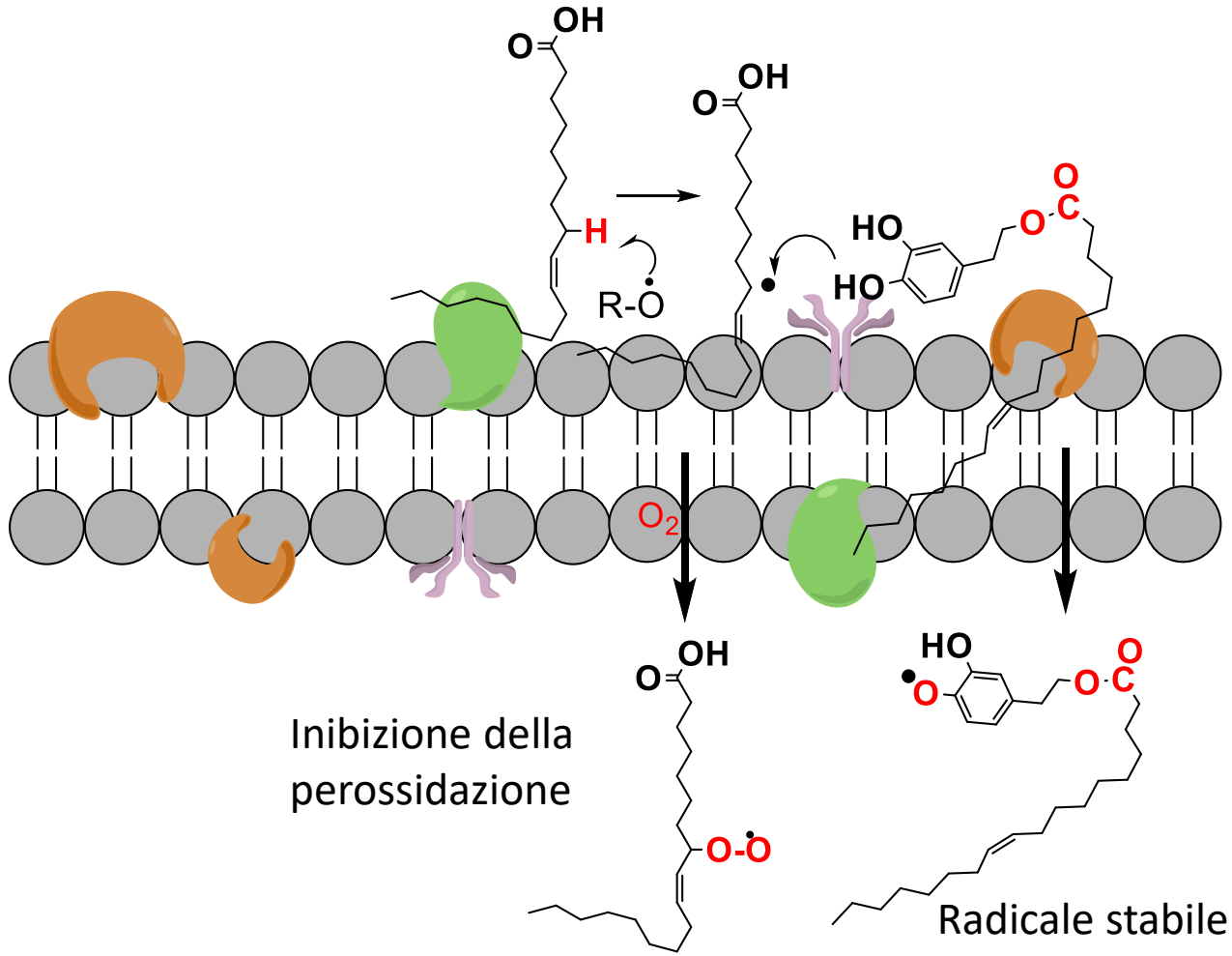


Acido oleico

- H_2O



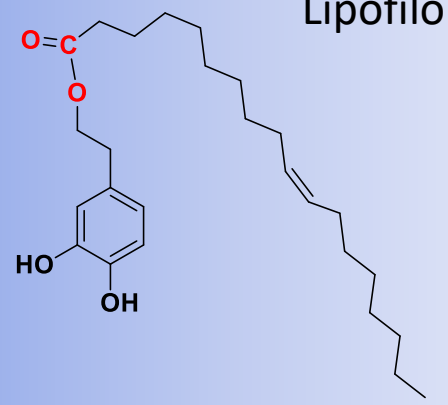
Lipasi da *Candida Antarctica*



Inibizione della perossidazione

Radicale stabile

Lipofilo



Idrossitirosolo estere

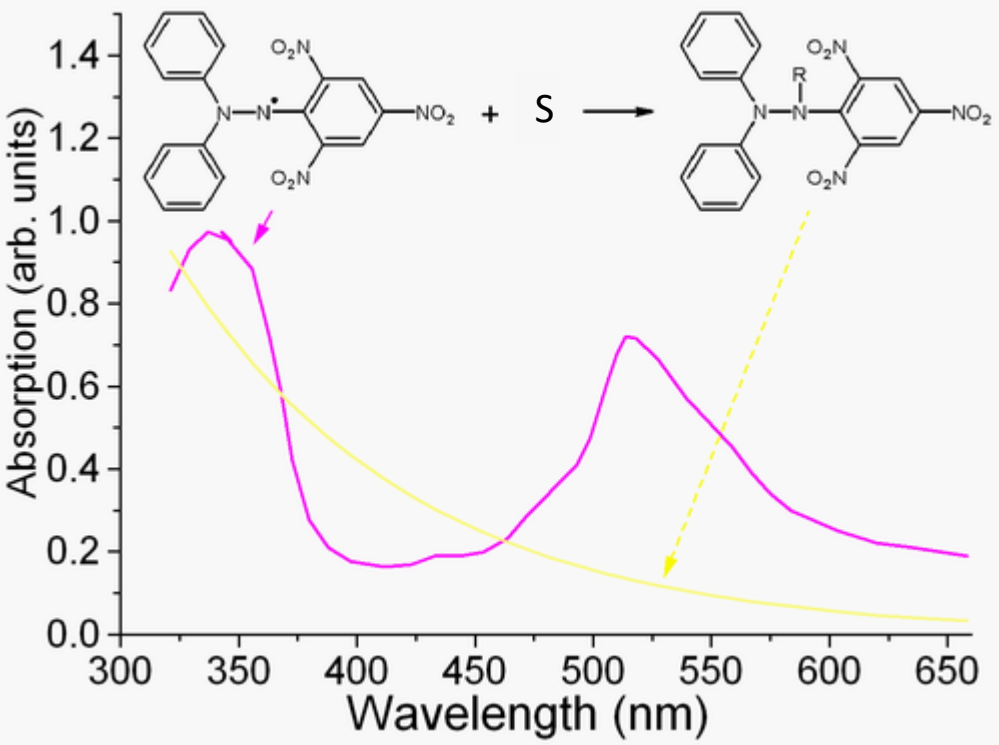
Antiossidante selettivo
Antivirale a largo spettro

BMCVolume 23, Issue 17, 1 September 2015, Pages 5345-5351

Carbon nanotubes supported tyrosinase in the synthesis of lipophilic hydroxytyrosol and dihydrocaffeoyl catechols with antiviral activity against DNA and RNA viruses.

Giorgia Botta, Bruno Mattia Bizzarri, Adriana Garozzo, Rossella Timpanaro, Benedetta Bisignano, Donatella Amatore, Anna Teresa Palamara, Lucia Nencioni, Raffaele Saladino*

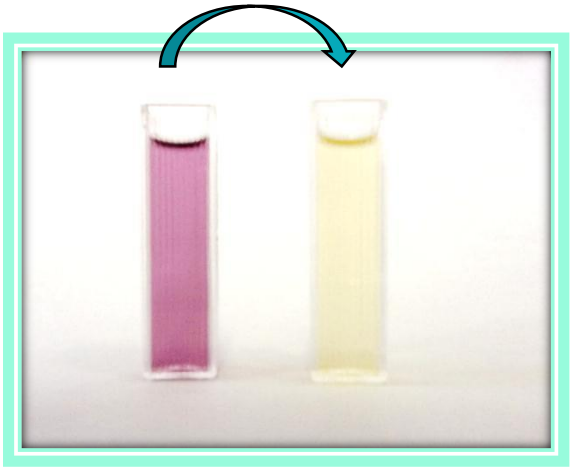
DPPH Assay



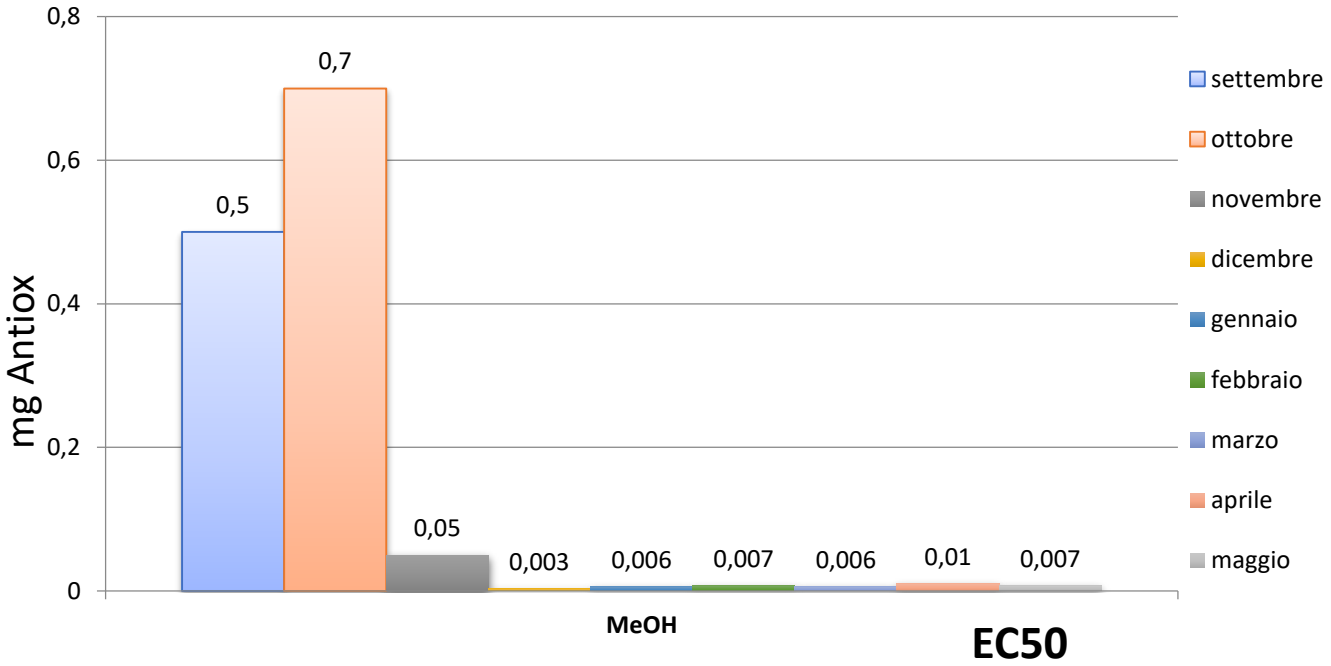
515 nm

DPPH
di(phenyl)-
(2,4,6-trinitrophenyl)
iminoazanium

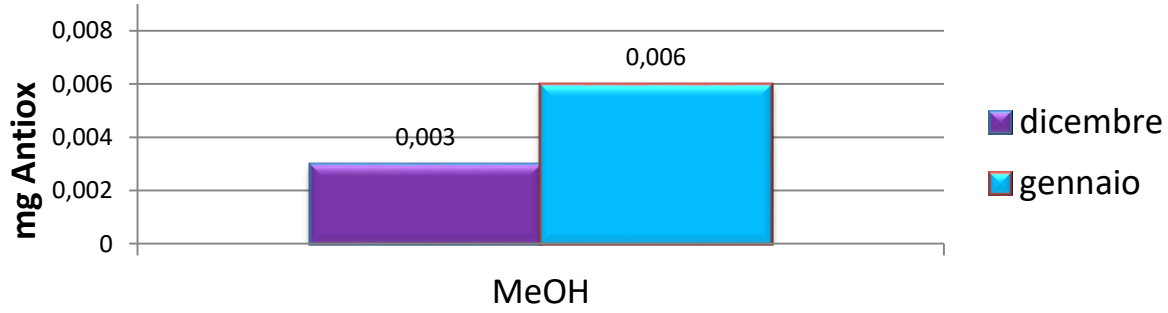
Time (min)



Results: white mud-polar fraction

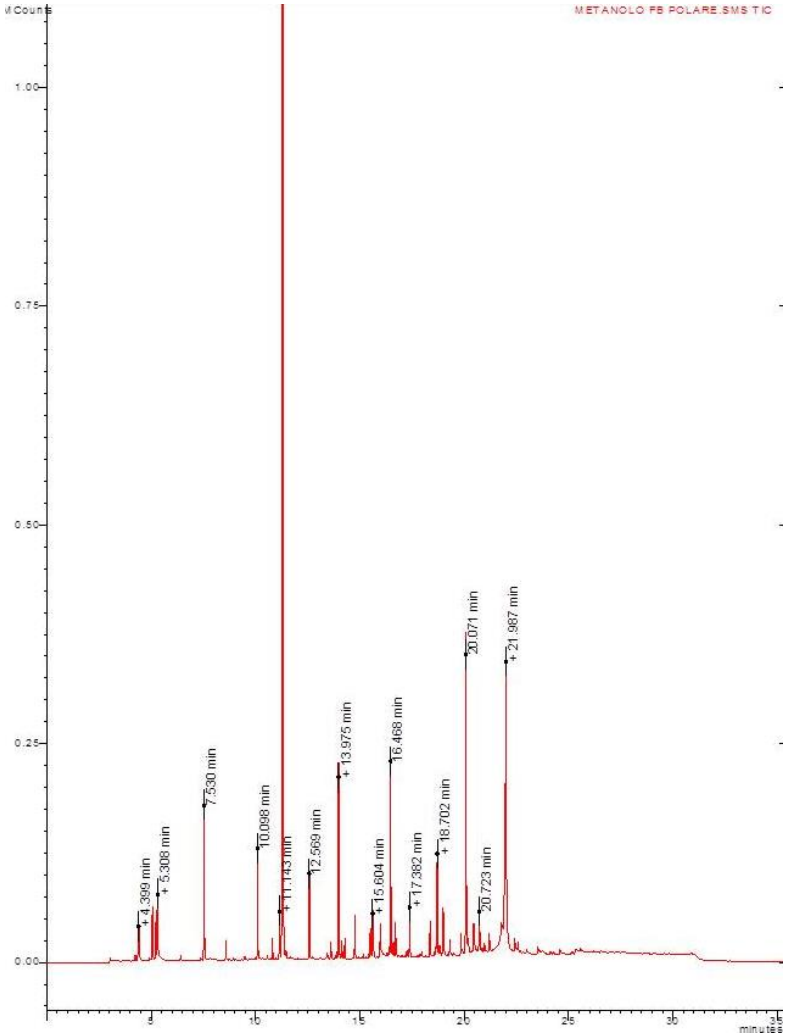


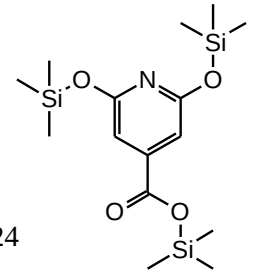
EC50



EC50 Vitamin C = 52µg/mL

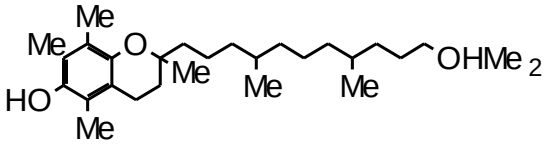
Antioxidant Activity Structure Relationships





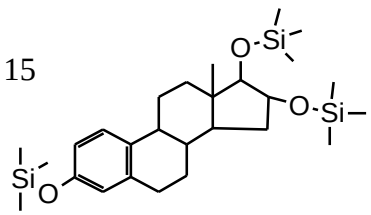
24

trimethylsilyl 2,6-bis(trimethylsilyloxy)isonicotinate
Chemical Formula: $C_{15}H_{29}NO_4Si_3$
Molecular Weight: 371,65



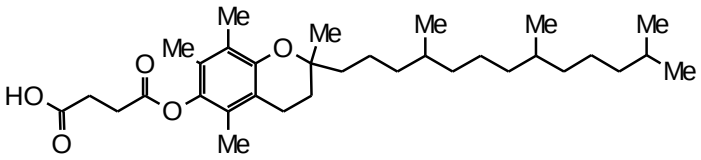
16

a-Tocopherol vitamin E
Chemical Formula: $C_{28}H_{50}O_3$
Molecular Weight: 434,69



15

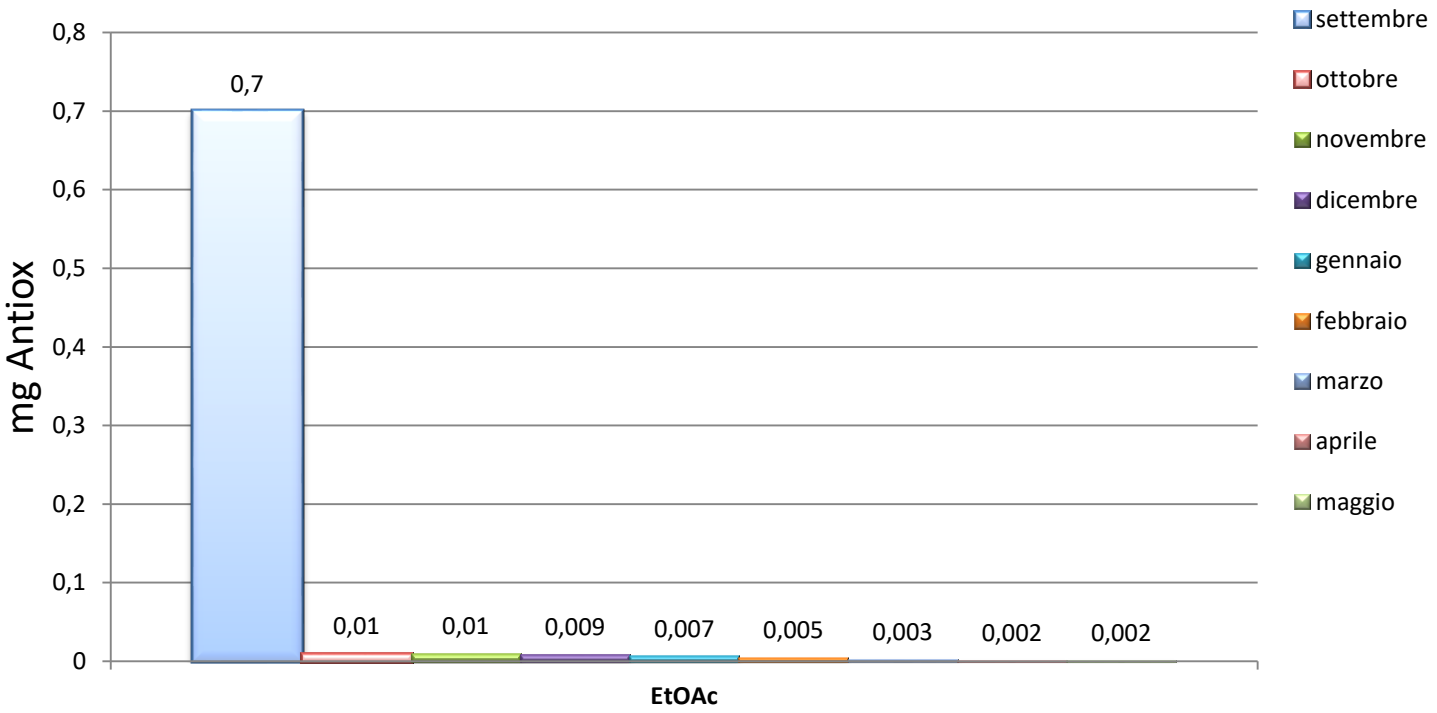
silane (steroide)
Chemical Formula: $C_{27}H_{48}O_3Si_3$
Molecular Weight: 504,92



23

4-oxo-4-(2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yloxy)butanoic acid
Chemical Formula: $C_{33}H_{54}O_5$
Molecular Weight: 530,78

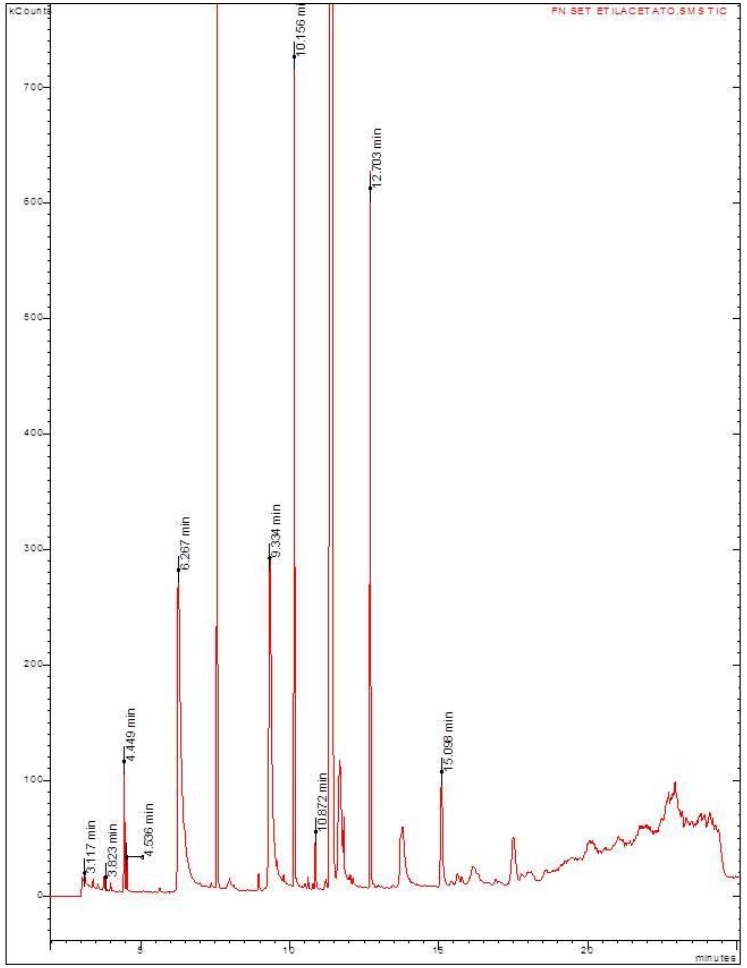
Results: black mud-polar fraction

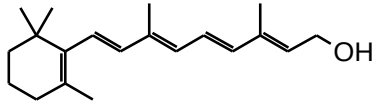


EC50 Vitamin C = 52µg/mL

700 >EC50 >0,7

Antioxidant Activity Structure Relationships

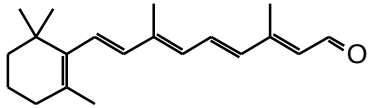




7 Retinol

Chemical Formula: $C_{20}H_{30}O$

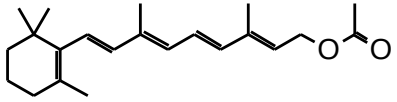
Molecular Weight: 286,45



3 Retinal, 9-cis-

Chemical Formula: $C_{20}H_{28}O$

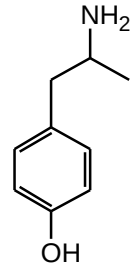
Molecular Weight: 284,44



12 Retinol, acetate

Chemical Formula: $C_{22}H_{32}O_2$

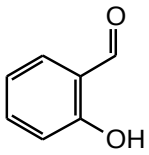
Molecular Weight: 328,49



2 4-(2-aminopropyl)phenol

Chemical Formula: $C_9H_{13}NO$

Molecular Weight: 151,21

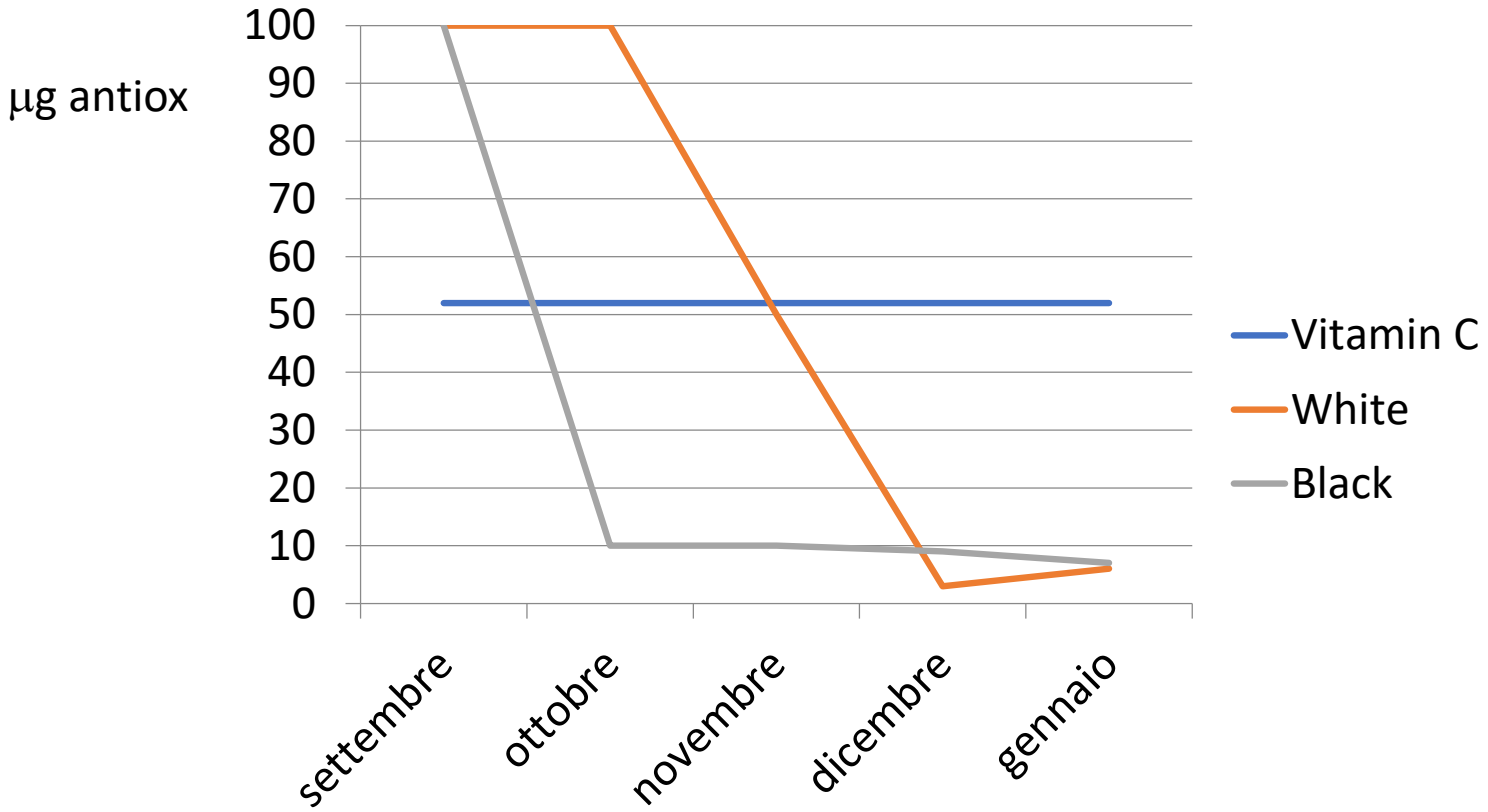


11 2-hydroxybenzaldehyde

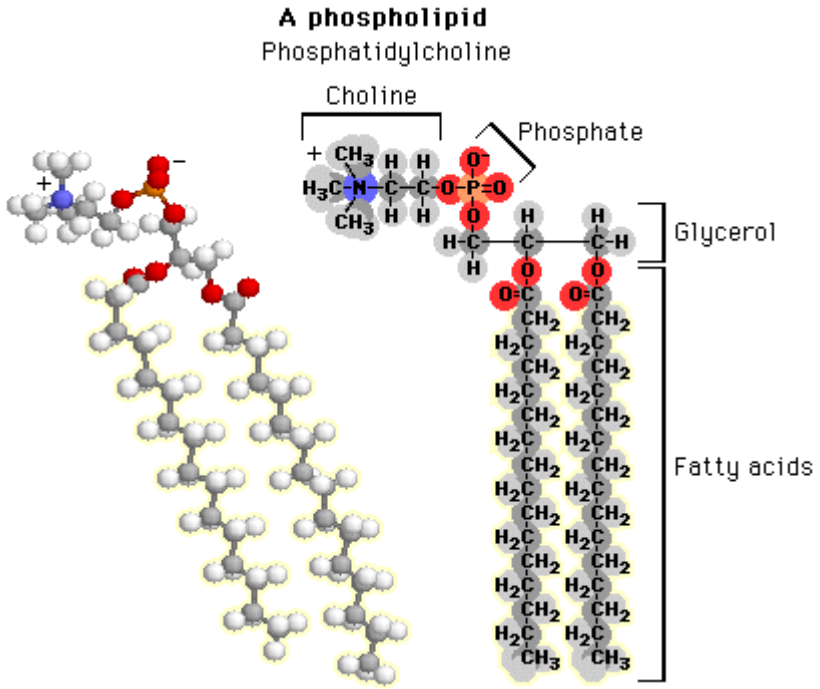
Chemical Formula: $C_7H_6O_2$

Molecular Weight: 122,12

White versus Black mud



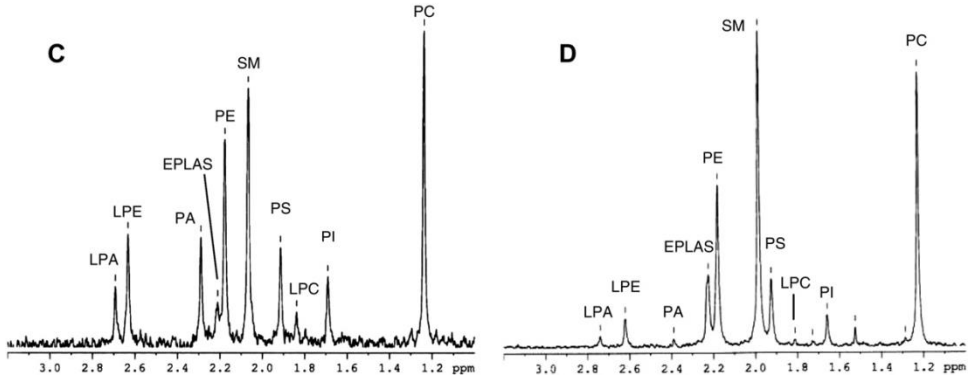
NMR of Lipid Molecules



-Polar head group: ^{31}P NMR

Natural abundance, ^{31}P: 100%
Spin: ½

- Acyl chains: ^{1}H NMR

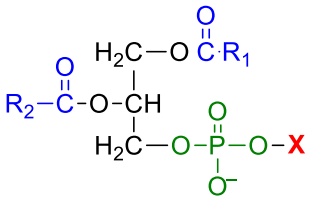


✓ Non destructive analytical method
✓ Qualitative and Quantitative method

Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), Phosphatidylserine (PS) ,
Phosphatidylinositol (PI),
Phosphatidic Acid (PA) ,
Sphingomyelin (SM) ,
Lysophosphatidic acid (LPA), Lysophosphatidylcholine (LPC), Lysophosphatidylethanolamine (LPE),
Phosphatidylethanolamine plasmalogen (EPLAS)

Fosfolipidi nei fanghi termali

The main phospholipids found in thermal muds were phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and sphingomielin (SM).

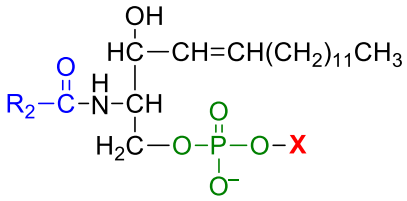


X phosphatidyl

—$CH_2CH_2NH_3^+$ choline

—$CH_2CH_2N(CH_3)_3^+$ ethanolamine

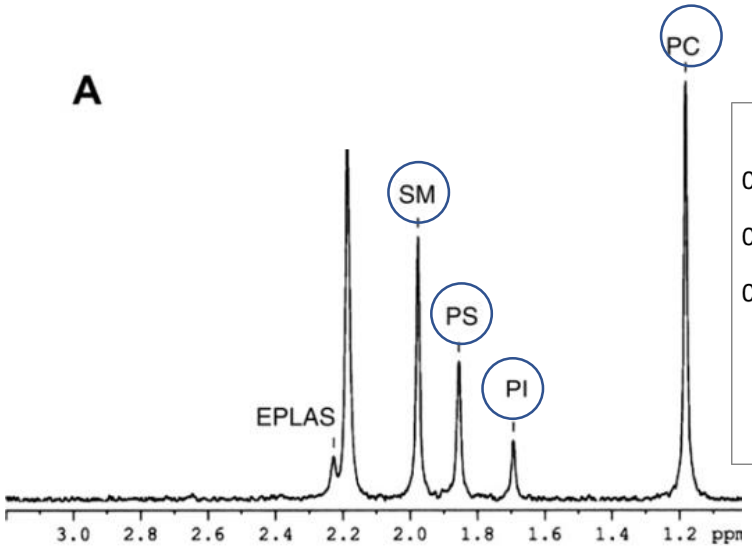
—CH_2CHCOO^- (with NH_3^+) serine

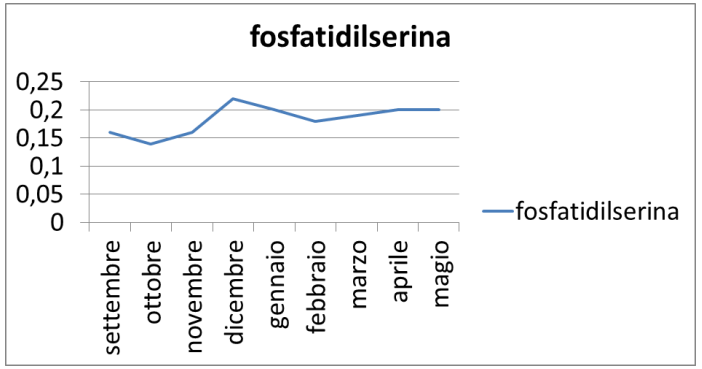


X sphingomyelin

—$CH_2CH_2NH_3^+$ choline

—$CH_2CH_2N(CH_3)_3^+$ ethanolamine





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Free radical scavenging capacity and protective effect of natural substances in peloids from the thermal spring pool Bagnaccio (Viterbo, Italy)

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Synopsis

Natural peloids from sulfurous thermal springs are largely used in cosmetic and pelotherapy for the treatment of different dermatological conditions, including skin aging, dermatitis, and other eczemas. The beneficial effects are correlated to mineralogical and other thermal properties, as well as to the presence of natural substances with specific antioxidant activity. Few data are available for the comparison between natural peloids and synthetic (i.e., artificially maturated) muds. In this context, the natural substances and antioxidant activity of natural white mud (WM) and dark mud (DM) peloids from the sulfurous thermal spring pool Bagnaccio (Viterbo, Italy) have been studied in detail to evaluate possible relationships between physico-chemical properties and therapeutic effect. A large panel of natural substances in WM and DM were characterized for the first time by ^{31}P-nuclear magnetic resonance and gas chromatography associated to mass spectrometry analysis. Polar fractions of WM and DM peloids were characterized by the presence of several bioactive natural compounds, showing high antioxidant activity and DNA protective effect, as evaluated by 2,2-diphenyl-1-picrylhydrazyl assay, and hydrogen peroxide–induced DNA breakage in the alkaline comet assay. The antioxidant activity and DNA protective effect could be attributed to radical scavenging rather than a modulatory effect on the induced DNA repair, and are of order of intensity higher than that reported for synthetic muds.

INTRODUCTION

Peloids are thermal muds characterized by an inorganic matrix embedded with natural bioactive compounds, which are synthesized during the metabolism of growing microorganisms (1). The therapeutic use of peloids is called pelotherapy (2,3), and represents a typical procedure in cosmetic and medical hydrology for the treatment of different

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pathologies, including rheumatoid, osteoarticular and cutaneous diseases (skin aging, dermatitis, and other eczemas), usually associated to stimulatory, antiphlogistic, antioxidant, and analgesic effects (4–6). The activity of peloids is strictly correlated to their chemical composition, as well as to mineralogical and other thermal properties (7). During pelotherapy, the bioactive compounds can penetrate the skin by diffusion and electrophoresis, as demonstrated by the Franz-type cell model (8). In addition, the inorganic particles' transepidermal water loss (9) occlusion process induces the active transport of organic substances through the epidermal barrier (10). Among bioactive compounds, natural antioxidants prevent skin aging by specific radical scavenging and anti-inflammatory activities, while phospholipids improve formulation performances and are active ingredients largely used in cosmetic products (11–14). The thermal spring pool Bagnaccio (TSB) is related to Viterbo (Italy) geothermal area, which is fed by a circuit of groundwater derived from the carbonate aquifer, consisting of Mesozoic-Cenozoic Umbria–Marche succession of the Narni-Amelia chain, Spoleto, Martani Mountains, and Sabini Mountains in central Apennines (15). Detailed stratigraphic, structural, and hydrogeological data of the Viterbo geothermal area are provided in Supplemental #1 (Supplemental Figures 1–8). TSB produces two types of peloids: (a) black-gray mud (dark mud, DM), and (b) white mud (WM). Both peloids are known for their analgesic, anti-inflammatory, osteoarticular, and neuro-hormonal activities that affect the local sites of application, and in general, the whole organism (16). Beneficial effects on health of WM and DM are historically documented (17). The WM peloid shows light brown or yellow-orange pseudo-micellar particles, containing white and pigmented (blue, red-orange, and green) microflora of *Mastigocladus laminosus*, *Oscillatoria cortiana*, *Spirulina subtilissima,* and *Phormidium laminosus* (18). The DM peloid is devoid of granules, and the pigmented component (brown-blue) is abundant and homogeneously mixed in the clay matrix. This peloid is rich in diatoms *Navicula gracilis* and *Cymbella lanceolata* van Heurck, algae (*M. laminosus*), and fragments of hyphae of *Oscillatoria animalis* (18). Few reports are available dealing with the analysis of the organic composition and antioxidant activity of natural peloids, mainly due to sulfur interference in common analysis procedures (19,20). We report here the identification of bioactive compounds in DM and WM peloids by gas chromatography associated to mass spectrometry (GC-MS) analysis through sulfur interference suppression and ^{31}P-nuclear magnetic resonance (^{31}P-NMR). Polar fractions of WM and DM peloids were characterized by the presence of several bioactive natural compounds, showing high antioxidant activity and DNA protective effect, as evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and hydrogen peroxide (H_2O_2)–induced DNA breakage in the alkaline comet assay.

MATERIALS AND METHODS

All commercial products and solvents were purchased in the highest purity grade available and were used as such. Organic solvents (*n*-heptane, cyclohexane, diisopropyl ether, ethyl acetate (EtOAc), acetone, methanol (MeOH), and pyridine), as well as deuterated chloroform ($CDCl_3$), Cr(III) acetylacetonate, and triphenyl methane, were reagent grade and were supplied by Aldrich (Milan, Italy). The chemical constituents were identified by liquid ion chromatography (IC 761 Metrohm, Bern, Switzerland), and determinations of minor and trace elements were carried out using quadrupole inductively coupled plasma mass-spectrometry (ICP-MS) analysis (Thermo X Series II, Thermo Finnigan, Milan, Italy). The mineralogical composition of peloids was measured by diffraction analysis (Goniometer

Seifert MZIV q/2q, Seifert Analytical, Berlin, Germany) and chemical analysis (ICP-MS, Thermo X Series II).

PREPARATION OF POLAR AND NONPOLAR FRACTIONS OF DM AND WM PELOIDS: GENERAL PROCEDURE

WM and black mud (BM) peloids were centrifuged (Rotofix 32A, Hettich, Berlin, Germany; 6000 rpm for 20 min) to remove water, dried (48 h of exposure to air), and subjected to trituration in a porcelain mortar. For the preparation of the polar fractions, the sample (5.0 g) was treated in soxhlet with *n*-heptane (100 ml) at 40°C for 24 h, followed by sequential extraction with 200 ml of EtOAc at 60°C, acetone at 80°C, or MeOH at 80°C (the amount of polar extracts and the extraction yield for DM and WM peloids are given in Table I).

The organic fractions were dried under high vacuum and analyzed by GC-MS. For the preparation of the nonpolar fractions, the appropriate sample (5.0 g) was placed in 250-ml glass beaker and macerated with MeOH (100 ml) for 24 h at 25°C under orbital stirring at 200 revolutions/min. The residual sludge was centrifuged (Rotofix 32A; 6000 rpm for 15 min), and the recovered solid was suspended in *n*-heptane (200 ml) under orbital stirring at 25°C for 14 h (200 revolutions/min). After centrifugation (Rotofix 32A Centrifuge, 6000 rpm for 20 min), the organic phase was concentrated (up to a final volume of 1.5 ml) and loaded on a chromatography column (25 cm high with a diameter of 1.5 cm), previously packed with silica gel (SiO_2). The purification was performed in isocratic conditions with *n*-heptane, cyclohexane, and diisopropyl ether, monitoring the eluate by thin-layer chromatography (TLC). For each eluent the purification was continued until complete elution of the organic compounds as evaluated by ultraviolet (UV)-visible and phosphomolybdic acid detection. The three final organic phases, namely *n*-heptane, cyclohexane, and diisopropyl ether, were concentrated and analyzed by GC-MS (the amount of nonpolar extracts and the extraction yield for DM and WM peloids are given in Table II).

ANALYSIS OF POLAR AND NONPOLAR DM AND WM FRACTIONS BY GC-MS

The analyses were performed on the appropriate sample (10 mg) dissolved in EtOAc (1.0 ml) in the presence of 6-methoxy purine (0.1 mg) as internal standard, by using GC Varian 3900 (Varian-Agilent, Milan, Italy), associated with a mass-spectra analyzer Varian Saturn 2000 (Varian-Agilent, Milan, Italy). Specifications are as follows: column: FactorFour capillary column VF-5ms; 30 m × 0.25 mm and software: Varian MS Work-Station-System-Control

Table I
Extraction Yield of DM and WM Polar Fractions[a]

Sample	Amount of extract (mg)	Extraction yield (%)
DM-EtOAc	160	3.2
DM-Acetone	80	1.6
DM-MeOH	60	1.2
WM-EtOAc	38	0.8
WM-MeOH	50	1.0
WM-MeOH	40	0.8

[a]The extraction yield refers to 5.0 g of starting material.

Table II
Extraction Yield of DM and WM Nonpolar Fractions[a]

Sample	Amount of extract (mg)	Extraction yield (%)
DM-Heptane	20	0.4
DM-Cyclohenane	55	1.1
DM-Diisopropyl ether	50	1.0
WM-Heptane	33	0.7
WM-Cyclohenane	35	0.7
WM-Diisopropyl ether	30	0.6

[a]The extraction yield refers to 5.0 g of starting material.

version 6.9. Method: (a) mass range from 50 to 800 m/z, (b) trap temperature 150°C, (c) manifold temperature 80°C, and (d) transfer line temperature 247°C; injection volume 1 µl, splitting 10%. Specific data of the program analysis are provided in Table III.

To identify the main organic components, two strategies were followed. First, the spectra of identifiable peaks were compared with commercially available electron mass spectrum libraries such as National Institute of Standards and Technology (NIST) (NIST-Fison, Manchester, UK). In this latter case, spectra with at least 93–97% similarity were chosen. Second, GC-MS analysis was repeated using commercially available standard compounds.

^{31}P-NMR ANALYSIS OF PHOSPHOLIPIDS: GENERAL PROCEDURE

The appropriate peloid (20 g) was extracted with $CHCl_3$/MeOH (2:1 v/v ratio), and the solvent was evaporated under reduced pressure. The crude was extracted with $CHCl_3$/MeOH/KCl (Folch method) to concentrate phospholipids in the organic phase. A solvent mixture of pyridine and $CDCl_3$ (1.6:1.0 v/v) was prepared under anhydrous conditions. Triphenyl methane was used as internal standard at a concentration of 0.1 mol/l in the aforementioned solvent mixture. Cr(III) acetylacetonate (15 mg) was added as relaxation agent to this standard solution. The sample (100 mg) was dissolved in the solvent solution (0.5 ml). ^{31}P-NMR spectra were recorded on a Bruker (400 MHz, Milan, Italy) spectrometer, and phospholipids were assigned on the basis of the comparison of the chemical shift with commercially available samples, and when necessary by spiking with predetermined quantities of commercial samples. Typically, the sample was analyzed during 180 min of acquisition time (equivalent to a mean value of 10,000 scans).

ANTIOXIDANT ACTIVITY

Test systems and culture conditions. L5178Y TK+/− clone (3.7.2C) mouse lymphoma cells were obtained from ATCC (CRL-9518™). Generation time, plating efficiency, and

Table III
Set of Data for the GC-MS analysis of DM and WM fractions

Temperature	Rate (°C/min)	Hold (min)	Total time (min)
50	–	3	3
280	10	5	31

absence of mycoplasma were checked at regular intervals. Stocks of the L5178Y cells were stored in liquid nitrogen, and subcultures were prepared from the frozen stocks for experimental use. Cells were grown in RPMI 1640 supplemented with 10% heat-inactivated horse serum, 2 mM L-glutamine, and antibiotics (100 IU/ml penicillin and 100 IU/ml streptomycin) and incubated at 37°C in a 5% carbon dioxide (CO_2) atmosphere and 100% nominal humidity. Chinese hamster ovary (CHO) cells were obtained from Prof. A. T. Natarajan (State University of Leiden, the Netherlands). The cell lines were derived from the CHO isolated from an explant of the ovary of the Chinese hamster (Cricetulus griseus, 2n = 22). The CHO cell line is particularly useful for this kind of studies because of its stable karyotype (modal number is 21 chromosomes), short cell cycle (12–14 h), and its high plating efficiency. Stocks of CHO cells were stored in liquid nitrogen, and subcultures were prepared from these stocks for experimental use. Cultures were grown as monolayer cultures in Ham's F-10 medium (Gibco BRL, Thermo Fisher, Milan, Italy) supplemented with 15% fetal bovine serum, 4 mM L-glutamine, and antibiotics (50 IU/ml penicillin and 50 IU/ml streptomycin). All incubations were at 37°C in 5% CO_2 atmosphere and 100% nominal humidity.

Cytotoxicity evaluation. Approximately 24 h before treatment, exponentially growing cells were detached by trypsin action, and an appropriate number of 75-cm^2 plastic cell culture flasks containing 15 ml of complete culture medium were individually inoculated with 2.0×10^6 cells. Test compounds were dissolved in dimethyl sulfoxide (DMSO) immediately before treatment and added to the culture medium such that the final concentration of solvent did not exceed 1%. The assay was performed using a set of at least six dose levels for each test compound spaced by a factor of 2 (1.0–526 µg/ml), and cell cultures were treated for 3 h. At the end of the treatment, the cultures were washed twice with phosphate-buffered saline, trypsinized, and diluted to obtain an estimated number of 2×10^3 cells/ml. A volume of 100 µl of each cell suspension was plated in each of three 60-mm tissue culture petri dish to assess the viability of the cells. Plates were incubated for at least 6 days before scoring. After incubation, colonies were stained with a 10% aqueous Giemsa solution, and the number of colonies were scored by hand.

Comet assay. Cultures of mouse lymphoma cells at a concentration of 1×10^6 cells/ml were treated for 3 h at 37°C in 5% CO_2 atmosphere and 100% nominal humidity, with each sample at a single dose level selected as the dose levels that reduced the relative cloning efficiency (RCE) of CHO cells to approximately 50% over the concurrent vehicle control values of cultures (Table IV). At the end of the treatment, 10 µl of each cell suspension was added to 65 µl of 0.7% (w/v) low melting point agarose (Bio-Rad Laboratories, Milan,

Table IV
Dose Levels for Different Fractions

Compound	Dose level (µg/ml)
WM-Acetone	16.0
DM-Acetone	8.0
WM-EtOAc	8.0
DM-EtOAc	8.0
WM-MeOH	128.0
DM-MeOH	64.0

Italy) and sandwiched between a lower layer of 1% (w/v) normal melting agarose (Bio-Rad Laboratories, Milan, Italy). For each compound, two sets of three slides each were prepared. An aliquot of 50 µl of H_2O_2 (0.25 µM) was added to one set, while PBS was added to the parallel set. The slides were kept at 4°C for 5 min and then immersed in lysing solution (2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris, pH 10) containing 10% DMSO and 1% Triton X-100 (ICN Biomedicals Inc., Irvine, CA) at 4°C overnight.

Slides were then randomly placed in a horizontal gel electrophoresis apparatus with fresh alkaline electrophoresis buffer (300 mM NaOH, 1 mM Na2EDTA, pH > 13) and incubated for 25 min at 4°C to allow for DNA unwinding and expression of alkali-labile sites. Electrophoresis was performed at 4°C for 15 min at 30 V (1 V/cm) and 300 mA. After electrophoresis, slides were immersed in 0.3-M sodium acetate in ethanol for 30 min. Slides were then dehydrated in an alcohol series (2 min at 70%, 85%, and 100%) and air-dried. Slides stained with 20 µg/ml ethidium bromide in the presence of antifade reagent immediately before analysis were examined at 40× magnification using an automated image analysis system (Comet Assay III; Perceptive Instruments, Suffolk, UK) connected to a fluorescence microscope (Zeiss Axioskop 2, Milan, Italy). DNA damage was quantified from the tail moment (TM) values. A number of 50 cells from each slide (150 cells in total) were analyzed per experimental point.

RESULTS

MINERALOGICAL COMPOSITION

Four samples of DM and three samples of WM (namely, FS I–IV and FC II–IV, respectively) were collected from the floor of the pool Bagnaccio during four representative periods of the year: winter (sample I), spring (sample II), summer (sample III), and autumn (sample IV). The particle size was determined by wet sieving and sedimentation methods (21), while the triangle of Shepard was used for the particle size classification (22). The inorganic constituents were identified by liquid ion chromatography, and minor and trace elements were detected by quadrupole ICP-MS. The mineralogical composition was defined by both diffraction analysis (Goniometer Seifert MZIV q/2q) and chemical analysis (ICP-MS). Results for DM and WM peloids are given in Tables V and VI, respectively. Samples FS II and FS IV were silty sands, while FS I and FS III were silt and sand, respectively (Table V).

The percentage of clay was quite homogeneous with a standard deviation lower than that calculated for the surroundings sand and silt. The grain size composition of WM was rather heterogeneous (Table VI), with a slight predominance of sand and silt fractions (sandy silt, silty sand, sandy silt clay).

The diagram of Shepard, percentages of sand, silt, and clay, respectively, are given in Supplemental #2; (Supplemental Figures 9–12). The cumulative curves (Supplemental #2, Supplemental Figure 13) of WM and DM were well sorted, in agreement with values lower than 100 of the uniformity coefficient U, given by the ratio between the particle diameter at 60% and the diameter at 10% (Table VII).

The DM peloid consists of alumina silicates of kaolinite and montmorillonite type, while WM peloid of microcrystalline calcium sulfate. Among minor elements, relatively high

Table V
Results of the Textural and Chemical-Physical Analyses of the DM Peloid

DM	FS I	FS II	FS III	FS IV	Mean	Standard deviation
Sand	3.3	45	36.4	48	33.2	20.5
Silt	78.4	36.8	40.6	39.5	48.8	19.8
Clay	18.3	18.2	23	12.5	18.0	4.3
Texture	Silt	Silty sand	Sandy silt	Silty sand		
Humidity (%)	144	146	148	143	145	2.2
N tot (%)	5	15	18	9	11.8	5.9
pH	4	5.7	6.6	5.1	5.4	1.1
Li	8.7	6.8	7.9	9.1	8.1	1.0
V	13.7	15.2	18.1	22.1	17.3	3.7
Mn	15.2	18.2	22.1	18.1	18.4	2.8
Fe	2969	3550	3489	3780	3447.0	342.4
As	6.6	12.1	11.9	12.2	10.7	2.7
Rb	27.7	32.3	35.1	40.1	33.8	5.2
Sr	54.5	60.1	65.2	70.2	62.5	6.7
Cs	17.3	18.2	19.5	21.5	19.1	1.8
Ba	21.2	21.2	20.1	18.9	20.4	1.1
Pb	7.1	8.2	9.2	11.2	8.9	1.7
U	1.2	2.1	3.1	2.8	2.3	0.8
Al	6700	8960	9250	8970	8470.0	1187.6

The minor and trace elements are reported in mg/kg.

values of aluminum and iron were observed, followed by strontium, rubidium, barium, cesium, manganese, vanadium, arsenic, lithium, lead, and uranium. The composition and other general properties of the TSB are detailed in Supplemental #3, Supplemental Table A.

Table VI
Results of the Textural and Chemical–Physical Analyses of the WM Peloid

WM	FC II	FC III	FC IV	Mean	Standard deviation
Sand	49.1	49.1	15.5	41.7	24.6
Silt	39	39	72.4	48.6	22.9
Clay	11.9	11.9	12.1	9.8	2.8
Texture	Silty sand	Sand silt clay	Sandy silt		
Humidity (%)	145	150	155	150	5
pH	4.2	6.2	4.1	4.6	1.1
N tot (%)	8	11	18	12.3	5.1
Li	7.7	7.9	6.5	7.4	0.8
V	12.4	11.4	12.9	12.2	0.8
Mn	14.4	13.3	11.1	12.0	1.7
Fe	2865	2975	3100	2980	117.6
As	8.6	10.6	12.1	10.4	1.8
Rb	29.9	28.2	28.6	28.9	0.9
Sr	56.8	55.9	70.9	61.2	8.4
Cs	16.3	18.5	14.1	16.3	2.2
Ba	19.3	22.4	21.5	21.1	1.6
Pb	6.3	8.5	5.7	6.8	1.5
U	1.5	2.5	1.8	1.9	0.5
Al	6870	7150	7320	7113.3	227.2

The minor and trace elements are reported in mg/kg.

Table VII
Diameter of the Grains Corresponding to 60% of Passing (D 60%) and 10% (D 10%) and Coefficient of Uniformity (U)

Sample	D 60% (mm)	D 10% (mm)	U
FS I	0.011	<0.002	7
FS II	0.125	<0.002	83
FS III	0.04	<0.002	27
FS IV	0.08	<0.002	53
FC II	0.18	<0.002	120
FC III	0.18	<0.002	120
FC IV	0.018	<0.002	12

DETERMINATION OF BIOACTIVE COMPOUNDS IN DM AND WM PELOIDS

Natural compounds produced during microbiological growth in thermal muds are used in the benign effects in cosmetic applications and pelotherapy. Structural activity relationships between the organic composition and biological effects cannot be easily defined due to the high complexity of the system. Nevertheless, useful insights can be provided by identification of specific compounds with well-known biological effects, such as antioxidant, radical scavenging, and anti-inflammatory properties. In this context, the presence of sulfur in natural peloids originates interferences in GC-MS analysis, fully overlapping the peaks of organic compounds and increasing the chromatogram baseline. We avoided this side effect by treating DM and WM samples with appropriate solvents followed by chromatographic purification and soxhlet extractions, by a slight modification of the procedure reported for the muds of San Ðiego de los Baños (Pinar del Rio, Cuba) (14). Sulfur-free nonpolar (*n*-heptane, cyclohexane, and diisopropyl ether) and polar (EtOAc, acetone, and MeOH) fractions were obtained and analyzed by GC-MS using 6-methoxy purine as internal standard (DM and WM gas chromatograms of diisopropyl ether, acetone, and MeOH fractions are shown in Supplemental #4, Supplemental Figures 14–19). To identify the chemical structure of the main components, two strategies were followed. First, the spectra of identifiable peaks were compared with commercially available electron mass spectrum libraries such as that of NIST-Fison. Spectra with at least 97% similarity index were chosen. Second, GC-MS analysis was repeated using commercially available standard compounds. In Tables VIII and IX are reported the compounds identified in DM. The m/z values and peak abundances for selected compounds are reported in Table X and selected mass-fragmentation spectra in Supplemental #5. For the analysis of the polar fraction, the DM sample (5.0 g) was treated with *n*-heptane to remove sulfur, followed by sequential extraction with EtOAc, acetone, and MeOH (the extraction yields are given in Table I). The fractions (10 mg) were analyzed using GC-MS (Table VIII, entries 1–3). We identified alkanols, carboxylic acids (and ester derivatives), terpenoids, phenols, alkaloid (colchicine), and sulfur derivative (octathiocane). The highest amount of compounds was in EtOAc (8.98 mg), followed by MeOH (0.59 mg) and acetone (c.a. 0.2 mg). Carboxylic acids (and ester derivatives) are well known for their biological properties, including the ability to quench reactive radical species (23,24), as in the case of the inhibition of lipoxygenase-catalyzed lipid degradation (25). For this reason, they are used for the

Table VIII
Bioactive Compounds Identified in the Polar Fractions of the DM Peloid

Entry	Fraction	Class of compounds	Compound(s)	Yield[a]
1	EtOAc	Alkanols	n.d.	n.d.
		Acids and derivatives	Myristic acid	0.78
			Palmitic acid	0.90
			Stearic acid	0.62
			Palmitoleic acid	0.57
			Oleic acid	0.60
			Methyl stearate	1.68
			Methyl palmitate	2.23
			Methyl oleate	0.38
		Terpenoids	Retinoic acid	0.46
			Retinol (vitamin A)	0.40
			Retinol acetate	0.14
		Phenols	2-Hydroxybenzaldhyde	0.02
			Salicylic acid	0.04
			Thymol	0.02
		Alkaloids	Colchicine	0.02
2	Acetone	Alkanols	Tetradecan-1-ol	0.01
			Tridecan-1-ol	0.02
			Hexadec-9-en-1-ol	0.06
		Acids and derivatives	Palmitoleic acid	0.37
		Terpenoids	Retinoic acid	0.06
		Phenols	2,4-Dimethylphenol	0.02
		Alkaloids	Colchicine	6×10^{-3}
		Inorganic compounds	Octathiocane	0.01
3	MeOH	Alkanols	Tridecan-1-ol	0.05
			Pentadecan-1-ol	0.25
			Tetradecan-1-ol	0.03
		Acids and derivatives	Ricinoleic acid	0.02
		Terpenoids	Gibberellic acid	0.05
			Methyl retinoate	0.04
			Retinol (vitamin A)	0.21
		Phenols	Hydroquinone	0.01
			Gallaldehyde	0.20
			Gallic acid	0.27
		Alkaloids	Colchicine	0.01

[a]Yield is defined as milligram of compounds per gram of DM sample. n.d. not determined

care and health of the skin in several cosmetic formulations (26–28). A similar benign effect was observed for terpenoids (such as vitamin A and retinoic acid) in the treatment of cardiovascular (29), inflammatory, melanoma, and oxidative-induced (30–34) diseases.

Among phenols, salicylic acid derivatives of salicylaldehyde show scavenging properties against radical species (35–38). Gallic acid (3,4,5-trihydroxy benzoic acid), gallaldehyde (3,4,5-trihydroxybenzaldehyde), hydroquinone (1,4-dihydroxyphenol), and thymol (2-isopropyl-5-methylphenol) are known antioxidant compounds (39–41).

Colchicine is a potent antimitotic agent (42). Alkanols with long carbon chains (fatty alcohols) are used in cosmetics as gelling additives (43), skin conditioning agents (44), humectants, and emollients (45,46). For the analysis of the nonpolar fractions, the DM

Table IX
Compounds Identified in the Nonpolar Fractions of the DM Peloid

Entry	Fraction	Class of compounds	Compound(s)	Yield[a]
1	*n*-Heptane	Alkanes	Pentadecane	0.03
			Dodecane	0.15
		Alkenes	Tridec-1-ene	2.5×10^{-3}
			Tetradec-1-ene	4.6×10^{-3}
		Aromatic hydrocarbons	Ethylbenzene	8.2×10^{-4}
			o-Xylene	1.7×10^{-3}
			1,4-Diethylbenzene	4.0×10^{-3}
			1,2,4-Trimethylbenzene	1.7×10^{-3}
2	Cyclohexane	Alkanes	Decane	3.5×10^{-3}
			Undecane	0.18
			Dodecane	0.02
			Tridecane	0.06
			Pentadecane	0.02
			2,4-Dimethylhexane	0.01
		Alkenes	Dodec-1-ene	0.05
			Tridec-1-ene	0.05
			Hexa-2,4-diene	0.09
		Aromatic hydrocarbons	Ethylbenzene	0.02
			o-Xylene	0.02
			m-Xylene	0.04
		Alkanols	Undecan-1-ol	8.7×10^{-3}
			Tretradecan-1-ol	0.02
			2-Methyldecan-1-ol	0.01
			2-Butyloctan-1-ol	0.02
			2,7-dimethyloctan-1-ol	4.5×10^{-3}
			Oleyl alcohol	0.02
3	Diisopropyl ether	Alkanes	Decane	3.7×10^{-3}
			Dodecane	0.17
			Hexadecane	0.03
		Alkenes	Hexa-2,4-diene	0.01
			Tridec-1-ene	4.8×10^{-3}
		Aromatic hydrocarbons	Ethylbenzene	0.01
			o-Xylene	0.05
			p-Xylene	0.02
			1,2,3-trimethylbenzene	8.9×10^{-3}
		Alkanols	Tetradecan-1-ol	3.5×10^{-3}
			2-Butyl-octan-1-ol	1.7×10^{-3}
			2-Methyl-undecan-1-ol	1.5×10^{-3}

[a]Yield is defined as milligram of compounds per gram of DM sample.

sample (5.0 g) was washed with MeOH to remove sulfur, and successively extracted with *n*-heptane. The extract was purified on SiO_2 by isocratic elution (*n*-heptane, cyclohexane, and diisopropyl ether) to afford 125 mg of total crude (the extraction yields are given in Table II). Alkanes, alkenes, aromatic hydrocarbons, and alkanols were identified by GC-MS analysis (Table IX, entries 1–3).

The cyclohexane fraction showed the highest amount of compounds (0.67 mg), followed by diisopropyl ether (0.31 mg) and *n*-heptane (0.2 mg). As a general trend, the amount of compounds in the polar fraction was higher than that found in the nonpolar fraction.

Table X
Selected GC-MS of Organic Components in DM and WM Samples[a]

Compounds	m/z (%)
Alkanols	
Decan-1-ol (C10)	140 (7) [M-H_2O], 112 (26), 83 (64), 70 (87), 55 (100)[b]
Undecan-1-ol (C11)	154 (8) [M-H_2O], 126 (22), 111 (29), 83 (71), 69 (91), 55 (100)
Dodecan-1-ol (C12)	168 (13) [M-H_2O], 140 (25), 111 (31), 97 (50), 83 (82), 69 (91), 55 (100)
2-Butyl-octan-1-ol (C12)	140 (10) [M-46], 125 (16), 111 (20), 85 (31), 69 (50), 57 (100)
Tridecan-1-ol (C13)	182 (12) [M-H_2O], 139 (10), 111 (30), 83 (70), 69 (80), 55 (100)
Tetradecan-1-ol (C14)	214 (10) [M-H_2O], 140 (12), 111 (35), 83 (71), 69 (75), 55 (100)
Octadecan-1-ol (C18)	252 (21) [M-H_2O], 224 (19), 209 (5), 125 (29), 111 (38), 83 (100)
Icosan-1-ol (C20)	298 (2) [M], 280 (24) [M-H_2O], 252 (15), 238 (7), 224 (9), 210 (9), 43 (100)
Aromatic hydrocarbons	
Ethylbenzene	106 (32) [M], 91 (100) [M-CH_3], 77 (11), 65 (13), 51 (12)
m-Xylene	106 (71) [M], 91 (100) [M-CH_3], 77 (18), 65 (11), 51 (19), 39 (24)
p-Xylene	106 (75) [M], 91 (100) [M-CH_3], 77 (13), 65 (7), 51 (10), 39 (10)
1,4-Diethyl benzene	134 (55) [M], 119 (100) [M-CH_3], 105 (83), 91 (25), 77 (12)
1,2,4-Trimethyl benzene	220 (35) [M], 105 (100) [M-CH_3], 91 (14), 77 (16), 65 (10)
Carboxylic acids and ester derivatives	
Butyl acetate (C6)	73(24), 61 (25), 56 (43), 43 (100)
Myristic acid (C14)	228 (42) [M], 185 (20), 129 (51), 73 (100)
Palmitic acid (C16)	256 (22) [M], 213 (14), 129 (31), 73 (87), 60 (81), 43 (100)
Stearic acid (C18)	284 (31) [M], 255 (11), 241 (14), 185 (18), 129 (32), 43 (100)
Ricinoleic acid (C18)	280 (21) [M], 264 (2), 184 (24), 166 (37), 98 (68), 55 (100)
Methyl palmitate (C17)	270 (22) [M], 238 (7) [M-CH_3OH], 227(19), 87 (74), 74 (100)
Methyl oleate (C19)	296 (5) [M], 264 (15) [M-CH_3OH], 222 (16), 180 (20), 83 (71), 69 (80), 55 (100)
Methyl stearate (C19)	298 (14) [M], 267 (4) [M-CH_3OH], 255 (10), 143 (18), 87 (68), 74 (100)
Terpenoids	
Retinol (vitamin A)	284 (100) [M], 269 (12) [M-CH_3], 173 (62), 159 (51), 145 (48), 119 (67)
Retinoic acid	300 (100) [M], 285 (27) [M-CH_3], 256 (22) [M-CO_2], 185 (28), 145 (47)
Retinol acetate	328 (16) [M], 268 (100) [M-COOH], 253 (24) [M-COOH-CH_3], 197 (29), 145 (67), 119 (63)
Methyl retinoate	314 (100) [M], 299 (14) [M-CH_3], 255 (23), 177 (20), 159 (18), 119 (26)
Gibberellic acid	346 (8) [M], 328 (14) [M-H_2O], 300 (15), 284 (13), 152 (28), 136 (100), 121 (61)
Limonene	136 (21) [M], 121 (22) [M-CH_3], 107 (21) [M-2xCH_3], 93 (45), 68 (100)
β-Carotene	536 (100) [M], 444 (23), 430 (8), 378 (9), 268 (13), 105 (64)
α-Tocopherol	430 (35) [M], 205 (8), 165 (100), 149 (5), 136 (7), 121 (9)
Phenols	
Salicylic acid	138 (90) [M], 120 (100) [M-H_2O], 92 (83), 64 (27), 39 (23)
Gallic acid	170 (45) [M], 152 (100) [M-H_2O], 124 (37), 106 (28), 95 (13), 48 (31)
Gallaldehyde	154 (100) [M], 136 (9) [M- H_2O], 125 (14), 108 (13), 79 (18), 51 (12)
Phenol	94 (100) [M], 66 (44), 55 (8), 39 (33)

Table X
Continued

Compounds	m/z (%)
Hydroquinone	110 (100) [M], 81 (37), 55 (26), 39 (24), 27 (21)
2,4-Dimethylphenol	122 (90) [M], 107 (100) [M-CH_3], 103 (8), 91 (23), 77 (38)
Thymol	150 (29) [M], 135 (100) [M-CH_3], 115 (9), 91 (18), 77 (8)
Alkaloids	
Colchicine	399 (54) [M], 371 (46) [M-H_2O], 340 (8) [M-CH_3CONH_2], 312 (100), 297 (51), 281 (46), 269 (28)

[a]Mass spectroscopy was performed by using a Varian apparatus. The abundance of peak is reported in parenthesis.
[b]Analytical tools in experimental.

Alkanes and alkenes have no significant antioxidant properties. However, some long carbon chain derivatives (C8–C10) are applied in cosmetics (or drugs) to make the formulations homogeneous (47), and are components of vegetable extracts, such as propolis (48). The absence of specific petroleum biomarkers, hopanes, homohopanes, steranes, and terpanes (49), suggests that the main sources of long chain alkanes in DM are plant wax compounds (50). The polar and nonpolar fractions of the WM peloid were identified as previously reported for the DM sample (Tables XI and XII). The extraction yields are reported in the Materials and Methods.

The m/z values and peak abundances are provided in Table X. In the polar fractions, we identified alkanes, alkanols, aromatic hydrocarbons, phenols, alkaloids, terpenoids, acids (and ester derivatives), and two sulfur derivatives (hexathione and octathione). The highest amount of compounds was detected in acetone (1.63 mg), followed by MeOH (0.72 mg) and EtOAc (0.19 mg) (Table XI). Terpenoids were the main component (for a total amount of 0.45 mg). Among the acid derivatives, the presence of 2-amino-4-mercaptobutyric acid (homocysteine) suggests the incorporation of sulfur (i.e., an abundant component in natural peloids) in a secondary metabolite. Homocysteine stimulates the antioxidant element–mediated expression in macrophages (51), and inhibits Cu^{2+}-dependent oxidation of human low-density lipoproteins (52). Tocopherols (vitamin E) are characterized by high antioxidant activity (53) and are used in prevention and therapy of aging (54), inflammatory (55) and cancer (56) diseases.

Similarly, β-carotene shows high antioxidant activity and radical scavenging properties in UV solar radiation–mediated damage (57), as well as in the prevention of melanoma (58). The antioxidant and anticancer properties of limonene (one of the main component of essential oils) are widely reported (59). About the composition of the nonpolar fractions, we identified alkanes and alkenes, besides to minor amounts of alkanols and carboxylic acids and esters derivatives. The highest amount of compounds was in diisopropyl ether (0.94 mg), followed by cyclohexane (0.58 mg) and heptane (0.10 mg). It is interesting to note that, irrespective to the nature of the sample, DM and WM peloids showed an amount of bioactive compounds several order of magnitude higher than that previously reported for mud samples produced by artificial procedures (i.e., synthetic clay maturated in thermal water for several months) (8). Moreover, most of the natural substances with antioxidant activity (terpenoids, phenols, and fatty acids) were concentrated in the polar fractions.

Table XI
Compounds Identified in the Polar Fractions of the WM Peloid

Entry	Fraction	Class of compounds	Compound(s)	Extraction yield
1	EtOAc	Alkanes	Dodecane	0.01
			Nonadecane	1.9×10^{-3}
			2,3-Dimethylundecane	0.03
			3,7,11-Trimethyldecane	4.3×10^{-3}
		Alkanols	Dodecan-1-ol	4.8×10^{-3}
			2-Methylhexadecan-1-ol	0.02
			2-Butyl-ocatan-1-ol	5.7×10^{-3}
		Aromatics hydrocarbons	*p*-Xylene	2.3×10^{-3}
		Phenols	Phenol	0.01
			2-Hydroxybenzaldehyde	0.01
			Salicylic acid	0.03
		Alkaloids	Colchicine	0.04
		Terpenoids	Limonene	3.2×10^{-3}
		Acids and derivatives	Butyl acetate	0.02
			2-amino-4-mercaptobutyric ac.	3.4×10^{-3}
2	Acetone	Alkanes	Dodecane	0.05
		Alkenes	isoprene	0.02
			Heicos-10-ene	0.01
			Hexadec-9-en-1-ol	0.02
		Alkanols	Octan-1,2-diol	0.01
			Icosan-1-ol	0.22
			5-Methylicosan-1-ol	0.16
			Nonadecan-1-ol	0.05
		Alkaloids	colchicine	0.01
		Terpenoids	β-Carotene	0.14
			α-Tocopherol	0.50
		Acids and derivatives	Oleic acid	0.04
			Dioctyl adipate	0.30
			Hex-2-enylhexanoate	0.10
3	MeOH	Alkanes	Dodecane	0.01
		Alkenes	Nonadec-1-ene	0.23
			Henicos-10-ene	0.21
		Alkanols	Icosan-1-ol	0.12
		Alkaloids	Colchicine	0.01
		Terpenoids	α-Tocopherol (vitamin E)	0.05
			δ-Tocopherol (vitamin E)	0.06
		Miscellanea	Isonicotinic acid	0.03
		Inorganic compounds	Hexathione	n.d
			Octathione	n.d

[a]Yield is defined as milligram of compounds per gram of WM sample. n.d. not determined

ANALYSIS OF PHOSPHOLIPIDS BY ^{31}P-MNR SPECTROSCOPY

Phospholipids improve the drug delivery and therapeutic efficacy in pelotherapy (60). ^{31}P-NMR spectroscopy has been used for the analysis of phospholipids in extracts of biological tissues (61), and oils (62), with or without sample purification (63). Pioneering studies of the composition of phospholipids in peloids of the spring pool Bagnaccio have been previously reported only by use of TLC (13). To apply ^{31}P-NMR spectroscopy for the analysis of DM and WM peloids, the samples were treated as reported for the analysis of

Table XII
Compounds Identified in the Three Nonpolar Fractions of the WM Peloid

Entry	Fraction	Class of compounds	Compound(s)	Extraction yield
1	*n*-Heptane	Alkanes	Nonane	6.2×10^{-3}
			Decane	2.8×10^{-3}
			Dodadecane	0.01
			Pentadecane	0.01
			Hexadecane	0.01
			Octadecane	2.0×10^{-3}
			Icosane	1.0×10^{-3}
			3,5-Dimethyloctane	8.8×10^{-3}
		Alkenes	Oct-1-ene	1.8×10^{-3}
			Dodec-1-ene	2.2×10^{-3}
			Tridec-1-ene	4.6×10^{-3}
			Pentadec-1-ene	0.02
			Tetradec-1-ene	$2.2\ 10^{-3}$
			Nonadec-1-ene	2.1×10^{-3}
		Alkanols	Undecan-1-ol	7.0×10^{-3}
			Tetradecan-1-ol	2.2×10^{-3}
			Pentadecan-1-ol	2.3×10^{-3}
			Octadecan-1-ol	2.2×10^{-3}
			Tridecan-1-ol	0.01
2	Cyclohexane	Alkanes	Decane	0.06
			Undecane	0.08
			Tridecane	0.01
			Pentadecane	0.04
			Hexadecane	0.02
			2,4-Dimethylhexane	0.01
			3,5-Dimethyloctane	0.01
		Alkenes	Tridec-1-ene	0.06
			Pentadec-1-ene	0.11
		Alkanols	Tridecan-1-ol	0.01
			2-propenyl-pentan-1-ol	0.10
			Octadecan-1-ol	0.02
		Miscellanea	Dodecanal	0.05
3	Diisopropylether	Alkanes	Dodecane	0.01
			Hexadecane	0.02
			Octadecane	0.09
			Icosane	0.05
			Tetracosane	0.13
			Octacosane	0.12
		Alkanols	Tetradecan-1-ol	0.06
			Octadecan-1-ol	0.04
		Acids and derivatives	Enoic acid	0.02
			Dodecanoic acid	0.05
			Methyl oleate	0.01
			Isopropyl palmitate	0.20
			Tetradecyl tetradecanoate	0.09

[a]Yield is defined as milligram of compounds per gram of WM sample.

soybean emulsions (64). The analysis was performed in $CDCl_3$, MeOH, and cesium-EDTA (1:1:1 w/w ratio; total volume 1.5 ml), in the presence of triphenyl methane as internal standard.

Table XIII
Amount of PC, PS, PE, and SM Identified in DM for Sampling Performed at Different Months

Compound	September[a]	November	January	March	May
PC	0.2	0.16	0.25	0.15	0.19
PS	0.16	0.16	0.22	0.18	0.2
PE	0.12	0.08	0.08	0.14	0.10
SM	0.04	0.04	0.03	0.06	0.03

[a]The amount is defined as milligram of phospholipid per gram of starting DM.

The use of cesium-EDTA was necessary to remove the metal ions that may interfere with the relaxation times of phospholipids. Phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), and sphingomyelin (SM) were the main phospholipids in DM (in a total amount of 0.52 mg/g), while they were not detected in WM. Among the recent applications, PC shows hepatoprotective effect in oxidative-stress-mediated organic solvent liver damage (65), and it is applied in bipolar radiofrequency treatment of localized fat deposits (66). PS is involved in specific drug delivery systems (67). Table XIII reports the amount of phospholipids identified in DM for sampling performed at different months in the year. As a general trend, the amount of phospholipids was relatively stable, the PC and PS being isolated in the highest amount (0.25 and 0.22 mg/g, respectively) in January, PE and SM in March (0.13 and 0.06 mg/g, respectively).

ANTIOXIDANT ACTIVITY

Determination of the antioxidant activity by DPPH radical scavenging analysis. The *in vitro* antioxidant activity of DM and WM peloids was evaluated by the DPPH radical scavenging analysis (68). In brief, the appropriate fraction was dissolved in EtOH (0.01–100 mg/ml) and added to freshly prepared DPPH solution (6×10^{-5} M in EtOH). The decrease in absorbance (475 nm) was determined at different times until the reaction reached a plateau.

Table XIV
DPPH Radical Scavenging Properties of Polar and Nonpolar Fractions of DM and WM Peloids, Respectively

Entry	Sample	IC_{50}
1	DM-EtOAc	0.11
2	DM-Acetone	0.20
3	DM-MeOH	2.0×10^{-3}
4	DM-Heptane	>95
5	DM-Cyclohexane	>93
6	DM-Diisopropyl ether	>90
7	WM-EtOAc	0.13
8	WM-Acetone	0.19
9	WM-MeOH	1×10^{-3}
10	WM-Heptane	>95
11	WM-Cyclohexane	>93
12	WM-Diisopropyl ether	>90

Each experiment was performed in triplicate.

Table XV
Analysis of Colony-Forming Activity for WM-Acetone

Test compound	Dose level (μg/ml)	Mean of colonies	RCE
DMSO	1	317	100
	1.0	311	98
	2.0	310	98
WM-Acetone	4.0	276	87
	8.0	200	63
	16.0	171	54
	32.0	10	3

The kinetic of the process was analyzed for each concentration tested, and the percentage of DPPH remaining at the steady state was estimated. This value was used to calculate the IC_{50} (defined as the concentration of substrate mg/ml that causes 50% loss of DPPH activity). Results are reported in Table XIV. The polar fractions of DM and WM showed a similar antioxidant activity (Table XIV, entries 1–3 vs. 7–9), higher than that observed for the corresponding nonpolar fractions (Table XIV, entries 1–3 vs. 4–6). In particular, EtOAc was the most active fraction in DM (IC_{50} 2.0×10^{-3} mg/ml), and MeOH in WM (IC_{50} 3.0×10^{-3} mg/ml).

Evaluation of cytotoxic potential. The cytotoxic potential was evaluated in CHO cells only for the polar fractions, using the clonogenic assay (CA). CA enables an assessment of the differences in reproductive viability (capacity of cells to produce progeny, i.e., a single cell to form a colony of 50 or more cells) between control untreated cells and cells after treatment (69). Normally, only a hundred or few hundred cells are inoculated. Each viable cell grows and forms a colony. After a suitable incubation time (5–8 days), colonies are stained and counted manually. Cloning efficiency is calculated as percentage (%) of colonies from all inoculated cells. Cytotoxicity was determined by measuring the RCE after treatment compared with cloning efficiency of solvent control cultures. The assay was performed using a set of at least six dose levels for each extract, spaced by a factor of 2.0. DMSO was used as solvent. Results obtained indicated that after a 3-h treatment, dose level of 32.0 μg/ml proved to be toxic (RCE <20%) for all tested extracts, with the exception of WM-MeOH and DM-MeOH for which toxic levels were reached at 562.0 and 128.0 μg/ml, respectively (Tables XV–XX).

Genotoxic and antioxidant activity in cultured mammalian cells. The antioxidant activity of polar and nonpolar fractions of DM and WM peloids was further evaluated in

Table XVI
Analysis of Colony-Forming Activity for DM-Acetone

Test compound	Dose level (μg/ml)	Mean of colonies	RCE
DMSO	1	298	100
	1.0	292	98
	2.0	265	89
DM-Acetone	4.0	224	75
	8.0	143	48
	16.0	54	18
	32.0	12	4

Table XVII
Analysis of Colony-Forming Activity for WM-EtOAc

Test compound	Dose level (μg/ml)	Mean of colonies	RCE
DMSO	1%	287	100
	1.0	281	98
	2.0	250	87
WM-EtOAc	4.0	215	75
	8.0	135	45
	16.0	54	18
	32.0	12	4

mouse lymphoma L5178Y (TK+/−) cells, to have a more realistic scenario of the complex interaction within the cell in biological systems. To this end, the antioxidant activity was assessed by the ability to reduce the extent of DNA breakage induced by H_2O_2, using a slightly modified version of the alkaline comet assay as previously proposed (70).

The genotoxic activity was evaluated comparing the extent of DNA breakage (TM value) in the cells treated with each individual test compound and the concurrent solvent control, while their antioxidant potential was assessed by the ability to reduce the extent of DNA breakage induced by H_2O_2 at 0.25 μM for 5 min. TM is defined as the product of the tail length and the fraction of total DNA in the tail, and is a measure of both the smallest detectable size of migrating DNA (reflected in the comet tail length) and the number of relaxed/broken DNA fragments (represented by the intensity of DNA in the tail). Both for genotoxic and antioxidant activity, each polar fraction was assayed at a single dose level selected as a concentration that reduced the RCE of CHO cells to approximately 50% over the concurrent vehicle control cultures (Tables XV–XX). Selection of dose levels was performed in previous experiments. For genotoxicity, the results indicated the absence of increase in the DNA migration (as measured by TM values), after treatment with any fraction at the selected dose levels (Figure 1). These data suggest that samples were devoid of genotoxic activity under the reported experimental conditions. For the antioxidant activity (Figure 1), a marked protection against oxidative DNA breakage induced by H_2O_2 alone (TM value of 23.07), was observed for WM-MeOH, which reduced the TM value of H_2O_2 to 34%. Moderate protection was also noted for DM-acetone and DM-MeOH, for which the TM value of H_2O_2 alone was reduced to 79%

Table XVIII
Analysis of Colony-Forming Activity for DM-EtOAc

Test compound	Dose level (μg/ml)	Mean of colonies	RCE
DMSO	1%	332	100
	1.0	324	98
	2.0	327	98
DM-EtOAc	4.0	282	85
	8.0	186	56
	16.0	110	35
	32.0	41	12

Table XIX
Analysis of Colony-Forming Activity for WM-MeOH

Test compound	Dose level (μg/ml)	Mean of colonies	RCE
DMSO	1%	322	100
	1.0	316	98
	2.0	312	97
	4.0	309	96
	8.0	311	97
	16.0	293	91
WM-MeOH	32.0	258	80
	64.0	248	77
	128.0	171	53
	256.0	90	28
	562.0	0	0

and 53%, respectively. These data are in accordance with the results of DPPH analysis, suggesting that the in vivo antioxidant properties could be attributed to radical scavenging rather than a modulatory effect on the induced DNA repair. In accordance with this hypothesis, the time lapse between treatment with H_2O_2 and processing of cells for comet assay was approximately 10 min, a gap clearly insufficient for DNA repair events to take place.

CONCLUSIONS

Analytical studies (ionic liquid chromatography, ICP-MS, GC-MS, and ^{31}P-NMR) allowed the identification of mineralogical properties as well as organic composition of DM and WM peloids from TSB. Different natural substances have been identified, including compounds with antioxidant and anti-inflammatory activity, such as phenols, terpenoids, long-chain carboxylic acids, and ester derivatives. Irrespective to the nature of the sample (i.e., DM vs. WM peloid), the polar fractions showed the highest antioxidant activity in both DPPH and comet assays, the nonpolar fractions being inactive. Since polar fractions are also characterized by the highest concentration of natural

Table XX
Analysis of Colony-Forming Activity for DM-MeOH

Test compound	Dose level (μg/ml)	Mean of colonies	RCE
DMSO	1%	307	100
	1.0	301	98
	2.0	285	93
	4.0	267	87
	8.0	234	76
DM-MeOH	16.0	220	72
	32.0	187	61
	64.0	160	52
	128.0	34	11

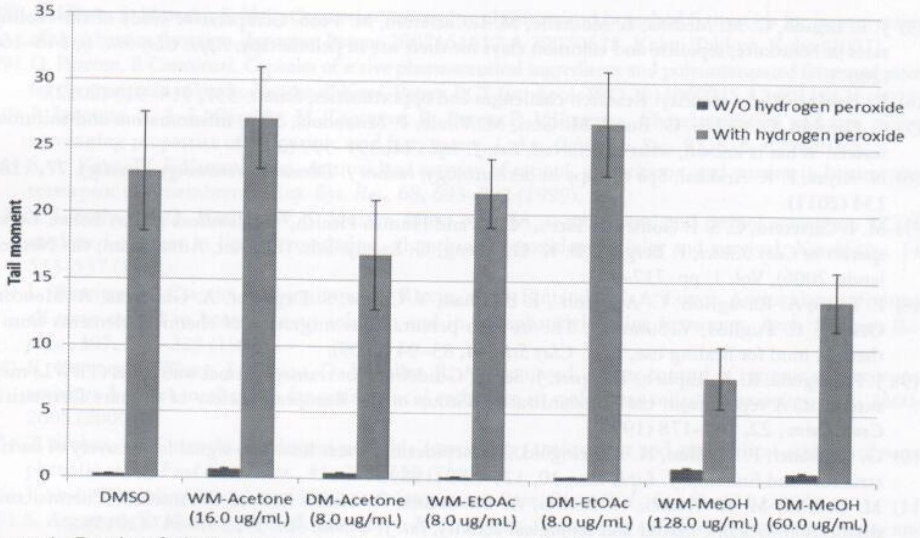

Figure 1. Results of alkaline comet assay for genotoxic and antioxidant properties of polar fractions of DM and WM peloids.

substances, a possible correlation between the antioxidant activity and the chemical composition can be suggested. Moreover, synergic effects are expected to be operative, as in the case of the increased antioxidant activity in the lipid membrane in the presence of mixture of vitamin A and α-tocopherol (vitamin E). This study improves previously reported data, since we demonstrated for the first time that the antioxidant activity and protective DNA effect of natural muds, which are characterized by unique environmental situation being naturally matured in the thermal water of TSB, are of order of intensity higher than synthetic muds (11,12). Moreover, the antioxidant activity could be attributed to radical scavenging rather than a modulatory effect on the induced DNA repair. These data further highlight the use of natural peloids for therapeutic and cosmetic purposes.

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Osservazioni sulla componente organica dei fanghi termali: indagini morfoistochimiche e biochimiche sui componenti lipidici dei fanghi delle Terme dei Papi (Laghetto del Bagnaccio, Viterbo).

Basi chimiche per l'interpretazione
di azioni biologiche e terapeutiche dei fanghi termali.

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