|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Table s2. Polysaccharides, fatty acids, and other compounds of chaga and their purification/identification** | | | | | | |
| **Compound** | **Molecular formula** | **Extraction Method** | **Qualification Method** | **Purification Method** | **Production regions** | **Reference** |
| **Polysaccharides** | | | | | | |
| Glycoprotein (230 kDa) | - | Water, 3 h, 80℃ | SEC | Alcohol precipitation, AEC (DEAE-Sepharose fast flow column), SEC (SepharoseCL-6B column), dialysis |  | Huang et al. (2012) |
| Proteoglycan (40kDa) | - | Water, 2 h, 100℃, two times | HPSEC (refractive index, UV, and MALLS detectors), AEC, and FT-IR | Liquid-liquid extraction |  | Liu et al. (2019) |
| α-Linked fucoglucomannan (1000 kDa) | - | Water, 6 h, 121℃ | SEC | Alcohol precipitation, AEC (DEAE-cellulose column), SEC (Toyopearl HW65F column), dialysis |  | Kim et al. (2006) |
| Purified fractions of polysaccharide (93 kDa) | - | Water, 3 h, 80℃ | GC and HPSEC | Alcohol precipitation, AEC (DEAE-Sepharose CL-6B column), SEC (sepharose CL-6B column), dialysis |  | Fan et al. (2012) |
| Purified fractions of polysaccharide (122 kDa) | - | Water, 80 min, 75 ℃, ultrasonication | SEC | Deproteination (Sevag reagent), alcohol precipitation,  DEAE-52 cellulose column, dialysis |  | Ma et al. (2012);  Zhang et al. (2013) |
| Purified fractions of polysaccharide (32.5 kDa) | - | Water, 2.5 h, 60℃ | SEC | Anion-exchange DEAE cellulose column and SEC (Sephadex G-200) |  | Hu et al. (2016) |
| Purified fractions of polysaccharide (111.9 kDa) | - | Water, 2 h, 90℃ | UV, IR spectra, HPSEC | Alcohol precipitation, DEAE-52 column, SEC (Sephadex G-100) |  | Han et al. (2019) |
| Purified homogeneous polysaccharide fraction (37.354kDa) | - | Water, 2.5 h, 60℃ | FT-IR, HPSEC, 1H-NMR/13C-NMR | Deproteination (Sevag reagent), alcohol precipitation, AEC (DEAE cellulose column), Sephadex G-200 gel |  | Hu et al. (2017) |
| Neutral polysaccharides (60-73 kDa) | - | Water, 2 h, 100℃, two times | SEC-MALLS, IR spectra, 1H-NMR/13C-NMR, and GC-MS | AEC (ANX Sepharose™ 4 Fast Flow), SEC (Superose® 6 column), dialysis |  | Wold et al. (2018) |
| Acidic polysaccharides (melanin-polysaccharide complex) (10-31 kDa) | - | AEC (ANX Sepharose™ 4 Fast Flow), SEC (Hiload™ 16/60 Superdex™ 200 column), dialysis |  |
| Alkaline polysaccharides (>450 kDa) | - | AEC (ANX Sepharose™ 4 Fast Flow), SEC (Sephacryl S-500 HR column), dialysis |  |
| **Alkaloids** | | | | | | |
| 3,3-Dimethyl-9-(propylamino)-3,4-dihydro-1(2H)-acridinone | C18H21N2O | Chloroform, 12 h, RT, three times | UPLC-Q-TOF-MSn | Silica gel column/HPLC (C18 column) | Mycelia culture | Geng et al. (2013) |
| 2-Butyl-3- (3-methylphenyl)-4(3H)-quinazolinone | C19H19N2O |
| 1-(4-Methyl-1-piperazinyl)-2-{[3-(2-methyl-1- piperidinyl)propyl]amino}ethanone | C16H31N4O |
| 1-{[2-(Diethylamino)ethyl]amino}-3-(4-methyl-1-piperazinyl)- 2-propanol | C14H31N4O |
| N-{(1S,2S)-1-benzyl-3-[1-(cyclohexylmethyl)hydrazino]-2-hydroxypropyl}-N2-[(2- methoxyethoxy)carbonyl]-L-valinamide | C26H43N4O5 |
| 1,1-Dimethyl-3,3-bis(2,2,6,6-tetramethyl-1-prop-2-en-1- ylpiperidin-4-yl)urea | C27H49N4O |
| 1-(3,6-Dihydropyridin-1(2H)-yl)-3-[3-(dimethylamino)propyl]urea | C11H21N4O |
| (2R,4S,5S,7S)-5-Amino-N-butyl-7-{4-[4-(dimethylamino)-butoxy]-3-(3-methoxypropoxy)benzyl}-4- hydroxy-2,8-dimethylnonanamide | C32H57N3O5 |
| 2,2-Bis[2,2,6,6-tetramethyl-1-(octyloxy)piperidin-4-yl]-hexanedioate | C40H73N2O6 |
| 3-(4-Cyclohexylbutyl)-6,11-dimethyl-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocine | C24H36N |
| **Fatty acids and other organic compounds** | | | | | | |
| 2-(1,4,4-Trimethylcyclohex-2-en-1-yl)ethyl acetate | C13H22O2 | HCl-water, 5 h, reflux; then hot ethyl acetate and methanol | IR spectra and GC-MS | - | Poland | Mazurkiewicz (2006) |
| 4-Oxopentanoic acid | C5H8O3 |
| Docosane | C22H46 |
| Hexatriacontane | C36H74 |
| O-Acetyl-all-*trans*-Retinol | C22H32O2 |
| Hexadecanoic acid | C16H32O2 |
| Heneicosane | C21H44 |
| Benzyl alcohol | C7H8O |
| Oxalic acid | C2H2O4 | Water or 70% ethanol, 2-24 h, 70-80℃ | LC | None | Russia, Finland, Thailand | Glamočlija et al. (2015) |
| Cinnamic acid | C9H8O2 |
| Isocitric acid | C6H8O7 | High-pressure steam, 35% methanol, 35% acetone, 30% water | LC-MS and GC-MS | Liquid-liquid extraction | South Korea | Ju et al. (2010) |
| 1-Dodecanol | C12H26O | Petroleum, 14 h, RT | GC-MS | - | Cultivated or wild chaga from China | Sun et al. (2011) |
| 2,10-Dimethyl-9-undecenol | C13H26O |
| Ethyl octadecanoate | C20H40O2 |
| Isopropyl linoleate | C20H38O2 |
| Ethyl oleate | C20H38O2 |
| Ethyl hexadecanoate | C18H36O2 |
| Ethyl dodecanoate | C14H28O2 |
| Ethyl tetradecanoate | C16H32O2 |
| Di-isobutyl phthalate | C16H22O4 |
| Di-iso-octyl phthalate | C24H38O4 |
| Ethyl pentadecanoate | C17H34O2 |
| Ethyl Heptadecanoate | C19H38O2 |
| 2,6,10,14-Tetramethyl heptadecane | C21H44 |
| 2,6,10,14-Tetramethyl pentadecane | C19H40 |
| Hexadecane | C16H34 |
| Octadecane | C18H38 |
| Heptadecane | C17H36 |
| Nonadecane | C19H40 |
| Dibutyl phthalate | C16H22O4 |
| Methyl-8,11-octadecadienoate | C19H34O2 |
| Ethyl linoleate | C20H36O2 |
| Pentadecanal | C15H30O |
| Linoleic acid | C18H32O2 |
| Suberic acid | C8H14O4 | MTBE, Soxhlet extractor, 27h | GC-MS (HP-5 MS sorbent column) | - | Russia | (Shcherbakov et al., 2022) |
| Lauric acid | C12H24O2 |
| Myristic acid | C14H28O2 |
| Pentadecanoic acid | C15H30O2 |
| Pentadecenoic acid | C15H28O2 |
| Palmitic acid | C16H32O2 |
| Palmitoleic acid | C16H30O2 |
| Hexadecanedioic acid | C16H30O4 |
| 2-Hydroxyhexadecanoic acid | C16H32O3 |
| Margaric acid | C17H34O2 |
| Heptadecenoic acid | C17H32O2 |
| Stearic acid | C18H36O2 |
| Oleic acid | C18H34O2 |
| Vaccenic acid | C18H34O2 |
| 9,11,13-Octadecatrienoic acid | C18H30O2 |
| Octadecanedioic acid | C18H34O4 |
| Nonadecanoic acid | C19H38O2 |
| Arachidic acid | C20H40O2 |
| Eicosanedioic acid | C20H38O4 |
| Heneicosanoic acid | C21H42O2 |
| Behenic acid | C22H44O2 |
| 2-Hydroxydocosanoic acid | C22H44O3 |
| Docosanedioic acid | C22H42O4 |
| Tricosanoic acid | C23H46O2 |
| Tricosenoic acid | C23H44O2 |
| 2-Hydroxytricosanoic acid | C23H46O3 |
| Lignoceric acid | C24H48O2 |
| 15-Tetracosenoic acid | C24H46O2 |
| 2-Hydroxytetracosanoic acid | C24H48O3 |
| Pentacosanoic acid | C25H50O2 |
| 16-Pentacosenoic acid | C25H48O2 |
| 2-Hydroxypentacosanoic acid | C25H50O3 |
| Montanic acid | C26H56O2 |
| Cerotic acid | C26H52O2 |
| 17-Hexacosenoic acid | C26H50O2 |
| 2-Hydroxyhexacosanoic acid | C26H52O3 |
| Heptacosanoic acid | C27H54O2 |
| Nonacosanoic acid | C29H58O2 |
| Melissic acid | C30H60O2 |
| Tricosanal | C23H46O |  |  |  |  |  |
| Benzaldehyde | C7H6O | HCl-water, 5 h, reflux; then hot ethyl acetate and methanol | IR spectra and GC-MS | - |  |  |
| 1-Octen-3-ol | C8H16O | Hydrodistillation | GC and GC-MS | - | Unknown | Kahlos (1994) |
| Linolenic acid | C18H30O2 278 | Hexane | TLC, GLC, and GC-MS | - | Mycelia culture | Kahlos et al. (1989) |
| (2S)-2-[(1S)-1-Phenylethyl]- 3,6-dihydro-2H-pyran | C13H15O | Chloroform, 12 h, RT, three times | LC-Q-TOF-MSn, | Silica gel column/HPLC | Mycelia culture | Geng et al. (2013) |
| 1,6-Dideoxy-3,4-O-(1,5,9-trimethyl-decylidene)-Dmannitol | C19H37O4 |
| (1S,4aR,5R,8aS)-5-[(1R)-5-Hydroxy-1,5-dimethylhexyl]-4a-methyldecahydronaphthalen-1-ol | C19H35O2 |
| Ribitol | C5H12O5 |  |  |  |  | Im et al. (2017) |
| Glucitol | C6H14O6 | 95% Ethanol, 24 h, RT, 5 times | MS and 1H-NMR/13C-NMR | Liquid-liquid extraction, silica gel column | Japan | Shin et al. (2001a) |
| TGly-Cys | C16H20N4O4S | Pure water and 70% ethanol, 30 °C, 12 h | HPLC, LC-MS, automatic protein sequencer | Preparative HPLC (C18 column), ultrafiltration, Sephadex G-10 column | South Korea | Hyun et al. (2006) |
| Phenylalanine | C9H11NO2 | 50% Ethanol, 24 h, RT | HPLC | Sephadex LH-20 column | Mycelia Culture | Zheng et al. (2008) |
| Tyrosin | C9H11NO3 |
| Purified melanin fractions (56-60 kDa or 100-120 kDa or more) | - | NaOH-water, 2 h, boiling | SEC (Toyopearl HW-65 resin column) | SEC (Sephadex G-75 column) | Cultured mycelia | Babitskaya et al. (2000) |
| Purified melanin fractions (2-20 kDa or 90-100 kDa or more) | - | 50-95% ethanol, 2 h, 100℃; then water, 1 h, 100℃; then KOH-water, 1-3 h, 20℃ | IR spectra, 13C NMR | Ethanol precipitation, acid precipitation, Sephadex G-100 column | Russia | Olennikov et al. (2012) |
| Purified melanin-polysaccharide (< 10 kDa, ~5% polysaccharide) | - | Water, 2 h, boiling, three times | HPSEC (diol-300 column) | Ethanol precipitation, dialysis, acid precipitation | Norway | Wold et al. (2020) |
| Purified polysaccharide-melanin complex (10-31 kDa, ~4.2-9.7% melanin) | - | Water, 2 h, boiling, 2 times | SEC-MALLS, IR spectra, 1H-NMR/13C-NMR, and GC-MS | AEC (ANX Sepharose™ 4 Fast Flow); SEC (Hiload™ 16/60 Superdex™ 200 column); dialysis | Norway | Wold et al. (2018) |
| Crude melanin | - | Water, 10 h, 70℃ or microwave-assisted extraction | - | Acid precipitation | Mycelia culture | Burmasova et al. (2019); Parfenov et al. (2019) |
| UPLC-Q-TOF-MS/MS:  ultra-high-performance liquid chromatography-quadrupole time-of-flight tandem mass spectrometry; HPLC: high performance liquid chromatography;  MALLS: multi-angle laser light scattering; SEC: size exclusion chromatography; HPSEC: high performance size exclusion chromatography; AEC: anion-exchange chromatography; HPAEC: high-performance anion-exchange chromatography | | | | | | |

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