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SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

## List of references supporting the assessment of *Aesculus hippocastanum* L., cortex

Draft – Revision 1

**The European Medicines Agency acknowledges that copies of the underlying works used to produce this monograph were provided for research only with exclusion of any commercial purpose.**

- Ambrogini P, Cuppini R, Bruno C and Bombardelli E. Effects of proanthocyanidin on normal and reinnervated rat muscle. *J. Biol. Res. – Bol. Soc. It. Biol. Sper.* 1995, 71(7-8): 227-234
- Appeldoorn MA, Vincken J-P, Gruppen H, Hollman PCH. Procyanidin dimers A1, A2, and B2 are absorbed without conjugation or methylation from the small intestine of rats. *J. Nutr.* 2009; 139: 1469–1473
- Bate-Smith EC. Phytochemistry of proanthocyanidins. *Phytochemistry* 1975, 14 (4): 1107-1113 (abstr.)
- Bézanger-Beauquesne L, Pinkas M, Torck M. Les plantes dans la thérapeutique moderne, 1975, 55-58
- Bézanger-Beauquesne L, Pinkas M, Torck M and Trotin F. *Aesculus hippocastanum* L. Plantes médicinales des régions tempérées. Maloine, 1980, 2<sup>e</sup> ed., 164-165
- Blaschek W. Wichtl – Teedrogen und Phytopharmaka 6 Aufl. Wissenschaftliche Verlagsgesellschaft Stuttgart 2016, 332-334
- Bombardelli E, Morazzoni P and Griffini A. *Aesculus hippocastanum* L. *Fitoterapia* 1996, 67:483–511
- Boullard B. Plantes médicinales du monde. Réalités et croyances. Paris, éditions ESTEM, 2001
- Bzeouich JM, Mustapha N, Maatouk M, Ghedira K, Ghoul M, Chekir-Ghedira L. Genotoxic and anti-genotoxic effects of esculin and its oligomer fractions against mitomycin C - induced DNA damages in mice. *Regulatory Toxicology and Pharmacology* 2016; 82: 48-52
- Calapai G, Miroddi M, Minciullo PL, Caputi AP, Gangemi S, Schmidt RJ. Contact dermatitis as an adverse reaction to some topically used European herbal medicinal products – part 1: *Achillea millefolium*-*Curcuma longa*. *Contact Dermatitis* 2014, 71(1): 1-12
- Cals-Grierson M-M. Modulation of activity of the adipocyte aquaglyceroporin channel by plant extracts. *International Journal of Cosmetic Science* 2007, 29:7-14
- Cerda B, Ceron JJ, Tomas-Barberan FA, Espin JC. Repeated oral administration of high doses of the

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- pomegranate ellagitannin punicalagin to rats for 37 days is not toxic. *J. Agric. Food Chem.* 2003, 51: 3493-3501
- Chen S-C and Chung K-T. Mutagenicity and antimutagenicity studies of tannic acid and its related compounds. *Food and Chemical Toxicology* 2000, 38: 1 - 5
- Comaish J.S., Kersey P.J. Contact dermatitis to extract of horse chestnut. Short communications, 1980
- Ding Y, Zhang B, Zhou KY, Chen MC, Wanga MM, Jia YY, Song Y, Li YW, Wen AD. Dietary ellagic acid improves oxidant-induced endothelial dysfunction and atherosclerosis: Role of Nrf2 activation. *International Journal of Cardiology* 2014, 175: 508–514
- ECHA report on Aesculin: <https://echa.europa.eu/pl/substance-information/-/substanceinfo/100.007.744>
- Engelmann A, Hübner F, Rzeppa S, Hump H-U. Intestinal metabolism of two A-type procyanidins using the pig cecum model: detailed structure elucidation of unknown catabolites with Fourier Transform Mass Spectrometry (FTMS). *J. Agric. Food. Chem.* 2021; 60: 749-757
- Fleurentin J. Marronnier d'Inde (*Aesculus hippocastanum* L.). Plantes médicinales: traditions et thérapeutiques. Ed. Ouest-France 2008, 24-25
- FoodDB, Showing Compound Proanthocyanidin A2 (FDB016778), <https://foodb.ca/compounds/FDB016778>, access 11.2022
- Fournier P. *Marronnier d'Inde – Aesculus hippocastanum* L. Le livre des plantes médicinales et vénéneuses de France Paris: P. Lechevalier : 1948, T.2, 475-479
- Gagić T, Knez Ž, Škerget M. Subcritical water extraction of horse chestnut (*Aesculus hippocastanum*) tree parts. *J. Serb. Chem. Soc.* 2021; 86(6): 603-613
- García-Villalba R, Espín J C, Tomás-Barberán FA. Chromatographic and spectroscopic characterization of urolithins for their determination in biological samples after the intake of foods containing ellagitannins and ellagic acid. *Journal of Chromatography A* 2016, 1428: 162–175
- Garnier G, Bézanger-Beauquesne L et Debraux G. *Hippocastanacées - Aesculus hippocastanum* L. *Marronnier d'Inde. Ressources médicinales de la flore française.* Paris : Vigot : 1961 T.1, 668-674
- Girre L. Marronnier d'Inde. Traditions et propriétés des plantes médicinales: histoire de la pharmacopée. Privat, 1997, 191
- Gonzalez-Barrio R, Truchado P, Ito H, Espín J C, Tomas-Barberan F A. UV and MS identification of urolithins and nasutins, the bioavailable metabolites of ellagitannins and ellagic acid in different mammals. *J. Agric. Food Chem.* 2011, 59:1152-1162
- Heck AM, DeWitt BA, Lukes AL. Potential interactions between alternative therapies and warfarin. *Am J Health-Syst Pharm.* 2000, 57:1221-1230
- Hughes JDM, Pratt MD. Allergic Contact Dermatitis and Autoeczematization to Proctosedyl Cream and Proctomyxin Cream. *Case Reports in Dermatology* 2018; 10:238-246 (publ. online: October 24, 2018 by S. Karger AG Basel; DOI: 10.1159/000486475, access 14.06.2022)
- Kandi S & Charles AT. Measurement, correlation, and thermodynamic properties for solubilities of bioactive compound (–)-epicatechin in different pure solvents at 298.15 K to 338.15 K. *Journal of Molecular Liquids* 2018, 264: 269–274
- Kaneko T, Tahara S, Takabayachi F. Suppression of lipid hydroxide-induced oxidative damage to cellular DNA by Esculetin. *Biol. Pharm. Bull.* 2003, 26(6):840-844

Kaneko T, Tahara S, Takabayashi F, Harada N. Inhibitory effect of esculin on oxidative DNA damage and carcinogenesis induced by N-nitrosobis(2-oxopropyl)amine in hamster pancreas. *BioFactors* 2004; 22: 83-85

Kaneko T, Tahara S, Takabayashi F. Inhibitory effect of natural coumarin compounds, esculin and esculin, on oxidative DNA damage and formation of aberrant crypt foci and tumors induced by 1,2-dimethylhydrazine in rat colons. *Biol Pharm. Bull.* 2007, 30(11): 2052-2057

Khar'kov Y. K, Mbarga MJA, Martynenkova VA, Podoprigora IV, Volina GE, Madina MA, Ait AA. Assessment of antimicrobial activity of ethanolic and aqueous extracts of *Aesculus hippocastanum* L. (horse chestnut) bark against bacteria isolated from urine of patients diagnosed positive to urinary tract infections. *Front. Biosci. (Schol Ed)* 2022; 14(2): 11 available online <https://doi.org/10.31083/j.fbs1402011>, access 09.09.2022

Kong L, Zhou J, Wen Y, Li J, Cheng CHK. Aesculin possesses potent hypouricemic action in rodents but is devoid of xanthine oxidase/dehydrogenase inhibitory activity. *Planta Medica* 2002, 68:175-178 (Letter to editor)

Kostova IN, Iossifova T. Chemical components of *Fraxinus ornus* bark - Structure and biological activity. *Studies in Natural Products Chemistry* 2002, 26: 313-349 available via Google Book 27.09.2022

Larrosa M, Garcia-Conesa T, Espin JC, Tomas-Barberan FA. Ellagitannines, ellagic acid and vascular health. *Molecular Aspects in Medicine* 2010; 31:513-539

Leclerc H. Précis de phytothérapie, 5è ed, 1976, 100-101

Lee A-Y. Allergic contact dermatitis from dibucaine in Proctosedyl ointment without cross-sensitivity. *Contact Dermatitis (Short Communications)* 1998; 39: 261

Lee W-J, Ou H-C, Hsu W-Ch, Chou M-M, Tseng J-J, Hsu S-L, Tsai K-L and Sheu W H-H. Ellagic acid inhibits oxidized LDL-mediated LOX-1 expression, ROS generation, and inflammation in human endothelial cells. *Journal of Vascular Surgery* 2010, 52(5): 1290-1300

Les médicaments à base the plants. AFSSAPS - Agence Francaise de Securite Sanitaire des Produits de Sante. *Cahiers de l'Agence* No 3, 1998.

Leung AY and Foster S. Horsechestnut. *Encyclopedia of common natural ingredients used in food, drugs and cosmetics.* John Wiley 1996, 304-306

Li C-X, Li J-C, Lai J, Liu Y. The pharmacological and pharmacokinetic properties of esculin: A comprehensive review. *Phytotherapy Research* 2022, 36: 2434-2448

Li W, Wang Y, Wang X, Zhang H, He Z, Zhi W, Liu F, Niu X. astroprotective effect of esculin on ethanol-induced gastric lesion in mice. *Fundamental and Clinical Pharmacology* 2016, Ed. Online, doi: 10.1111/fcp.12255 access 14.06.2022

Matysik G, Głowniak K, Soczewiński E, Garbacka M. Chromatography of esculin from stems and bark of *Aesculus hippocastanum* L. for consecutive vegetative periods. *Chromatographia* 1994, 38 (11/12): 766-769

Médicaments a base de plants, avis aux fabricants concernant les demandes d'autorisation de mise sur le marché. Ministère des Affaires Sociales et de la Solidarité 1990, *Bulletin Officiel* No 90/22 bis

Moorthy M, Khoo JJ, Palanisamy UD. Acute oral toxicity of the ellagitannin geraniin and a geraniin-enriched extract from *Nephelium lappaceum* L rind in Sprague Dawley rats. *Heliyon* 2019, 5, e02333 available at: <https://doi.org/10.1016/j.heliyon.2019.e02333>, access 09.09.2022

- Naaz F, Abdin MZ, Javed S. Protective effect of esculin against prooxidant aflatoxin B1-induced nephrotoxicity in mice. *Mycotoxin Res.* 2014; 30: 25-32
- Nagy M. Human poisoning from horse chestnuts. *JAMA*, 1973, 226 (2 Oct 8) 213
- Niu X, Wang Y, Li W, Zhang H, Wang X, Mu Q, He Z, Yao H. Esculin exhibited anti-inflammatory activities in vivo and regulated TNF- $\alpha$  and IL-6 production in LPS-stimulated mouse peritoneal macrophages in vitro through MAPK pathway. *International Immunopharmacology* 2015, 29: 779-786
- Ollier C. Marronnier d'Inde. Conseil en phytothérapie. Groupe Liaisons santé, 2000,86
- Owczarek A, Kłys A, Olszewska MA. A validated <sup>1</sup>H qNMR method for direct and simultaneous quantification of esculin, fraxin and (-)-epicatechin in *Hippocastani cortex*. *Talanta* 2019, 192: 263-269
- Owczarek A, Olszewska AM. Development and validation of UHPLC-PDA method for simultaneous determination of bioactive polyphenols of horse-chestnut bark using numerical optimization with MS Excel Solver. *Journal of Pharmaceutical and Biomedical Analysis* 2020; 190: 113544 publication online (<https://doi.org/10.1016/j.jpba.2020.113544> access 14.06.2022)
- Papoutsis Z, Kassi E, Chinou I, Halabalaki M, Skaltsounis LA and Moutsatsou P. Walnut extract (*Juglans regia* L.) and its component ellagic acid exhibit anti-inflammatory activity in human aorta endothelial cells and osteoblastic activity in the cell line KS483. *British Journal of Nutrition* 2008, 99: 715-722
- Paris RR, Moyses H. Abrégé de matière médicale, Tome 1, 1981; 147-150
- Pencheva I, Kostova I, Konstantinov S, Naidenova E, Karaivanova M, Manolov I. Cardioprotective efficacy of new esculin metal complexes. *Acta Pharm. (Zagreb)* 1998, 48: 127-131
- Piowarski JP, Kiss AK, Kozłowska-Wojciechowska M. Anti-hyaluronidase and anti-elastase activity screening of tannin-rich plant materials used in traditional Polish medicine for external treatment of diseases with inflammatory background. *Journal of Ethnopharmacology* 2011, 137: 937-941
- Piowarski JP, Granica S, Stefańska J, Kiss AK. Differences in metabolism of ellagitannins by human gut microbiota ex vivo cultures. *J. Nat. Prod.* 2016, 79(12): 3022-3030
- Quercetin on <https://pubchem.ncbi.nlm.nih.gov/compound/Quercetin>
- Quercitrin on <https://pubchem.ncbi.nlm.nih.gov/compound/Quercitrin#section=3D-Conformer>
- Rehman SU, Kim IS, Kang KS, Yoo HH. HPLC Determination of Esculin and Esculetin in Rat Plasma for Pharmacokinetic Studies. *Journal of Chromatographic Science* 2015, 53: 1322-1327
- Rios ERV, Rocha NFM, Venancio ET, Moura BA, Feitosa ML, Cerqueira GS, Soares PMG, Woods DJ, de Sousa FCF, Leal LKAM, de França Fonteles MM. Mechanisms involved in the gastroprotective activity of esculin on acute gastric lesions in mice. *Chemico-Biological Interactions* 2010; 188: 246-254
- Rozentsvit A, Vinokura K, Samuel S, Li Y, Martin Gerdes A, Carrillo-Sepulveda M A. Ellagic acid reduces high glucose-induced vascular oxidative stress through ERK1/2/NOX4 signaling pathway. *Cell Physiol Biochem* 2017;44:1174-1187
- Schötz K, Erdelmeier C, Koch E. Principal component analysis of metabolites in urine from rats treated with procyanidinA2 and two oligomeric procyanidin fractions from cranberry. *Planta Med* 2015; 81(16) : PW\_170 (conference poster)
- Sekiya K, Okuda H, Arichi S. Selective inhibition of platelet lipooxygenase by esculetin. *Biochimica et Biophysica Acta* 1982, 713:68-72

Senatore F, Mścisz A, Mrugasiewicz K and Gorecki P. Steroidal constituents and anti-inflammatory activity of the horse chestnut (*Aesculus hippocastanum* L.) bark. *Boll. Soc. It. Biol Sper.* 1989, LXV(2), 137-141

Stanić G, Jurišić B, Brkić D. HPLC analysis of esculin and fraxin in horse-chestnut bark (*Aesculus hippocastanum* L.). *Croatica Chemica Acta* 1999; 72(4): 827-834.

The Merck Index, Tenth Edition, Ed. Martha Windholz. Merck & Co. Inc. Rahway, N.J., USA; p. 534-535

Tianzhu Z & Shumin W. Esculin inhibits the inflammation of LPS-induced acute lung injury in mice via regulation of TLR/NF- $\kappa$ B pathways. *Inflammation* 2015, 38(4): 1529-1536

Tubaro A, Del Negro P, Ragazzi E, Zampiron S, Della Loggia R. Anti-inflammatory and peripheral analgesic activity of esculin in vivo. *Pharmacological Research Communications* 1988, 20(SV):83-85

Uwaifo AO. The mutagenicities of seven coumarin derivatives and a furan derivative (nimbolide) isolated three medicinal plants. *Journal of Toxicology and Environmental Health* 1984, 13:521-530

Varier KM, Thangarayan S, Chinnasamy A, Balakrishnan G, Paulose R. Comparative analysis of potentiality of esculin and hinokitol (B-thujaplicin) as anti-parkinsonism drugs: a pilot in silico study. *International Journal of Pharmacy and Pharmaceutical Sciences* 2017, 9(1): 108-115

Wang YW, Zhao M, Ou Y, Zeng B, Lou X, Wang M. Metabolic profile of esculin in rats by ultra high performance liquid chromatography combined with Fourier transform ion cyclotron resonance mass spectrometry. *Journal of Chromatography B* 2016, 1020:120-128

Yang S, Zhang Y, Li W, You B, Yu J, Huang X, Yang R. Gut microbiota composition affects procyanidin A2-attenuated atherosclerosis in ApoE<sup>-/-</sup> mice by modulating the bioavailability of its microbial metabolites. *J. Agric. Food Chem.* 2021; 69: 6989-6999

Yang X, Tomás-Barberán F A. Tea is a significant dietary source of ellagitannins and ellagic acid. *J. Agric. Food Chem.* 2019; 67: 5394-5404