



REFERENCE SUBSTANCES FOR HERBAL PRODUCTS

As one of the leading manufacturers internationally, PhytoLab offers over 1,500 extensively documented herbal reference substances of all classes of natural compounds. Our portfolio currently includes a total of 21 anthocyanins and 7 anthocyanidins.

General properties

Anthocyanins are water-soluble secondary plant metabolites that can occur in all parts of higher plants, including leaves, stems, roots, flowers and fruits. The name derives from the Greek words *άνθος* (*anthos*) = flower and *κυανός* (*kyanos*) = blue. Depending on pH-value, anthocyanins may also appear orange, red and purple. In nature, anthocyanins are responsible for making bright-colored flowers and fruits attractive to pollinators or animals. Anthocyanins also act as a “sunscreen” and protect cells from damage due to exposure to UV-light. They may also act as antioxidants in the cell vacuoles. The antioxidant properties have been confirmed *in vitro*, although this effect is most likely limited *in vivo* due to low bioavailability of the intact anthocyanins. However, anthocyanin metabolites may have an indirect effect.

Plant origin

Plants rich in anthocyanins are e.g. blueberry, cranberry, and bilberry (*Vaccinium sp.*); raspberry and blackberry (*Rubus sp.*); blackcurrant (*Ribes nigrum*); cherry and plum (*Prunus sp.*); chokeberry (*Aronia sp.*); elderberry (*Sambucus sp.*); strawberry (*Fragaria sp.*); grape (*Vitis sp.*); eggplant (*Solanum melongena*); black soybean (*Glycine max* L. Merr.). Some less known anthocyanin-containing varieties of common foodstuffs exist, e.g. purple potatoes (*Solanum tuberosum*), purple corn (*Zea mays*) or purple carrots (*Daucus carota*).

Currently available phyproof® anthocyanins & anthocyanidins

Reference Substance	Product #
Cyanidin	80022
Cyanidin 3-arabinoside	89614
Cyanidin 3,5-diglucoside	89615
Cyanidin 3-galactoside	89463
Cyanidin 3-glucoside	89616
Cyanidin 3-(6“malonylglucoside)	85728
Cyanidin 3-rutinoside	80577
Cyanidin 3-sambubioside	89617
Cyanidin 3-sophoroside	80579
Delphinidin	89625
Delphinidin 3,5-diglucoside	89626
Delphinidin 3-galactoside	89506
Delphinidin 3-glucoside	89627
Delphinidin 3-rutinoside	80735
Delphinidin 3-sambubioside	82249
Dracorhodin	83497
Malvidin	80083
Malvidin 3,5-diglucoside	89727
Malvidin 3-galactoside	80600
Malvidin 3-glucoside	89728
Pelargonidin	80084
Pelargonidin 3,5-diglucoside	80334
Pelargonidin 3-glucoside	89753
Peonidin	80085
Peonidin 3,5-diglucoside	80335
Peonidin 3-glucoside	89754
Petunidin	80225
Petunidin 3-glucoside	89755

phyproof® Reference Substances

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Structural properties

Anthocyanins belong to the class of natural compounds known as flavonoids. Their 15-carbon skeleton consists of two phenyl rings and one heterocyclic ring containing a positively charged oxygen atom. In nature, usually carboxylate anions of water-soluble acids would act as counter ions, while the pure compounds are most frequently isolated as chloride salts. The most common anthocyanins exhibit hydroxyl functions in positions 3, 5, 7 and 4'. **The anthocyanidins are the aglycones of the anthocyanins**, which most often bear a sugar moiety bound to position 3. Structural variation is usually achieved by the substitution pattern in the B-ring and differences in the glycosidic profile.

Pharmacopoeias

In **European Pharmacopoeia**, a specification for total content of anthocyanins, calculated as cyanidin 3-glucoside, is given in the monographs for fresh bilberry fruit and fresh bilberry fruit dry extract, refined and standardised. The latter monograph also specifies a maximum limit for five anthocyanidins, calculated as cyanidin, and requests a certain chromatographic profile to confirm identity. While a photometric assay for anthocyanins is used in the monograph for fresh bilberry fruit, an HPLC method identifying 15 anthocyanins is used in the dry extract monograph. A minimum content of procyanidins, expressed as cyanidin, is given in the monograph on hawthorn berries.

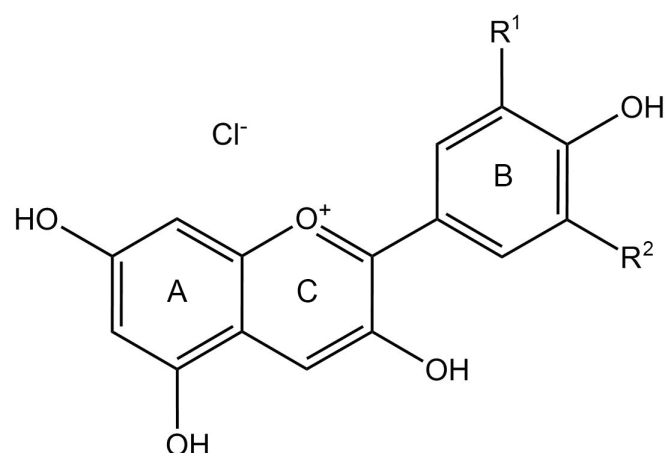
In **United States Pharmacopoeia**, the dietary supplements monograph on powdered bilberry extract specifies a minimum content of anthocyanins, calculated as cyanidin 3-glucoside, and a maximum limit for anthocyanidins, calculated as cyanidin. Requirements on chromatographic profiles including peak intensities of various anthocyanins are given. A total of 15 anthocyanins are described in the HPLC chromatogram. The monograph on European elder berry dry extract specifies a minimum content of anthocyanins, calculated as the sum of the chloride salts of cyanidin 3-sambubioside 5-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-sambubioside and cyanidin 3-glucoside on the anhydrous basis. A maximum limit for anthocyanidins, calculated as anhydrous cyanidin chloride,

is given as well. Requirements on chromatographic profiles including peak intensities of various anthocyanins are given.

Reference Substances

For a reliable quantitative analysis of anthocyanins & anthocyanidins well characterized reference substances are essential. Currently we offer 28 anthocyanins & anthocyanidins, among them most of the compounds mentioned in the respective EP or USP monographs. All of PhytoLab's phyproof® Reference Substances are supplied together with a comprehensive certificate of analysis, and the majority of them are even certified as primary reference standards. For all anthocyanins & anthocyanidins characterized as primary reference substances we quantify as well the counter ion. It is consequently considered as an impurity in the calculation of the absolute content, which refers to the pure anthocyanin or anthocyanidin only. For up-to-date information on prices and specifications please contact us or visit our webshop at phyproof.phytolab.com.

Structure of common anthocyanidins



Anthocyanidin	R ₁	R ₂
Cyanidin	OH	H
Delphinidin	OH	OH
Malvidin	OCH ₃	OCH ₃
Pelargonidin	H	H
Peonidin	OCH ₃	H
Petunidin	OH	OCH ₃

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