

www.cropwatch.org



THE FIRST TRULY INDEPENDENT WATCHDOG FOR THOSE
WORKING WITH NATURAL AROMATIC MATERIALS

E: info@cropwatch.org T: ++44 (0)7771 872 521

Notes on Minor Oils.

Ground Ivy (*Glechoma hederacea* L.) Essential Oil.

Copyright ©Tony Burfield July 2010 v 1.02.

Ground Ivy (syn. Alehoof or Creeping Charlie): *Glechoma hederacea* L.

syn. *Nepeta glechoma*. Benth.

syn. *Nepeta hederacea* (L.) Trevir.

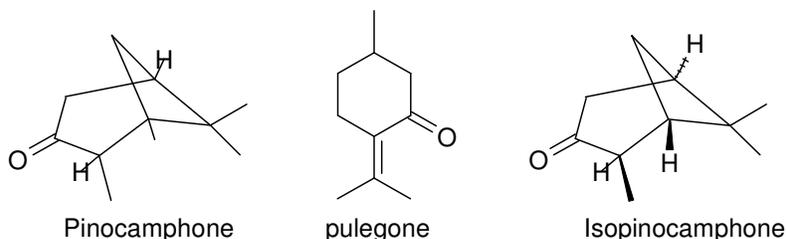
Glechoma is a genus of up to 47 species. *Glechoma hederacea* L., commonly known as Ground Ivy, is a creeping perennial herb which grows from 20-50 cm. in height, spreading by stolons or from seed, and preferring the damp shady areas of woods and waste ground. The herb is native to Europe & S.E. Asia, and has been introduced into US & New Zealand, being characterized by round, fan-shaped leaves with toothed edges, and small funnel-shaped bluish to purple flowers which appear in the early Spring. The aerial parts of the herb were commonly used as an ingredient for ale-making in Europe (for flavour, brew clarification, and to extend the keeping qualities) prior to the introduction of hops in the 15th Century (hence the name 'alehoof' together with the fact that the strongly odoured essential oil is released from the bruised/trampled herb by the hooves of wandering farmstock). This author described the odour of the crushed flowering herb as minty, bitter herbaceous, with strong distinct grapefruit-zest colouration, green, with dominant germacrene-like dank notes (Burfield 2000).

Various subspecies are also described: the hairier *G. hederacea* ssp. *hirsuta* (Waldst & Kit) F. Herman (aka *G. hirsuta* Delias 1983) with its pale blue corolla, is found in S. Europe, whilst *G. hederacea* ssp. *grandis* (A. Gray) Hara is distinguished in E. Asia. North American subspecies include *G. hederacea* ssp. *micrantha* Moricand (for further detail see Hutchings & Price 1999).

Uses & Safety.

Essential oil from the aerial parts of the herb is not commercially available. The leaves of the herb have historically been used for salads, beverage and soup-making in Europe, and the whole herb and decoctions has various uses in folk-medicine (Mills & Bone 2000), including for the treatment of upper respiratory congestion and catarrh, and as a tonic. The safety of the herb or its' extracts however have not been established, and the plant is reported to poison the cattle which have consumed it (MAFF 1984) – notwithstanding, the plant was made

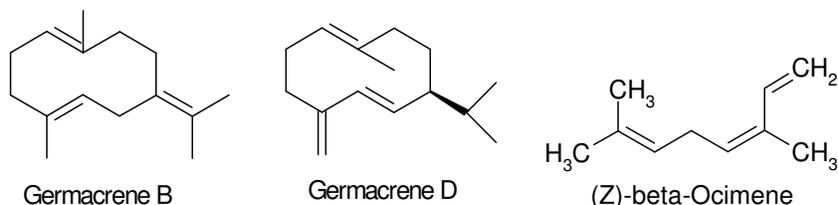
official in the British Herbal Pharmacopoeia (1990). It could be speculated that active compounds in the herb or extracts could include pulegone, pinocamphone & isopinocamphone, and (-)-pinocamphone was first confirmed in Ground Ivy (*Glechoma hederacea* L. subsp. *grandis* (A. Gray) Hara) by Hikino *et al.* (1962).



Pinocamphone has previously been linked with the neurotoxicity shown by hyssop oil from *Hyssopus officinalis* (Miller *et al.* 1980). It has been demonstrated that methanolic extracts of the aerial parts of *G. hederacea* which contain the tropane alkaloids hederacine A & B show cytotoxic effects against the colon cancer cell line (CaCo-2) (Kumarasamy *et al.* 2006). Studies by Singh *et al.* (2006) & by Wang *et al.* (2003) explore the properties of the potent insecticidal lectin Gleheda, occurring in the leaves of *G. hederacea*.

Composition.

Lawrence (1972) reported on the composition of the oil obtained in 0.01% yield from *G. hederacea* plants from Fredrickton, New Brunswick, finding the presence of germacrene D (19.4%), germacrene B (8.9%) and 1,8-cineole (6.2%), as well as (Z)- β -ocimene & β -elemene (8.9%).



Stahl & Datta (1972) report an oil obtained in 0.03-0.06% yield containing p-cymene, linalol, limonene, menthone, α -pinene, β -pinene, pinocamphone, pulegone, α -terpineol and glechomafuran. (-)-Pinocamphone was first confirmed in ground ivy (*Glechoma hederacea* L. subsp. *grandis* (A. Gray) Hara) by Hikino *et al.* (1962). More recently Mockutė & Judpentiėnė (2005) analysed a series of essential oils of *G. hederacea* from four locations in the Vilnius district of Lithuania, finding germacrene D (15.6–18.8%) to be the dominant compound in the oils. Other identified constituents included γ -elemene (9.7–16.0%), β -elemene (9.8–11.1%), iso-phytol + phytol (4.7–15.6%) and (Z)- β -ocimene (4.7–5.6%).

[N.B. All references cited above can be found in the bibliography below].

Bibliography for *Glechoma hederacea* L. Botany/ecology.

Hutchings M.J. & Price E.A.C. (1999) "Biological flora of the British Isles No. 205. *Glechoma hederacea* L. (*Nepeta glechoma* Benth., *N. hederacea* (L) Trev." *Journal of Ecology* **87**, 347-364).

Widén B. & Widén M. (2000) "Enzyme variation and inheritance in *Glechoma hederacea* (Lamiaceae), a diploidized tetraploid." *Hereditas*. **132**(3), 229-41. **Abstract.** The chromosome number of the polyploid species *Glechoma hederacea* was found to be $2n = 36$ in a sample of 93 ramets derived from 27 sites in N and C Europe. Variation in 10 enzymes was surveyed in material from S Sweden and S Czech Republic. The genetic control of variation was investigated using segregating progeny from crosses and self-fertilized heterozygous plants. The genetic analysis comprised 30 of 32 putative alleles detected in the geographical survey. Five loci (Aat-2, Tpi-1, Tpi-2, Pgd-2 and Mnr) behaved as isoloci with one copy of a locus being monomorphic for a common allele, the other di-allelic for a common allele and a variant allele. In four isoloci (Pgd-1, Pgi-2, Mdh-2 and Adh), both copies of the duplicated locus were polymorphic, with one allele common to both copies and with another allele unique for each copy except for Pgd-1 where both copies were tri-allelic. Three loci, Pgm-3, Skd-1 and Skd-2 were regarded as being non-duplicated. Segregation ratios for all enzyme loci were in close agreement with expectations based on disomic inheritance. Our data suggest that the tetraploid *G. hederacea* is a diploidized autotetraploid.

Chemistry & Composition.

Burfield T. (2000) *Natural Aromatic Materials – Odours & Origins* publ AIA Tampa (2000).

清水,唐沢,伝英,池田, 長守. (1969). 信州大学農学部紀要 6(1): 67-82(1969) "Mentha aquatica L. Studies on essential Oils of *Mentha aquatica* I-III." – see <https://soar-ir.shinshu-u.ac.jp/dspace/handle/10091/2254> **Abstract.** *M. aquatica* were imported from the following countries; Belgium, Plantentuin der Rijksuniversiteit to Gent; Belgium, Institut et Jardin de Botanique de l'Universite de Liege; France, Jardin Botanique Ville de Nantes; Italy, Istituto ed Orto Botanico dell' Univ. di Roma; Portugal Hortus Botanicus Coimbra. They were cultivated in Okayama University and Kurashiki Experimental Station for Agriculture. By analyzing the essential oils obtained from *aquatica* herbs by gas chromatography (column, SAIB 20% on Celite 545; column temp., 155°C; carrier gas, hydrogen; flow rate, 65 ml/min.), we divided *aquatica* mint into following three chemical strains. Linalool strain seems to be very similar to *Mentha citrata* from chemical and morphological point of view. Menthofuran in the second oil was identified by infrared spectrum, thin-layer chromatography (a purple red spot by vanillin sulfuric acid reagent) and Fluckiger reagent test. It will be surely identical with American *aquatica* reported by Reitsema. The essential oil obtained from third strain showed strong absorption at 1700 cm^{-1} and gave semicarbazone of m. p. 232°C in the yield of 30%. The oil regenerated from this semicarbazone by treatment with acid media showed two peaks in the gas chromatogram, the later of which was determined to coincide with the main peak

of aquatica original oil; retention time relative to (-) menthol was 0.88. This indicates that aquatica ketone is sensitive to acid and isomerizes to a stable isomer. The original ketone was isolated purely by silica column chromatography (first eluting solvent, petroleum ether, then followed by ether): b.p. 81°C/5mm., $[\alpha]^{15}_D$ -7.3°. By the reduction with lithium aluminum hydride, a borneol-like smelling crystal (m. p. 42~45°C) was obtained. From the elementary analysis of its 3, 5-dinitrobenzoate (Found: C, 58.70; H, 5.83; Calcd. for $C_{17}H_{20}N_2O_6$ =dinitrobenzoate of $C_{10}H_{18}O$: C, 58.61; H, 5.79%), the molecular formula for original ketone was established to be $C_{10}H_{16}O$. It showed no double bond in the infrared spectrum and gave pinonic acid by potassium permanganate oxidation (identified as semicarbazone of m. p. 204°C). From these results aquatica ketone seemed to be a bicyclic ketone such as pinocamphone or isopinocamphone. NMR spectrum of this ketone in deuterio-chloroform showed the presence of gem-dimethyl (singlets, 9.12 8.68 τ) and one methyl (doublet, 8.85, 8.73 τ , J=7 c. p. s.), which support isopinocamphone skeleton. Then, (-)-isopinocamphone was prepared by hydroboration of (-)-pinene, $[\alpha]^{15}_D$ -35° according to Brown's method; b. p. 91~105°C/20mm., after silica chromatography, $[\alpha]^{15}_D$ -8.8°. Infrared spectrum absorption and retention time of (-)-isopinocamphone were found to be completely identical with those of aquatica ketone. This is the first paper which shows the presence of (-)-isopinocamphone in the essential oil of the Genus *Mentha*, while (-)-pinocamphone was found in the oil of *Hyssopus officinalis* L. and ground ivy (*Glechoma hederacea* L. subsp. *grandis* (A. Gray) Hara). Then, oils of various interspecific hybrid clones between aquatica and several mint species such as *rotundifolia* (2n=24), *spicata* (48), *japonica* (48), *arvensis* var. *agrestis* (72) and *arvensis* var. *piperascens* (96) were analyzed by gas chromatography. The results obtained are shown in Table 4.

Henry D.Y., Gueritte-Voegelein F., Insel P.A., Ferry N., Bouguet J., Potier P., Sevenet T. & Hanoune J. (1987) "Isolation and characterization of 9-hydroxy-10-trans,12-cis-octadecadienoic acid, a novel regulator of platelet adenylate cyclase from *Glechoma hederacea* L. Labiatae." *Eur J Biochem.* **170**(1-2), 389-94. [Abstract](#). We have identified and characterized a fatty acid, (9S,10E,12Z)-9-hydroxy-10,12-octadecadienoic acid (9-HODE) as a regulator of adenylate cyclase activity of human platelet membranes. This fatty acid was isolated from a methanolic extract of the plant *Glechoma hederacea* L. Labiatae (commonly known as 'lierre terrestre', 'ground ivy' or 'creeping Charlie'); it was identified by nuclear magnetic resonance and mass spectroscopy. This compound increased basal adenylate cyclase activity in platelet membranes about threefold and had an EC50 of 10-20 microM. This increase in adenylate cyclase activity occurred without a temporal lag, was reversible, and represented an increase in Vmax without a substantial change in Km for ATP, Mg2+ or Mn2+. In addition, 9-HODE additively or synergistically increased platelet adenylate cyclase activity in response to guanosine 5'-[beta,gamma-imido]triphosphate and forskolin, but the fatty acid failed to alter inhibition of adenylate cyclase activity mediated by epinephrine (alpha 2-adrenergic receptor). Studies of the interaction of 9-HODE with activation of platelet adenylate cyclase activity mediated by prostaglandin E1

(PGE1) and prostaglandin D2 (PGD2) indicated that this fatty acid produced a parallel shift in the concentration/response curve of PGE1 and PGD2 without altering maximal response, which was substantially greater than that observed with 9-HODE alone. From these results, we conclude that 9-HODE appears to be a partial agonist at PGE1 and PGD2 receptors on human platelets. We believe that this is a novel example of a plant-derived fatty acid which acts on cells to regulate adenylate cyclase via prostaglandin receptors.

Hikino, Kusano & Takemoto (1962); *Proceedings of 6th Symposium of Chem. Perfumery, Terpenes and Essential oils*, p115 (1962).

Kikuchi M., Goto J., Noguchi S., Kakuda R. & Yaoita Y. (2008) "Glycosides from whole plants of *Glechoma hederacea* L." *J Nat Med.* **62**(4), 479-80. [Abstract](#). From dried whole plants of *Glechoma hederacea* L. (Labiatae), seven known glycosides were isolated and identified: (6R,7E,9R)-megastigma-4,7-dien-3-one 9-O-beta-D-glucopyranoside (1), apigenin 7-O-neohesperidoside (2), chrysoeriol 7-O-neohesperidoside (3), (+)-pinoresinol 4,4'-bis-O-beta-D-glucopyranoside (4), (+)-syringaresinol 4,4'-bis-O-beta-D-glucopyranoside (5), (+)-lariciresinol 4,4'-bis-O-beta-D-glucopyranoside (6), and (7R,8R)-threo-7,9,9'-trihydroxy-3,3'-dimethoxy-8-O-4'-neolignan 4-O-beta-D-glucopyranoside (7).

Kühn H., Wiesner R., Alder L. & Schewe T. (1989) "Occurrence of free and esterified lipoxygenase products in leaves of *Glechoma hederacea* L. and other Labiatae." *Eur J Biochem.* **186**(1-2), 155-62. [Abstract](#). Leaves of *Glechoma hederacea* L. and other Labiatae contain (9S,10E,12Z,15Z)-9-hydroxy-10,12,15-octadecatrienoic acid, (10E,12Z,15Z)-9-oxo-10,12,15-octadecatrienoic acid, (9S,10E,12Z)-9-hydroxy-10,12-octadecadienoic acid and (10E,12Z)-9-oxo-10,12-octadecadienoic acid in a ratio of 71/14/12/3 (by mass), predominantly esterified in the membrane ester lipids. The leaves contain the highest level of these products, whereas only small amounts were found in the stalk and the roots. The chemical structures of these compounds were established by ultraviolet and infrared spectroscopy, by co-chromatography with authentic standards on various types of HPLC columns including chiral-phase HPLC and gas chromatography/mass spectrometry. The stereochemical specificity indicates the enzymatic origin of the products, most probably via a lipoxygenase reaction. Freshly harvested specimens of *G. hederacea* L. contain only small amounts of hydroxy-polyenoic fatty acids. Air-drying causes a strong increase in the content of free and esterified (9S,10E,12Z,15Z)-9-hydroxy-10,12,15-octadecatrienoic acid. Up to 80% of the hydroxy fatty acids of the total lipid extracts were esterified in the cellular lipids. The data presented indicate that lipoxygenase products occur in the cellular ester lipids of *G. hederacea* L. and other Labiatae. The results are discussed in the light of a possible involvement of the lipoxygenase pathway in the natural senescence of leaves

B.M. Lawrence *et al.* (1972) "Terpenoid composition of some Canadian Labiatae" *Phytochem.* **11**, 2636-2638.

Mockutė D. & Judpėntienė A. (2005) "Chemical composition of essential oils of *Glechoma hederacea* L. growing wild in Vilnius district." *Chemija* **16**(3-4), 47-50. [Abstract](#). Wild plants of *Glechoma hederacea* L. were collected at full flowering in four localities of Vilnius district. Essential oils produced by hydrodistillation were analysed using GC and GC / MS methods. Germacrene D (15.6–18.8%) was the dominant compound in the oils. The other major constituents were γ -elemene (9.7–16.0%), β -elemene (9.8–11.1%), iso-phytol + phytol (4.7–15.6%) and (Z)- β -ocimene (4.7–5.6%). Sesquiterpene hydrocarbons comprised the largest (55.0–66.2%) part of the essential oils. Sesquiterpenoids with germacrane and elemene carbon skeletons formed about a half of the samples. Forty-two identified constituents made up 87.5–94.0% of the oils.

Radulović N., Đorđević N., Marija Marković M. & Palić R. (2010) "Volatile constituents of *Glechoma hirsuta* Waldst. & Kit. and *G. hederacea* L. (Lamiaceae)." *Bulletin of the Chemical Society of Ethiopia* **24**(1) (2010) [Abstract](#). The essential oils of two *Glechoma* species from Serbia have been analyzed by GC and GC/MS. Eighty eight and two hundred thirty eight constituents identified accounted for 90.6 and 86.6% of the total oils of *G. hirsuta* Waldst. & Kit. and *G. hederacea* L., respectively. In both oils the dominant constituent class was the terpenoid one, 75.7% in *G. hirsuta* and 47.4% in *G. hederacea*. 1,8-Cineole (42.6%) and spathulenol (7.4%) were the main constituents of *G. hirsuta* oil while palmitic (13.3%) and linoleic acids (9.3%) alongside with germacrene D (7.3%) were the major ones of *G. hederacea* oil. The relative percentage of the sesquiterpene fraction (19.5%) and fatty acid derived compounds (7.6%) distinguished nicely *G. hirsuta* from *G. hederacea*. Additionally, oxygenated sesquiterpenes (16.9%) dominated the oil of *G. hirsuta*, while the reversed situation was noted for *G. hederacea* oil (the hydrocarbon sesquiterpenes amounted to only 2.6%). The results obtained provide a rationale for the parallel ethnopharmacological usage of *G. hirsuta* and *G. hederacea*. This is the first report on the composition of *G. hirsuta* oil.

Stahl E. & Datta SN (1972) "New sesquiterpenoids of the ground ivy (*Glechoma hederacea*)." *Justus Liebigs Ann Chem* **757**, 587-592.

Yamauchi H, Kakuda R, Yaoita Y, Machida K, Kikuchi M. (2007) "Two new glycosides from the whole plants of *Glechoma hederacea* L." *Chem Pharm Bull* (Tokyo). **55**(2), 346-7. [Abstract](#). Two new glycosides, 7S,7'S,8R,8'R-icariol A(2)-9-O-beta-D-glucopyranoside (1) and 4-allyl-2-hydroxyphenyl 1-O-beta-D-apiosyl-(1-->6)-beta-D-glucopyranoside (2), were isolated from the dried whole plants of *Glechoma hederacea* L. (Labiatae) together with four known compounds, cistanoside E (3), dihydrodehydrodiconiferyl alcohol 4-O-beta-D-glucopyranoside (4), apigenin 7-O-beta-D-glucuronopyranoside (5) and luteolin 7-O-beta-D-glucopyranoside (6). The structures of the new compounds were elucidated on the basis of chemical and spectral analysis.

Zieba J. (1973) "Isolation and identification of flavonoids from *Glechoma hederacea* L." *Pol J Pharmacol Pharm.* **25**(6), 593-7.

Zieba J. (1973) "Isolation and identification of non-heteroside triterpenoids from *Glechoma hederacea* L." *Pol J Pharmacol Pharm.* **25**(6), 587-92.

Safety.

MAFF Poisonous Plants in Britain London HMSO (1984) p139.

Miller Y. *et al.* (1980) "Étude de la toxicité d'huiles essentielles végétales du commerce essence d'hyssop et de sauge." *Médecine Légale Toxicologie* 23(1), 9-21.

Useful Properties

An H.J., Jeong H.J., Um J.Y., Kim H.M. & Hong S.H. (2006) "*Glechoma hederacea* inhibits inflammatory mediator release in IFN-gamma and LPS-stimulated mouse peritoneal macrophages." *J. Ethnopharmacol* **106**(3), 418-424, [Abstract](#). *Glechoma hederacea* (GH) is an herb widely used herb medicine for the treatment of a variety of pathologies. In this study, the effect of GH on interferon-gamma (IFN-gamma) and lipopolysaccharide (LPS)-induced production of nitric oxide (NO), interleukin (IL)-12p70, IL-12p40, tumor necrosis factor-alpha (TNF-alpha), and IL-6 were examined using mouse peritoneal macrophages. GH inhibits IFN-gamma/LPS-induced NO in a dose-dependent manner. The decrease in NO synthesis was reflected as a decreased amount of inducible NO synthase protein. We also found that GH inhibits pro-inflammatory cytokine, IL-12p70, and TNF-alpha production. However, GH increased IFN-gamma/LPS-induced IL-12p40 production. GH doesn't affect the IL-6 production. These findings mean that GH can be used in controlling macrophages mediated inflammation related disease.

Komprda T., Stohandlová M., Foltýn J., Pozdísek J. & Míka V. (1999) "Content of p-coumaric and ferulic acid in forbs with potential grazing utilization." *Arch Tierernahr.* **52**(1), 95-105. [Abstract](#). Content of p-coumaric (PCA) and ferulic (FA) acid was determined by the HPLC method in fourteen forbs with a potential utilization as forages (range of nutrient content per kg DM: 100 to 244 g CP, 339 to 528 g NDF and 180-369 g ADF. PCA and FA were determined after methanol extraction in four fractions: free phenolic acids extracted into either, ester-bound phenolic acids after alkaline hydrolysis, glycoside-bound phenolic acids after acid hydrolysis, and cell wall-bound phenolic acids after alkaline hydrolysis of the solid residue after the extraction with methanol. Cell wall-bound phenols were quantitatively the most important fraction (50% of total PCA and 47% of total FA, respectively). The differences among plant species in total PCA plus FA control were significant (F-value 775, $P < 0.01$). The range of total phenol content was 31.3 to 416.3 mg/100 g DM, the overall mean was 84 mg/100 g DM. Content of phenolic acids was correlated neither with ADF, NDF or ADL content ($R^2 = 1-3\%$, $P > 0.05$) nor with CP degradability ($R^2 = 3\%$ and $R^2 = 1\%$ for PCA and FA, respectively, $P > 0.05$). 95.4% and 30.9% of total PCA, and 98.3% and 72.5% of total FA disappeared in the rumen from the sample of *Glechoma hederacea* (species with the highest phenol content) and from the sample of *Galium aparine* (species with low phenol content), respectively, within the four hour incubation

interval. It is presumed that in comparison with grasses, PCA and FA concentration in tested forbs represents a much lower risk in potential ruminant nutrition.

Kumarasamy Y, Cox PJ, Jaspars M, Nahar L, Sarker SD. (2002) "Biological activity of *Glechoma hederacea*." **Fitoterapia**. **73**(7-8), 721-3. [Abstract](#). The n-hexane, dichloromethane and methanol extracts of the aerial parts of *Glechoma hederacea* have been screened for antibacterial and free radical scavenging activity. General toxicity (brine shrimp lethality assay) of these extracts has also been assessed.

Kumarasamy Y., Nahar L., Kong-Thu-Lin P., Jaspars M. & Sarker S.D. (2006) *Nat. Prod. Comm.* **1**, 33.

Mills S. & Bone K. (2000) *Principles and Practice of Phytotherapy*. Churchill Livingstone: London **2000**, p 216.

Milovanovic M, Zivkovic D, Vucelic-Radovic B. (2010) "Antioxidant effects of *Glechoma hederacea* as a food additive." *Nat Prod Commun.* **5**(1), 61-3. [Abstract](#). The antioxidant properties of *Glechoma hederacea* L. (Lamiaceae), of Serbian origin, were studied in respect to its potential use in foodstuffs. Ethanol-water (8:2, v/v) and purified ethyl acetate extracts of the plant were found to possess significant antioxidant activity. Tests were performed on two different substrates, prime steam pork lard and active-carbon-treated edible sunflower oil, using Schaal oven test storage conditions at 60 degrees C. The ethanol-water and purified ethyl acetate extracts of *G. hederacea* showed strong concentration-dependent antioxidant activity. On the contrary, under the Rancimat method conditions at 120 degrees C, the ethanol-water extract showed significantly stronger antioxidant activity, in comparison with the other tested extracts. All activities were compared with commercial antioxidants, such as BHA and a tocopherol mixture, respectively. For the first time, the activity of the flavonol quercetagenin was determined.

Singh T., Wu J.H., Peumans WJ., Rougé P., Van Damme E.J., Alvarez R.A., Blixt O. & Wu A.M. (2006) "Carbohydrate specificity of an insecticidal lectin isolated from the leaves of *Glechoma hederacea* (ground ivy) towards mammalian glycoconjugates." *Biochem J.* **1,393**(Pt 1), 331-41. [Abstract](#). Preliminary studies indicated that the potent insecticidal lectin, Gleheda, from the leaves of *Glechoma hederacea* (ground ivy) preferentially agglutinates human erythrocytes carrying the Tn (GalNAc α 1-Ser/Thr) antigen. However, no details have been reported yet with respect to the fine specificity of the lectin. To corroborate the molecular basis of the insecticidal activity and physiological function of Gleheda, it is necessary to identify the recognition factors that are involved in the Gleheda-glycotope interaction. In the present study, the requirement of high-density multivalent carbohydrate structural units for Gleheda binding and a fine-affinity profile were evaluated using ELLSA (enzyme-linked lectinosorbent assay) with our extended glycan/ligand collections, a glycan array and molecular modelling. From the results, we concluded that a high-density of

exposed multivalent Tn-containing glycoproteins (natural armadillo and asialo ovine salivary glycoproteins) were the most potent factors for Gleheda binding. They were, on a nanogram basis, 6.5×10^5 , 1.5×10^4 and 3.1×10^3 times more active than univalent Gal (galactose), GalNAc (N-acetylgalactosamine) and Tn respectively. Among mono- and oligo-saccharides examined, simple clustered Tn (molecular mass < 3000 Da) from ovine salivary glycoprotein was the best, being 37.5 and 1.7×10^3 times better than GalNAc and Gal respectively. GalNAc glycosides were significantly more active than Gal glycosides, indicating that the N-acetamido group at C-2 plays an important role in Gleheda binding. The results of glycan array support the conclusions drawn with respect to the specificity of Gleheda based on the ELLSA assays. These findings combined with the results of the molecular modelling and docking indicate the occurrence of a primary GalNAc α 1-binding site in the Gleheda monomer. However, the extraordinary binding feature of Gleheda for glycoproteins demonstrates the importance of affinity enhancement by high-density multivalent glycotopes in the ligand-lectin interactions in biological processes.

Wang W., Peumans W.J., Rougé P., Rossi C., Proost P., Chen J. & Van Damme E.J. (2003) "Leaves of the Lamiaceae species *Glechoma hederacea* (ground ivy) contain a lectin that is structurally and evolutionary related to the legume lectins." *Plant J.* **33**(2), 293-304. [Abstract](#). A novel lectin has been isolated and cloned from leaves of *Glechoma hederacea* (ground ivy), a typical representative of the plant family Lamiaceae. Biochemical analyses indicated that the *G. hederacea* agglutinin (Gleheda) is a tetrameric protein consisting of four subunits pairwise linked through an interchain disulphide bridge and exhibits a preferential specificity towards N-acetylgalactosamine. Cloning of the corresponding gene and molecular modeling of the deduced sequence demonstrated that Gleheda shares high sequence similarity with the legume lectins and exhibits the same overall fold and three-dimensional structure as the classical legume lectins. The identification of a soluble and active legume lectin ortholog in *G. hederacea* not only indicates that the yet unclassified Lamiaceae lectins belong to the same lectin family as the legume lectins, but also sheds a new light on the specificity, physiological role and evolution of the classical legume lectins.

Wang W., Hause B., Peumans W.J., Smagghe G., Mackie A., Fraser R. & van Damme E.J. (2003) "The Tn antigen-specific lectin from ground ivy is an insecticidal protein with an unusual physiology." *Plant Physiol.* **132**(3), 1322-34. [Abstract](#). Leaves of ground ivy (*Glechoma hederacea*) contain a lectin (called Gleheda) that is structurally and evolutionary related to the classical legume lectins. Screening of a population of wild plants revealed that Gleheda accounts for more than one-third of the total leaf protein in some clones, whereas it cannot be detected in other clones growing in the same environment. Gleheda is predominantly expressed in the leaves where it accumulates during early leaf maturation. The lectin is not uniformly distributed over the leaves but exhibits a unique localization pattern characterized by an almost exclusive confinement to a single layer of palisade parenchyma cells. Insect feeding trials demonstrated that Gleheda is a potent insecticidal protein for larvae of the Colorado potato beetle

(*Leptinotarsa decemlineata*). Because Gleheda is not cytotoxic, it is suggested that the insecticidal activity is linked to the carbohydrate-binding specificity of the lectin, which as could be demonstrated by agglutination assays with different types of polyagglutinable human erythrocytes is specifically directed against the Tn antigen structure (N-acetylgalactosamine O-linked to serine or threonine residues of proteins).