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Natural skin care activity from the Arctic

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Natural skin care activity from the Arctic

Plants from the Arctic have traditionally been used for promotion of health and beauty. Their activity is based on the secondary metabolites that the plants produce for protection against environmental stressors, such as UV radiation, cold, insects, and pathogens. In the North, the harsh conditions, especially the low temperature, promote the synthesis of flavonoids and other bioactive antioxidants.¹⁻³ This makes Arctic plants especially appealing as raw materials for active cosmetics ingredients.

Bioactive compounds and traditional uses of heather, cloudberry, roseroot, meadowsweet and juniper

Heather (*Calluna vulgaris*) flowers have traditionally been used as an anti-inflammatory and anti-bacterial remedy for treating urinary tract disorders, wounds, and rheumatic conditions. Beneficial effects reported in the scientific literature include antioxidant, anti-inflammatory, and anti-proliferative activities. Heather has a rich profile of bioactive compounds, including flavonols, catechins and procyanidins (condensed tannins), phenolic acids, phenols, and triterpenes. It is perhaps best known for containing arbutin, associated with effects on melasma (hypermelanosis of the face); ursolic acid, associated with anti-inflammation activity; and flavonols, with strong antioxidant and anti-inflammatory potential.^{4,5}

Cloudberry (*Rubus chamaemorus*) is characteristic of the bogs of Lapland. The yellow berries are rich in phenolic compounds, the ellagitannins, and their hydrolysis product ellagic acid, in particular.^{6,7} Polyphenol-rich extracts of cloudberry have exceptional anti-microbial properties, most likely due to ellagitannins and ellagic acid.^{6,8} Chemopreventive



Heather (*Calluna vulgaris*) flowers.

properties for ellagic acid have been reported as well.⁹ Cloudberry has traditionally been used for treatment of fever, and, because of its high vitamin C content, for treatment of scurvy.

Roseroot (*Rhodiola rosea*), also called Arctic or golden root, is known as an adaptogen, a substance that helps the body to counteract adverse physical, chemical, or biological stress factors. Studies suggest that intake of roseroot affects the central nervous system and may reduce the harmful effect of stress on memory and cognitive function.¹⁰ As reviewed by Khanum *et al.*,¹⁰ indications exist of beneficial effects of roseroot on physical work capacity and on cardiovascular and reproductive health. The characteristic bioactive compounds of roseroot include rosavins, salidroside,

and tyrosol. Roseroot is used for stimulating the nervous system, eliminating fatigue, and decreasing depression.¹⁰ For these properties, roseroot extracts are especially appealing as ingredients for anti-stress applications in cosmetics.

Meadowsweet (*Spiraea ulmaria/ Filipendula ulmaria*) is a plant found in damp meadows. It has been used for attenuation of inflammation, for rheumatic disorders, as an analgesic, for stomach disorders, and for its diuretic activity.¹¹ Meadowsweet is rich in flavonoids, and the presence of salicylates has been reported.^{11,12} Strong antibacterial activity has been reported in meadowsweet of Finnish origin.¹² The traditional use against inflammation is supported by studies indicating that meadowsweet has effects against activation of inflammatory pathways.¹³

Several parts of juniper (*Juniperus communis*), including the berries, branches with needles, bark, tar, and even

roots, have been used in folk medicine throughout history. The indications of use have been variable, from improving digestion to preventing infectious diseases.¹⁴ The water-soluble fraction of juniper is rich in phenolic compounds and has exceptionally strong antioxidant activity as assessed by several *in vitro* methods.¹⁵ The active compounds of the needle-like leaves of juniper include neolignan and flavonoid glycosides.^{16,17}

Water-based extracts as active ingredients for cosmetics

Water-based extracts of Arctic heather, cloudberry seed, roseroot, meadowsweet and juniper sprouts were manufactured for evaluation of their potential as ingredients in cosmetic products. The plants were

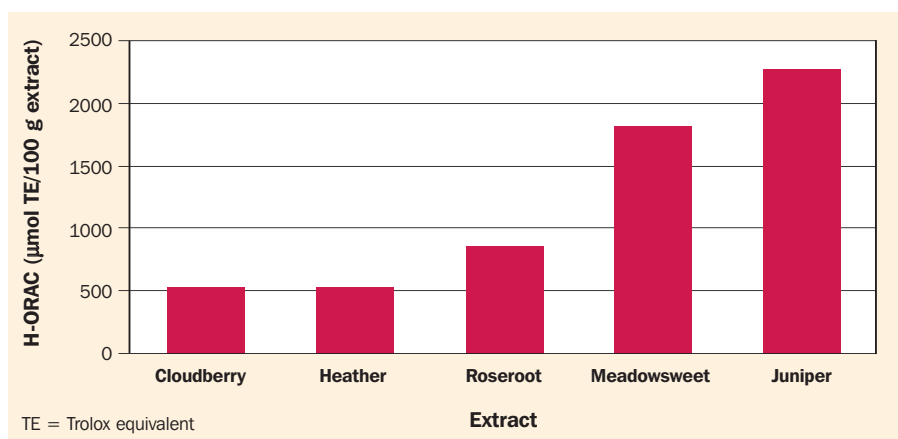


Figure 1: The peroxy radical scavenging activity of the Arctic glycerin–water-based plant extracts, corresponding to hydrophilic oxygen radical absorbance capacities (H-ORAC).

harvested in Northern Finland, near the latitude of the Arctic Circle. In the case of the cloudberry seeds and juniper sprouts, the extraction residue from supercritical CO₂ extraction was used as raw material for the water-based extractions. The dried plants were ground and extracted with a glycerin–water mixture (>50% glycerin, 10%-25% water), or butylene glycol–water (2:1 ratio). In both solvent mixtures, one kilogram of extract corresponded to approximately 0.07 kg of dried plant material. Glycerin–water extracts of heather, cloudberry seeds, roseroot, meadowsweet and juniper sprouts are members of the TruArctic cosmetic ingredient family of Aromtech Ltd in Tornio, Finland.

Polyphenols, antioxidant activity, allergens and tolerance

The polyphenols, antioxidant activity, allergens and skin and ocular tolerance were analysed from the glycerol-water extracts of the plants. The total polyphenol

content of three batches of each product was analysed via an UV-spectrophotometric method (ISO 14502-1:2005[E]). The extracts of juniper sprouts (polyphenol content: $0.38 \pm 0.03\%$), meadowsweet ($0.38 \pm 0.13\%$) and roseroot ($0.31 \pm 0.09\%$) had higher total concentrations of polyphenols than did cloudberry seed ($0.06 \pm 0.01\%$) and heather ($0.04 \pm 0.03\%$) extracts. The polyphenol content was reflected in the antioxidant activity of each extract (see Figure 1, showing antioxidant activity analysed by means of the method of Ahotupa *et al.*).¹⁸ The characteristic active compounds of roseroot (rosavins, salidroside, and tyrosol) and cloudberry (ellagic acid/ellagitannins) were found to be extracted to the glycerin-water extracts (MTT Agrifood Research Finland, in-house HPLC-UV/visible method). The flavonols quercetin and kaempferol were present in the meadowsweet extract (in-house HPLC-UV/visible method). All five extracts were non-irritant to the skin (patch test of

48 hours with 10 volunteers) and eyes (negligible cytotoxicity in ocular tolerance testing by neutral red release assay). Extracts of cloudberry, heather, and juniper were free of common allergens (22 allergens were tested for, in according with EC directives No 1223/2009; 2003/15/EC). Traces of allergens were found in the extracts of meadowsweet and roseroot. The amounts were, however, so small that they do not meet the thresholds of labelling requirements if the extracts are used in the final product in a concentration of $\leq 35\%$.

Anti-elastase activity of the water-soluble fractions of heather and cloudberry seeds

The activity of the hydrophilic fractions of heather and cloudberry seeds was studied in greater depth. The objective was to find out whether the water-soluble fractions of these plants affect the activity of the elastase enzyme and whether they have effects on inflammation. The studies were carried out at the Faculty of Pharmacy at the University of Pavia, in Italy.

Elastin is a skin protein that makes up the connective tissue, together with collagen and glycosaminoglycan, and it is responsible for the characteristic property of skin elasticity. The protein's fibres are arranged to form a network immersed in a gel matrix consisting of water, glycosaminoglycan, and fibroblasts (which are important for the synthesis of the other compounds). The presence of the network gives the tissue physical properties such as elasticity and firmness. The elasticity of the skin decreases with time for many reasons, the first cause being natural ageing. At about 40 years-of-age, the fibres suffer elongation and their immediate tendency

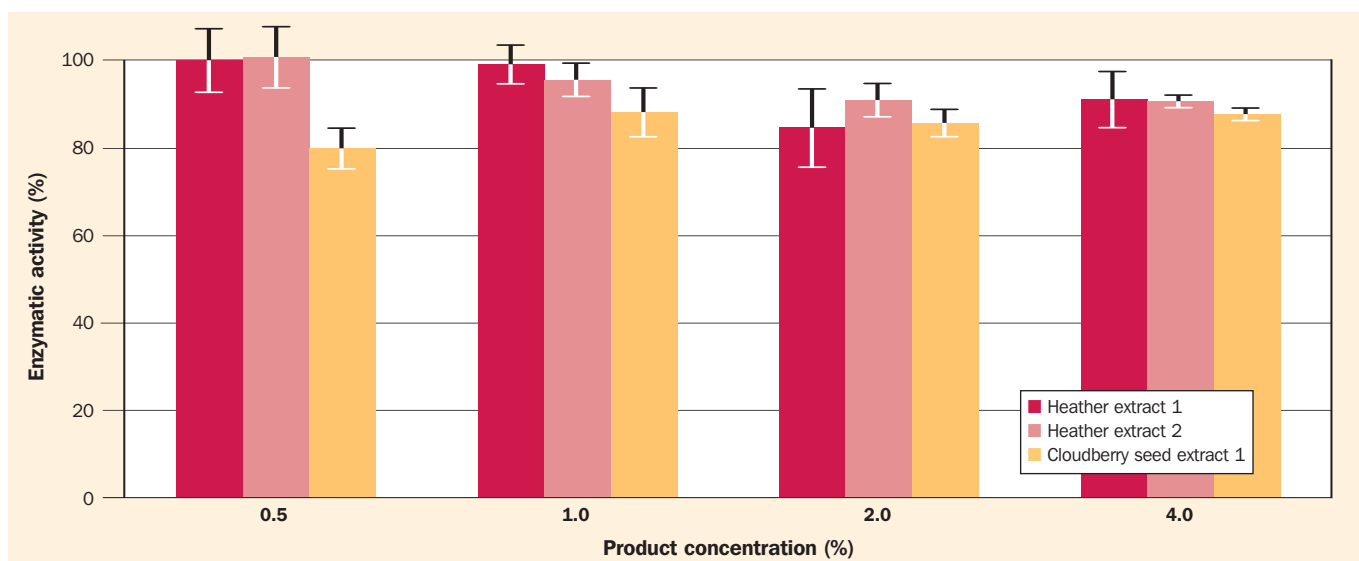


Figure 2: Effects of the hydrophilic extracts of heather and cloudberry seeds on the activity of porcine pancreatic elastase. Results are expressed as a mean of the recovered activity percentage with respect to elastase activity in the same solvent (solvent effect eliminated). The results suggest anti-elastase activity by heather and cloudberry seed extracts.

to return to their initial position decreases; moreover, the synthesis of the compounds and their turnover become very slow and the fibres grow more irregular and granular. Also the skin's enzymes, mainly elastase and collagenase, degrade the tissue's network. Other causes, including solar radiation, air pollution, rapid change in body weight, and skin disorders, are among the 'extrinsic agents'. Clearly, therefore, the problem of loss of elasticity is caused by agents of many types. For that reason, many ways to prevent this process have been explored, including reduction of free radicals, use of anti-elastase preparations, stimulation of fibroblast metabolism, and regulation of the dermal structure. The effects of skin enzymes can be reduced by means of an anti-elastase preparation: elastase is a serine-protease that is stored inactive in the pancreas, secreted in the intestine, and activated by another enzyme called trypsin. It is responsible for the fibre degradation.

Effects of hydrophilic extracts of heather and cloudberry seeds on porcine pancreatic elastase were investigated *in vitro*. The study was carried out using butylene glycol-water (2:1) extracts of heather flowers (heather samples from two separate sources) and cloudberry seeds (CO₂-extraction residue). Elastin activity was analysed spectrophotometrically by the method of Bieth, using Succ-Ala-Ala-Ala-para-nitroaniline (p-NA) as the enzymatic reaction substrate and monitoring the



Cloudberry (*Rubus chamaemorus*).

release of p-NA at 410 nm at 25 °C.¹⁹⁻²¹ The effects of the extracts were investigated at five concentrations: 0.5%, 1%, 2%, 4% and 8%. The effect of the solvent vehicle was analysed as well. All analyses were carried out in triplicate.

At concentrations of 8% and 16% the solvent (butylene glycol-water) had an inhibitory effect against elastase, the elastase activity levels being 75.9% and 69.6% of positive control, respectively. At concentration of 4% the effect of the solvent was minute, with the elastase activity being 97.2% of the control. Accordingly, at concentrations of ≤4% potential effects of the products on elastase would be expected to be derived from the active compounds of the plants, rather than from the solvent.

Both of the heather extracts and the cloudberry seed extract showed an inhibitory effect on elastase activity after elimination of the solvent effect (Fig. 2). The greatest anti-elastase activity was observed for cloudberry seed extract at a concentration of 0.5% (elastase enzyme activity: 80.3% of the control). For heather, the greatest anti-elastase effect was observed for extract 1 at 2% concentration (elastase enzyme activity: 85.1%). The results show anti-elastase activity by the hydrophilic fractions of heather flowers and cloudberry seeds in low concentrations.

Previously, ellagic acid and tannic acid were shown to protect dermal elastin from exogenous and endogenous enzymatic degradation by porcine pancreatic elastase, human leukocyte elastase, papain, and the UV-inducible matrix metalloproteinase-2.²² Both compounds were found to bind to pure elastin, which likely caused the protective effect. Procyanidins (condensed tannins) were found to inhibit the activity of porcine pancreatic elastase by interacting with the active site of the enzyme.²³ It is likely that the ellagic acid of cloudberry and the procyanidins of heather contributed to the effects observed in this study.

Anti-inflammatory activity of the water-soluble fractions of heather flowers

Inflammation is known to be a pivotal mechanism in the ageing of the human body, including the skin. Chronic self-

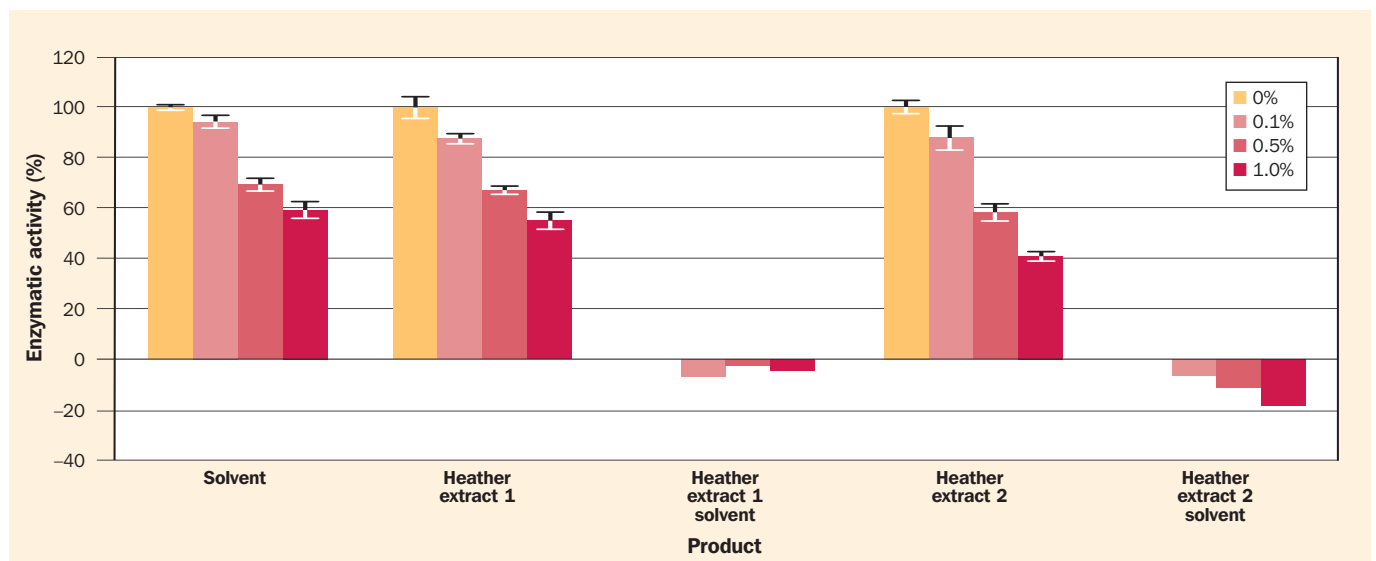


Figure 3: Effects of the hydrophilic extracts of heather on the production of TNF-α by LPS-stimulated macrophage cells at different concentrations of the products and the solvent (0%-1%). The concentration of TNF-α in the absence of added solvent of extract is normalised to 100%. The results suggest anti-inflammatory activity of heather extracts.



Juniper (*Juniperus communis*).



Meadowsweet (*Filipendula ulmaria*).

maintaining low-grade inflammation produces highly reactive oxygen species, which provoke the ageing process and skin changes associated with it.²⁴

The anti-inflammatory potential of the hydrophilic extracts of heather flowers and cloudberry seeds was analysed *in vitro* with the same extracts as for the anti-elastase study (see the previous subsection). An assay based on analysis of production of lipopolysaccharide (LPS)-induced tumour necrosis factor- α (TNF- α) by RAW264 macrophage cell line was used.^{25,26} The effect of extracts at four concentrations (0.1%, 0.5%, 1%, and 5%) was investigated. Control samples containing identical volumes of the vehicle were analysed for investigation of the solvents' effect. All of the analyses were performed in triplicate.

In a 5% concentration the solvents alone and the extracts were found to be cytotoxic for the macrophage cells. No cytotoxicity was observed in lower concentrations. Neither the extracts nor the solvents induced significant production of TNF- α in the unstimulated cells, suggesting that they do not perform pro-inflammatory activity. When the solvent effect was reduced, heather extracts in concentrations of 0.1% to 1.0% had a reducing effect on the production of TNF- α by the LPS-stimulated macrophage cells (Fig. 3). For cloudberry seed extract, no inhibitory effect on the production of TNF- α was observed. The results suggest anti-inflammatory potential by the water-soluble fractions of heather.

Conclusion

Hydrophilic fractions of Arctic heather, cloudberry, roseroot, meadowsweet and juniper are rich in bioactive compounds with antioxidant activity and a long history of use in the Northern naturopathy,

supported by observations of modern scientific research. The water-soluble extracts of the Arctic plants are safe and non-irritant ingredients for cosmetic applications. The anti-elastase activity of the hydrophilic parts of heather and cloudberry seeds and the anti-inflammation activity of heather further support their suitability for anti-ageing and soothing skin care applications



References

- Albert A, Sareedenchai V, Heller W, Seidlitz HK, Zidorn C. Temperature is the key to altitudinal variation of phenolics in *Arnica montana* L. cv. ARBO. *Oecologia* 2009; **160** (1): 1-8.
- Neugart S, Kläring H-P, Zietz M et al. The effect of temperature and radiation on flavonol aglycones and flavonol glycosides of kale (*Brassica oleracea* var. *sabelllica*). *Food Chemistry* 2012; **133** (4): 1456-65.
- Bilger W, Rolland M, Nybakken L. UV screening in higher plants induced by low temperature in the absence of UV-B radiation. *Photochem Photobiol Sci* 2007; **6** (2): 190-5.
- Monschein M, Neira JI, Kunert O, Bucar F. Phytochemistry of heather (*Calluna vulgaris* (L.) Hull) and its altitudinal alteration. *Phytochem Rev* 2010; **9** (2): 205-15.
- Simon A, Najid A, Chulia AJ, Delage C, Rigaud M. Inhibition of lipoxygenase activity and HL60 leukemic cell proliferation by ursolic acid isolated from heather flowers (*Calluna vulgaris*). *Biochim Biophys Acta* 1992; **1125** (1): 68-72.
- Määttä-Riihinen KR, Kamal-Eldin A, Törrönen AR. Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (family Rosaceae). *J Agric Food Chem* 2004; **52** (20): 6178-87.
- Häkkinen SH, Kärenlampi SO, Mykkänen HM, Törrönen AR. Influence of domestic processing and storage on flavonol contents in berries. *J Agric Food Chem* 2000; **48** (7): 2960-5.
- Puupponen-Pimiä R, Nohynek L, Meier C et al. Antimicrobial properties of phenolic compounds from berries. *J Appl Microbiol* 2001; **90** (4): 494-507.
- Päivärinta E, Pajari AM, Törrönen R, Mutanen M. Ellagic acid and natural sources of ellagitannins as possible chemopreventive agents against intestinal tumorigenesis in the Min mouse. *Nutr Cancer* 2006; **54** (1): 79-83.
- Khanum F, Bawa A, Singh B. *Rhodiola rosea*: A versatile adaptogen. *Comprehensive Reviews in Food Science and Food Safety* 2005; **4** (3): 55-62.
- Yildirim A, Turker AU. *In vitro* adventitious shoot regeneration of the medicinal plant meadowsweet (*Filipendula ulmaria* (L.) Maxim). *In Vitro Cell Dev Biol - Plant* 2009; **45** (2): 135-44.
- Rauha JP, Remes S, Heinonen M et al. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int J Food Microbiol* 2000; **56** (1): 3-12.
- Halkes SBA, Beukelman CJ, Kroes BH, van den Berg AJJ, Labadie RP, van Dijk H. *In vitro* immunomodulatory activity of *Filipendula ulmaria*. *Phytotherapy Research* 1997; **11** (7): 518-20.
- Piippo S. *Luonnon lääkeyrtit 3* (in Finnish). Hämeenlinna, Finland: Kustannusosakeyhtiö Tammi Oy, 2003.
- Dorman HJD, Hiltunen R. Antioxidant and pro-oxidant *in vitro* evaluation of water-soluble food-related botanical extracts. *Food Chemistry* 2011; **129** (4): 1612-8.
- Modnicki D, Labeledzka J. Estimation of the total phenolic compounds in juniper sprouts (*Juniperus communis* L., *cupressaceae*) from different places at the Kujawsko-Pomorskie province. *Herba Polonica* 2009; **55** (3): 127-31.
- Nakanishi T, Iida N, Inatomi Y et al. Neolignan and flavonoid glycosides in *Juniperus communis* var. *depressa*. *Phytochemistry* 2004; **65** (2): 207-13.
- Ahotupa M, Mäntylä E, Kangas L. Antioxidant



Roseroot (*Rhodiola rosea*).

properties of the triphenylethylene antiestrogen drug toremifene. *Naunyn Schmiedebergs Arch Pharmacol* 1997; **356** (3): 297-302.

- 19 Bieth J, Spiess B, Wermuth CG. The synthesis and analytical use of a highly sensitive and convenient substrate of elastase. *Biochem Med* 1974; **11** (4): 350-7.
- 20 Bieth J, Meyer JF. A colorimetric method for measuring the esterolytic activity of elastase. *Anal Biochem* 1973; **51** (1): 121-6.
- 21 Bieth J, Wermuth CG. The action of elastase on p-nitroanilide substrates. *Biochem Biophys Res Commun* 1973; **53** (2): 383-90.
- 22 Jimenez F, Mitts TF, Liu K, Wang Y, Hinek A. Ellagic and tannic acids protect newly synthesized elastic fibers from premature enzymatic degradation in dermal fibroblast cultures. *J Invest Dermatol* 2006; **126** (6): 1272-80.
- 23 Bras NF, Goncalves R, Mateus N, Fernandes PA, Ramos MJ, de Freitas V. Inhibition of pancreatic elastase by polyphenolic compounds. *J Agric Food Chem* 2010; **58** (19): 10668-76.
- 24 Giacomoni PU. Ageing, science and the cosmetics industry. The micro-inflammatory model serves as a basis for developing effective anti-ageing products for the skin. *EMBO Rep* 2005; **6** (Spec): S45-8.
- 25 Liu FQ, Liu Y, Lui VC, Lamb JR, Tam PK, Chen Y. Hypoxia modulates lipopolysaccharide induced TNF-alpha expression in murine macrophages. *Exp Cell Res* 2008; **314** (6): 1327-36.
- 26 Nagahira A, Nagahira K, Murafuji H et al. Identification of a novel inhibitor of LPS-induced TNF-alpha production with antiproliferative activity in monocyte/macrophages. *Biochem Biophys Res Commun* 2001; **281** (4): 1030-6.

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