Free radical-scavenging activity of sea urchins pigments by HPTLC-DPPH· method

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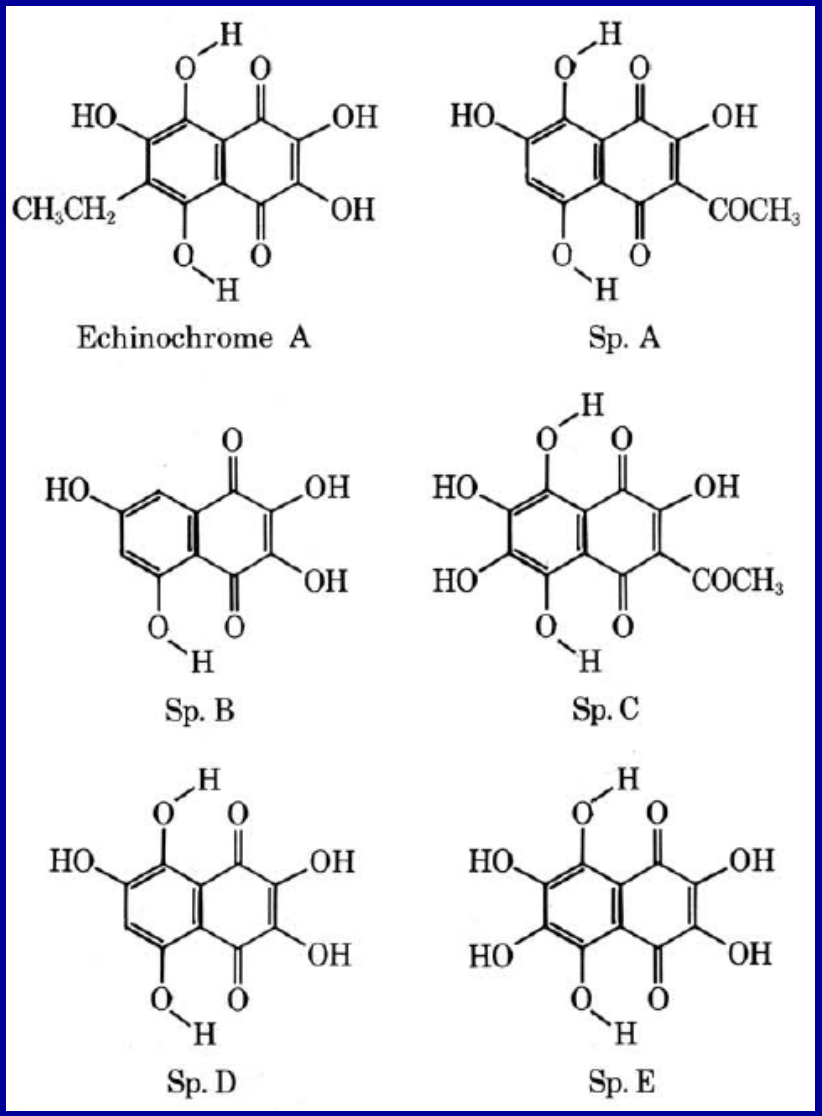
Sea urchins are major consumers in the shallow waters of the world oceans and often determine community structure. It is commonly found in northern waters all around the world including both the Pacific and Atlantic Oceans to a northerly latitude of 81 degrees.

Gonads of sea urchin are known as highly priced sushi foodstuff "Uni" in Japanese traditional food. After removal of gonads the residual shells with spines are dumped as waste.

The shells of sea urchins are known to contain various polyhydroxylated naphtoquinone pigments, spinochromes. The phenolic hydroxyl groups of this molecules suggested that they could participate in antioxidant activity as was observed in other well-known antioxidant polyphenols such as catechins or gallic acid.



A cardinal property of an antioxidant is the ability to scavenge free radicals. Free radicals are involved in the process of lipid peroxidation, are considered to play a fundamental role in several chronic diseases, and are implicated in the aging process. Due to the complexity of the composition of natural extracts, separating each antioxidant compound and studying it individually is costly and inefficient, notwithstanding the possible synergistic interactions among the antioxidant compounds in a mixture. Therefore, it is very appealing to researchers to have a convenient method for the quick quantitation of antioxidant effectiveness.

Benefits of HPTLC

- tolerates minimal sample preparation steps
- enables concentration during application by up to a factor of 10000
- enables parallel chromatography under identical environmental conditions
- enables multiple detection (UV-visible, FLD, MS)
- enables selective and simultaneous derivatization by a myriad of chemical reagents

Consolidating chromatographic separation and antiradical activity determination allows analysts to evaluate the antioxidant contribution of each component within a total extract.

TLC combined with DPPH• radical detection of antioxidants *in situ* was first introduced by Glavind and Holmer (1967). A similar method both on normal-phase (Cavin *et al.*, 1998) and on reversed-phase (Yrjonen *et al.*, 2003) TLC plates was used for screening antioxidants present in plant extracts.

But these methods did not offer a quantitative estimation of antiradical activity of each substance after separation of extract directly on the TLC plate.

Glavind J., Holmer G. 1967. *J Am Oil Chem Soc* 44: 539–542.
Cavin A. et al., 1998. *Planta Med* 64:393–396.
Yrjonen T. et al.2003. *J Am Oil Chem Soc* 80: 9 –14.

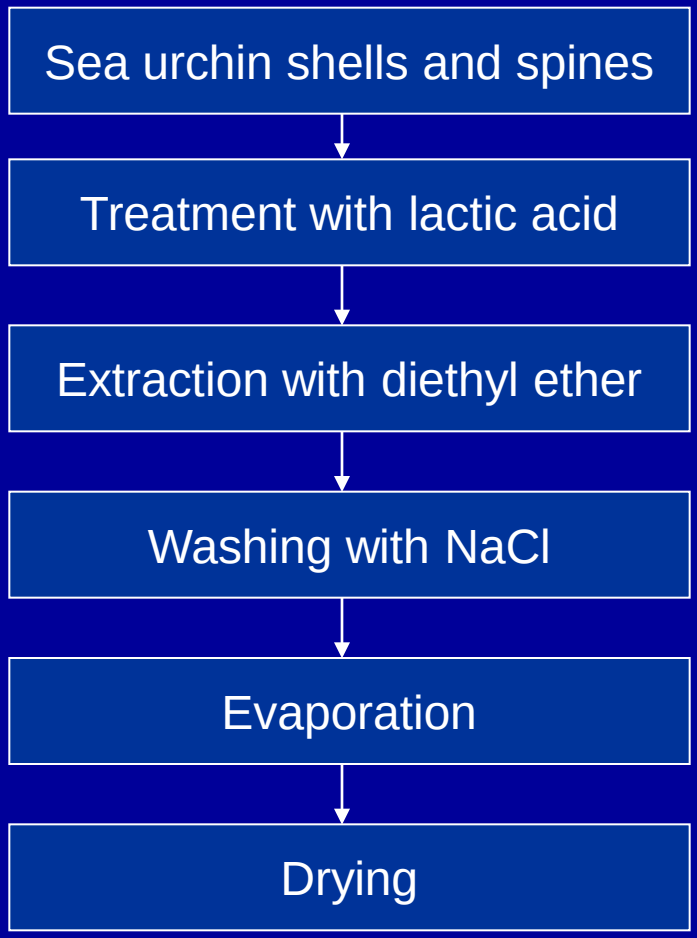
The aim:
separation of sea urchins pigments and quantification of antiradical activity *in situ* by HPTLC-DPPH$^{\bullet}$ method

Strongylocentrotus droebachiensis is commonly known as the green sea urchin because of its characteristic green color.

In our experiments sea urchins from Barents Sea were used.

The Barents Sea is a marginal sea of the Arctic Ocean, located north of Norway and Russia.

Pigments isolation



TLC conditions

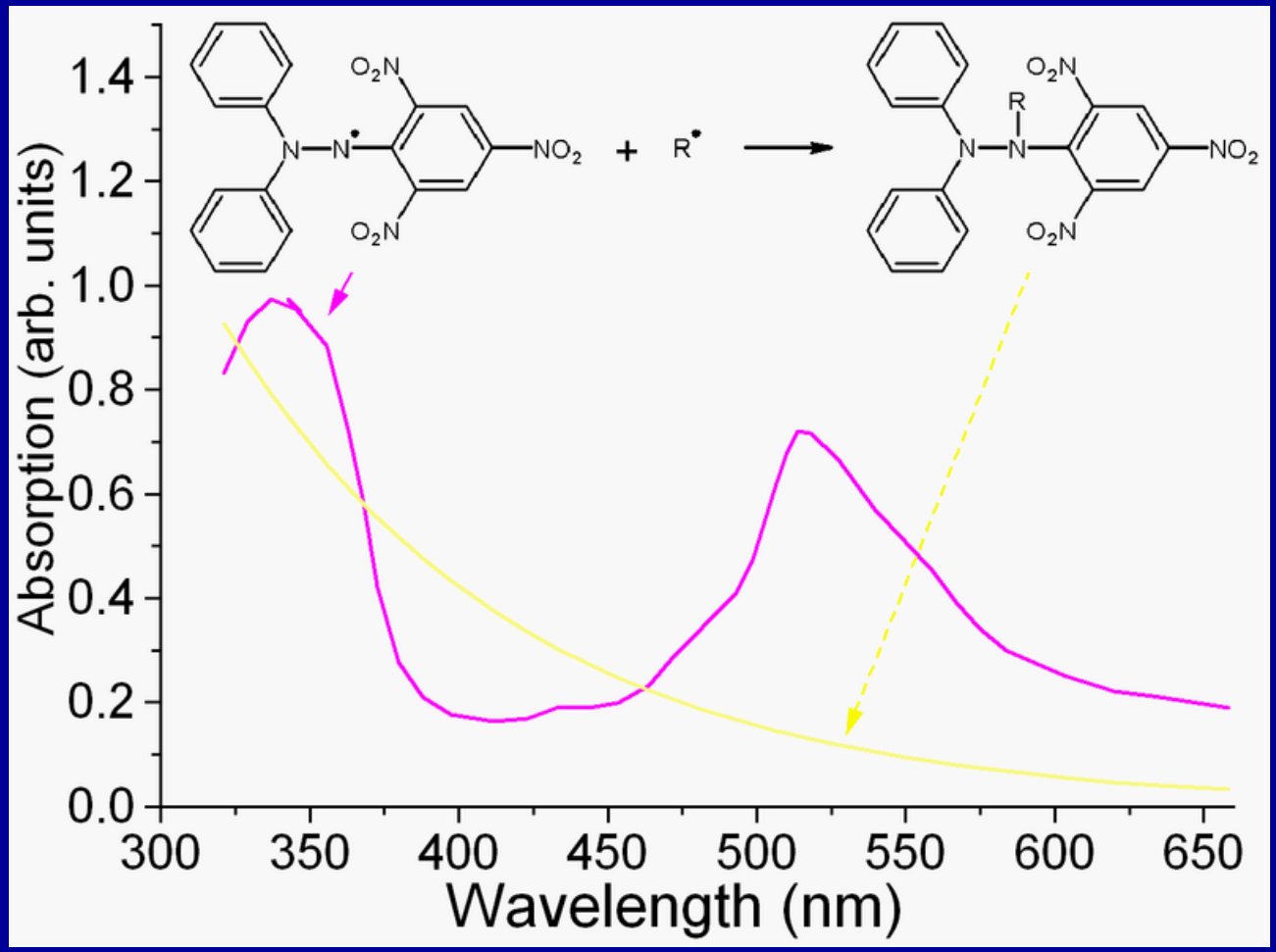
10 cm × 10 cm glass Silica gel 60F254 plates (Merck, Darmstadt, Germany) impregnated with EtOH solution of oxalic acid. Samples of pigments (2-20 µL) were applied by means of a CAMAG (Muttenz, Switzerland) Linomat V automated spray-on system fitted with a syringe as 6 mm bands.

TLC and DPPH• assay conditions

Mobile phase:
Methanol/Chlorophorm/Acetic acid/Water (11/50/5/2)
Visualization at λ=485 nm
Plates were dipped into a 0.5 mM solution of DPPH• in MeOH for 5 s, dried in darkness (23°C) for 90 s and than 30 s at 60°C.

Free radical-scavenging zones were identified as yellow areas against a light violet/purple background at λ=517 nm. ID_{50} were estimated by a nonlinear regression algorithm.

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH•) is a popular free radical used in assessing the radical-scavenging activity or antioxidant activity.



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Original Paper

Separation and evaluation of free radical-scavenging activity of phenol components of *Emblica officinalis* extract by using an HPTLC–DPPH• method

A new procedure has been developed to separate and quantify the free radical-scavenging activity of individual compounds from an *Emblica officinalis* extract based on the combination of HPTLC with a diode array detector (DAD) and postchromatographic DPPH• radical derivatization. Free gallic and ellagic acids and emblicanins A and B in
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Keywords:
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Short Communication

Separation and evaluation of free radical-scavenging activity of phenol components of green, brown, and black leaves of *Bergenia crassifolia* by using HPTLC-DPPH• method

A new procedure has been developed to separate and quantify the free radical-scav-
enging activity of individual compounds from green, brown, and black leaves of *Ber-*

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Phytochemical Analysis

Separation and Free Radical-scavenging Activity of Major Curcuminoids of *Curcuma longa* Using HPTLC-DPPH Method

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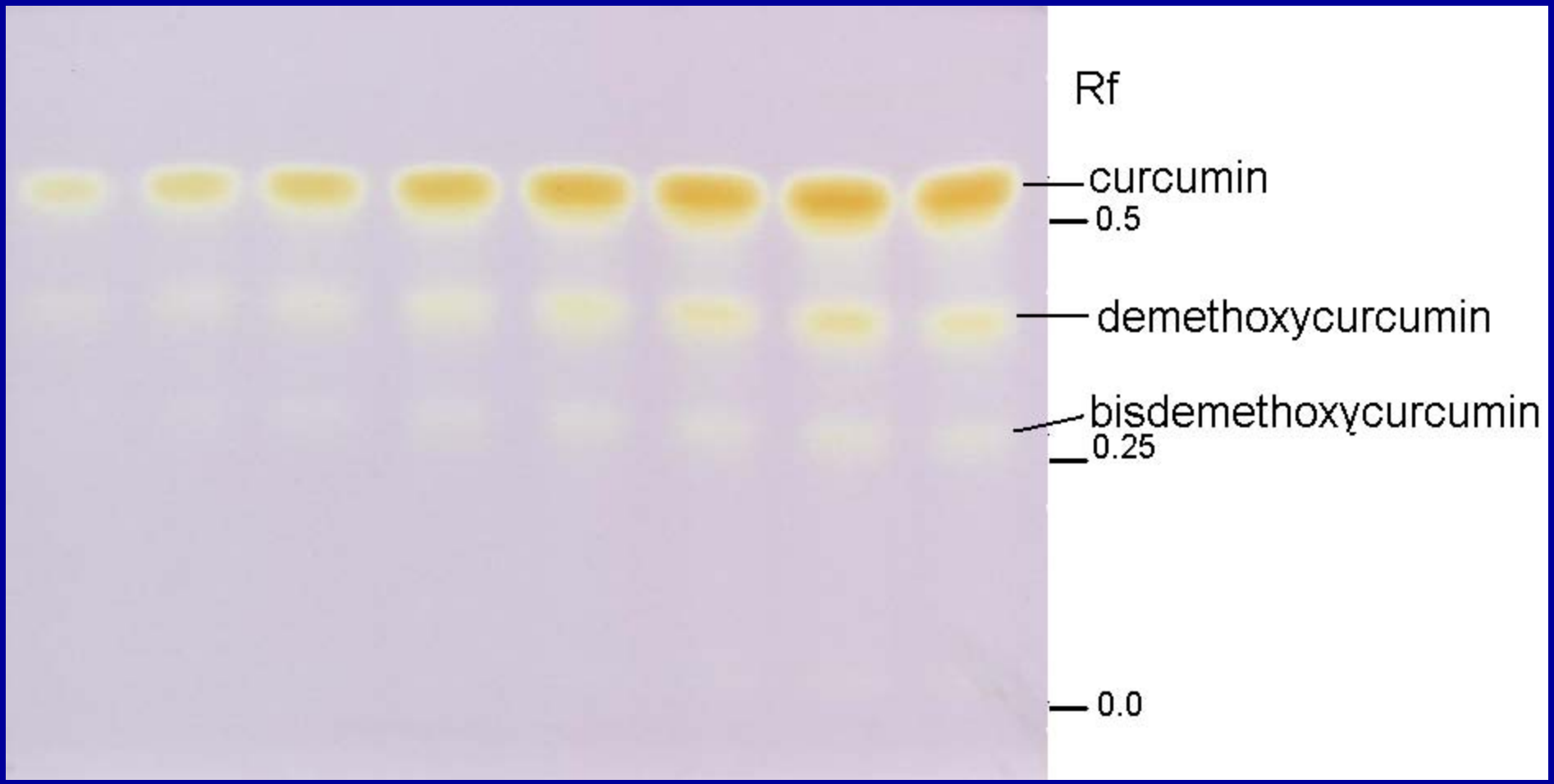
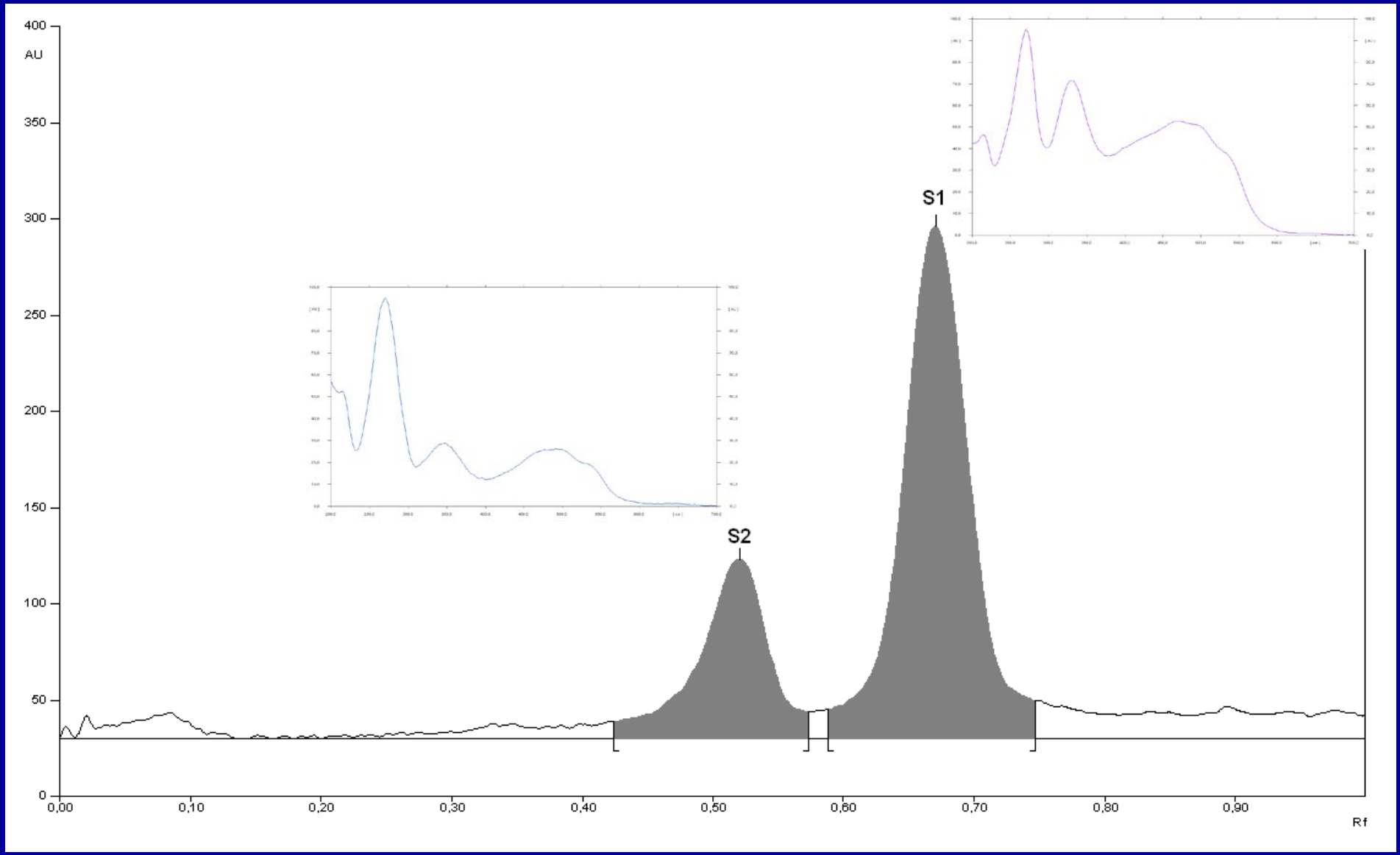
Abstract: A direct HPTLC assay was developed for the determination of total curcuminoids and three individual curcuminoids, curcumin, demethoxycurcumin and bisdemethoxycurcumin. In addition, a new procedure was developed to separate and quantitative the free radical-scavenging activity of individual compounds from the rhizome of *Curcuma longa* L. (Zingiberaceae) based on the combination of HPTLC with a diode array detector (DAD) and post chromatographic DPPH• radical derivatisation. It was established that both individual curcuminoids and the extract of *C. longa* were capable of scavenging DPPH• radicals. From the estimated ID_{50} values, it can be seen that the order of activity was curcumin > demethoxycurcumin > bisdemethoxycurcumin >> ascorbic acid. However, the ID_{50} values of curcuminoids were not significantly different. The data indicates the presence of a synergistic mechanism of antiradical activity of curcuminoids.



*Pozharitskaya O.N. et al. 2008. Separation and Free Radical-scavenging Activity of Major Curcuminoids of *Curcuma longa* Using HPTLC-DPPH Method. Phytochem. Anal., 19, 236-243

Results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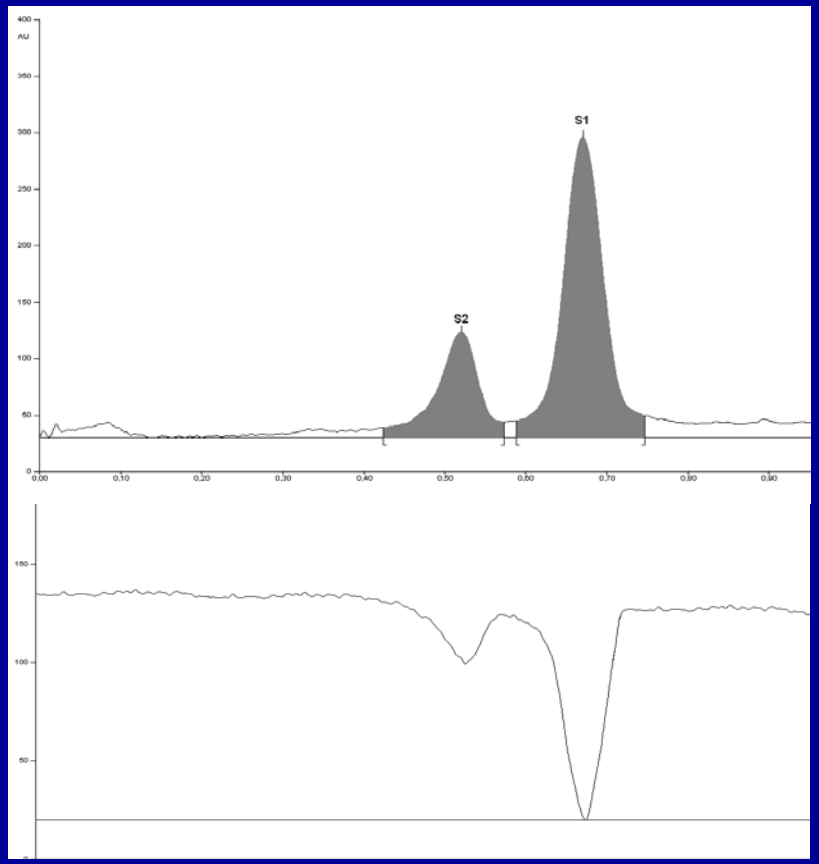
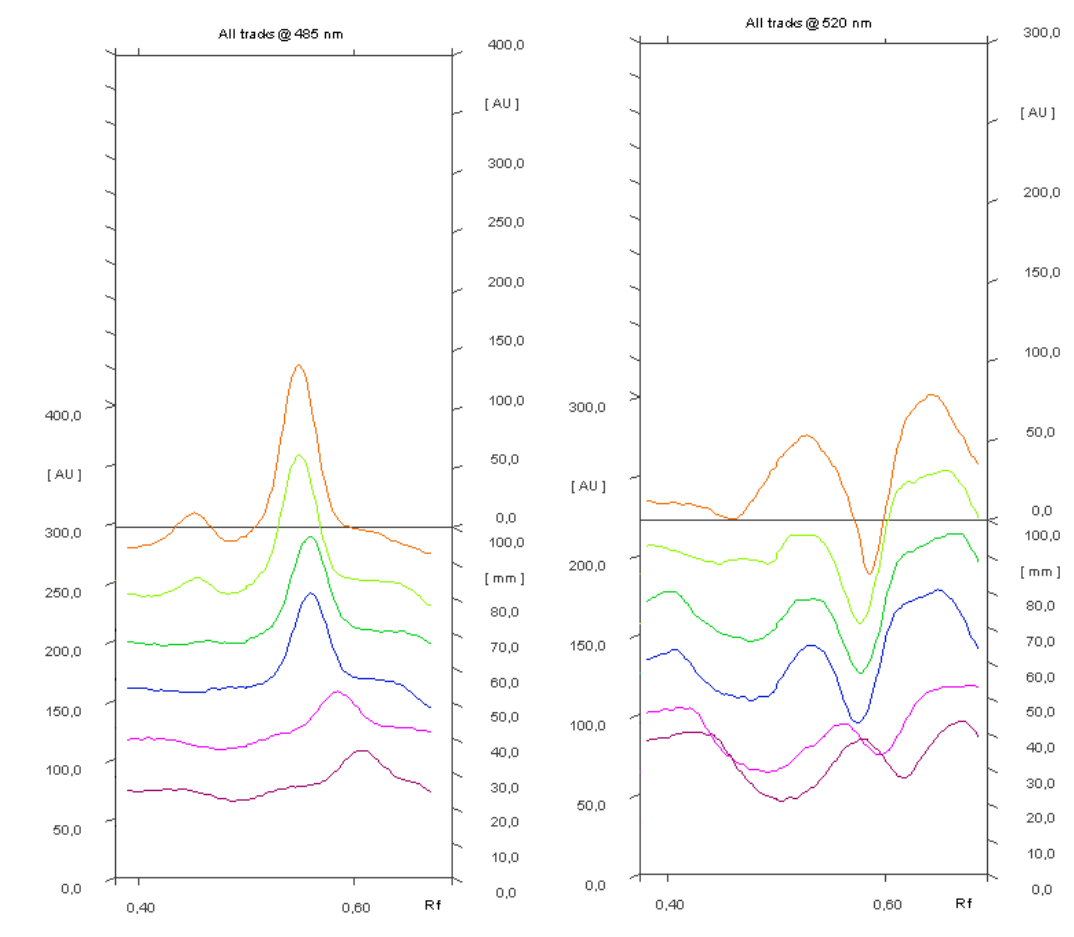
Results



HPTLC-PDA analysis (positive peaks) with detector responses at 485 nm overlaid and DPPH• radical quenching profile (negative peaks) at 517 nm for pigments

Results



Densitogram of pigments stained with 0.5 mM DPPH• solution for calculation of ID_{50}

Results

Compound	ID_{50}
S1	0.043 µg
S2	0.057 µg
Echinochrom A	0.134 µg

Conclusions

- Isolation and identification of active compounds within natural extracts is a difficult, long, and expensive process
- The use of HPTLC makes possible the application of natural extract solutions directly on the HPTLC plates without prior preparation steps
- Quantification of antiradical activity *in situ* with DPPH• radical with the use of postchromatographic derivatization of TLC plates, allowed to estimate the contribution of each component in the antioxidative activity of the total extract

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