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KARELINIA CASPIA L. O'SIMLIGI POLIFENOLLARI

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Annotatsiya:*O'zbekistonda keng tarqalgan, Asteraceae oilasiga mansub Karelinia caspia L. o'simligidan maqbul ekstraksiya usuli asosida polifenollar yig'indisi ajratib olindi. Olingan polifenollar yig'indisi zamonaviy Flash xromatografiya qurilmasi yordamida alohida fraksiyalarga ajratildi. Ajratilgan fraksiyalar BioBase BK-FD50S Pilot Freeze Dryer qurilmasida muzlatib quritildi. Olingan namunalarning tozalik darajasi HPLC 1260 yuqori samarali suyuqlik xromatografiyasi yordamida o'rganildi.Tozalik darajasi yuqori va miqdori ko'p bo'lgan beshta namunaning proton (^1H) va uglerod-13 (^{13}C) yadro magnit rezonansi (YaMR) spektrlari olindi hamda spektral ma'lumotlar asosida ularning tuzilishi aniqlandi. Natijada quyidagi birikmalar identifikatsiya qilindi:3,4,5-trigidroksibenzoy kislota (gallat kislota); 3,4-digidroksisinnam kislota (kofein kislota); 3,3',4',5,7-pentagidroksiflavon-3-O-ramnozilglukozid;4'-O-metil-8-γ,γ-dimetilallilkaempferol-3-ramnozid-7-glukozid; 1,2,3,4,6-penta-O-(3,4-digidroksi)-3,4,5-trigidroksibenzoil-β-D-glukopiranoza.*

Kalit so'zlar: *fenol kislota, flovonoidm tannin, UB, IQ, ^1H va ^{13}C YaMR spektr, QX, YuQX, YUSSX xromatografiya, BioBase BK-FD50S Pilot Freeze Dryer qurilma*

ПОЛИФЕНОЛЫ РАСТЕНИЯ KARELINIA CASPIA L.

Аннотация:*Из растения Karelinia caspia L., отиосящегося к семейству Asteraceae и широко распространёиного в Узбекистаие, оптимизированиым методом экстракции выделена сумма полифеиольиых соединений. Полученную сумму полифеиольиых соединений разделили иа отдельные фракции методом флэш-хроматографии. Выделеиные фракции лиофилизировали с использованием пилотиой лиофильиой сушилки BioBase BK-FD50S. Чистоту полученных образцов оценивали методом высокоэффективиой жидкостиой хроматографии (ВЭЖХ) на хроматографе Agilent 1260. Для пяти образцов, характеризующихся высокой степеиью чистоты и высоким содержаиием, были зарегистрированы спектры ядериого магиитиого резоиаиса (ЯМР) иа ядрах протоиа (^1H) и углерода-13 (^{13}C) и иа основаии полученных спектральиых даиных определили структуру исследуемых соединений. В результате были идеитифицированы следующие соединеиия: 3,4,5-тригидроксибеизойиая кислота (галловая кислота); 3,4-дигидроксикоричиая кислота (кофейиая кислота); 3,3',4',5,7-пеитагидроксифлавоп-3-О-рамиозилглюкозид; 4'-О-метил-8-γ,γ-диметилаллилкемпферол-3-рамиозид-7-глюкозид; 1,2,3,4,6-пеита-О-(3,4-дигидрокси)-3,4,5-тригидроксибеизоил-β-D-глюкопираноза.*

Ключевые слова: *фенольные кислоты, флавоноиды, танины, УФ, ИК, спектроскопия ЯМР ^1H и ^{13}C, БХ, ТСХ, ВЭЖХ, лиофильная сушилка BioBase BK-FD50S.*

POLYPHENOLS OF THE PLANT KARELINA CASPIA L.

Annotation:*A mixture of polyphenols was isolated from Karelinia caspia L. (family Asteraceae) a plant widely distributed in Uzbekistan—using an optimized extraction method. The obtained mixture was separated into individual fractions using a modern flash chromatography system. These fractions were then freeze-dried using a BioBase BK-FD50S Pilot Freeze Dryer. The purity of the samples was analyzed via high-performance liquid chromatography (HPLC 1260). Proton (^1H) and carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded for five samples of high purity and yield, and their structures were elucidated based on the spectral data. As a result, the following compounds were identified: 3,4,5-trihydroxybenzoic acid (gallic acid); 3,4-dihydroxycinnamic acid (caffeic acid); 3,3',4',5,7-pentahydroxyflavone-3-O-rhamnosylglucoside; 4'-O-methyl-8-γ,γ-dimethylallylkaempferol-3-rhamnoside-7-glucoside; and 1,2,3,4,6-penta-O-(3,4-dihydroxy)-3,4,5trihydroxybenzoyl-β-D-glucopyranose.*

Keywords: *phenolic acid, flavonoid, tannin, UV, IR, ^{1}H and ^{13}C NMR spectra, HPLC chromatography, BioBase BK-FD50S Pilot Freeze Dryer unit.*

Kirish.

O'simlik hujayralarida ko'plab birikmalar mavjud bo'lib, ulardan ba'zilari aminokislotalar, oqsillar va polisaxaridlar o'simliklarning o'sishi va rivojlanishi, ko'payishi uchun zaruriy birikmalar qatoriga kiradi. Bunday birikmalar o'simliklarning birlamchi metabolitlari hisoblanadi. Birlamchi metabolitlar bilan bir qatorda o'simliklar tomonidan ikkilamchi metabolitlar ham siztezlanadi. O'simliklar tomonidan sintezlanadigan ikkilamchi metabolitlar o'simlikning omon qolishi uchun muhum ahamiyat kasb etadi. Bu birikmalar qatoriga tanninlar flovonoidlar, alkaloidlar va terpenlar kirib, ular o'simliklarning ikkilamchi metabolitlari hisoblanadi. Garchi ushbu birikmalar o'simliklarda ma'lum vazifani bajarmasada, o'simliklarni tashqi ta'sirlardan himoya qilishda ularning ahamiyati kattadir. Hozirgi kunda dunyo va mahalliy olimlar tomonidan o'simliklarning kimyoviy tarkibi hamda biologik faolliklarini aniqlash bo'yicha keng miqyosda ilmiy tadqiqotlar olib borilmoqda[1,2].

Adabiyotlar tahlili

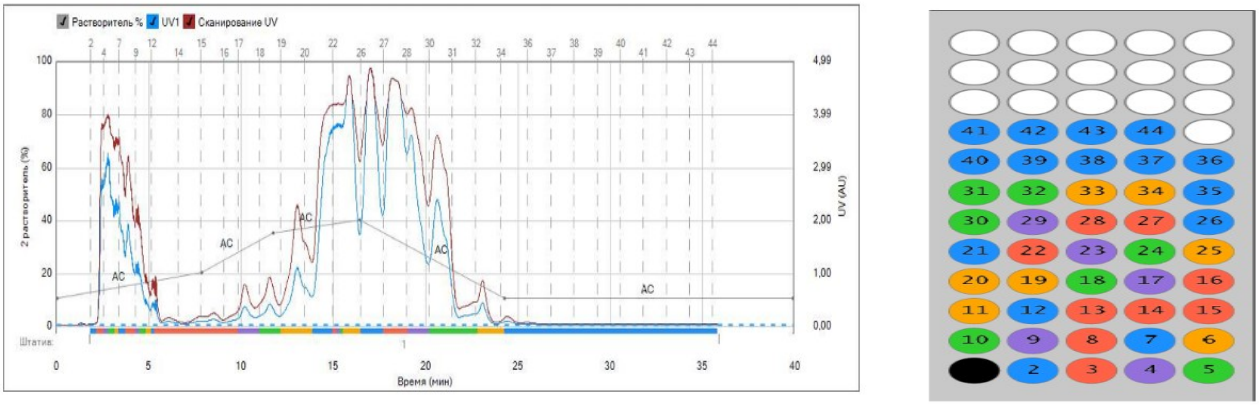
O'simliklar tarkibidan ajratib olingan biologik faol moddalar jumladan polifenol moddalar singari ikkilamchi metabolitlarning muhim xususiyatlari dastlab charm sanoatida "o'simlik tanninlari" [3] nomi ostida aniqlangan. Keyinchalik Emil Fisher va Karl Freydenberg ularni "o'simlik polifenollari" deb ataganlar, biroq o'sha paytda tahliliy vositalarning yetishmasligi sababli ularning keng tadqiqi cheklangan edi [4]. Bugungi kunga kelib bu sohada muammo qolmadi. Ko'plab ta'riflar berilib, qayta ko'rib chiqilgandan so'ng, polifenollarga hozirgi ma'noda shunday ta'rif beriladi: ular o'simliklarda hosil bo'ladigan va, asosan, himoya jarayonlarida ishtirok etadigan ikkilamchi metabolitlardir. Polifenollar o'simliklarda ko'pincha ortiqcha ultrabinafsha (UB) nurlanish yoki turli kasallik qo'zg'atuvchi mikroorganizmlardan himoya sifatida o'simlik turli organlarida sintezlanadi [5.]. O'simliklar ortiqcha UB nur, turli tashqi omillar, mikroblar yoki hayoti davomida uchraydigan turli stresslarga duch kelganda, ularda fenol birikmalarining miqdori ancha oshadi. Olib borilgan tadqiqotlar shuni ko'rsatadiki, o'simliklarda oziq moddalar yetishmaydigan tuproqda va yuqori haroratda o'sgan o'simliklarning turli organlarida ko'proq miqdorda ikkilamchi moddalar jumladan polifenol tabiatli birikmalar to'planadi.

Polifenollar fitokimyoviy moddalar bo'lib, ular inson salomatligiga foyda keltirish qobiliyatiga ega: surunkali kasalliklar yoki saratonni davolashda [6-7], yoki kuchli antioksidantlar sifatida qo'llaniladi. Ular inson oziqlanishidagi eng katta antioksidant manbai hisoblanadi va sog'lom turmushga hissa qo'shadi. Biroq ularning asosiy kamchiligi shundaki, biofoydalanishi past va tez so'riladi, natijada ko'p qismi siydik orqali chiqarib yuboriladi [8-10].

Karelinia caspia L. (*Asteraceae* oilasi) Markaziy Osiyo florasida keng tarqalgan o'simliklardan biri hisoblanadi. Uning halq tabobatida yallig'lanishga qarshi, antimikrob va antioksidant vosita sifatida qo'llanilishi haqida ma'lumotlar mavjud. Ammo ushbu o'simlikning polifenollari tarkibi, flavonoidlar va tanninlarining xilma-xilligi hamda biologik faolligi yetarlicha o'rganilmagan.

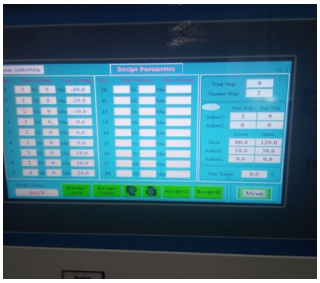
Tadqiqot metodologiyasi

Asteraceae oilasiga mansub *karelinia caspia L.* o'simligidan yuqori unum va sifatga ega bo'lgan polifenillar yig'indisi ajratib olindi**.** Ajratib olingan polifenollar yig'indisini sifat reaksiyalari olib borilganda fenol oksi kislotalar, flovonoidlar va tanninlar sinfiga mansub birikmalar bor ekanligi aniqlandi. Ushbu o'simlikdan ajratib olingan polifenollar yig'indisini zamonaviy Flash qurilmasi yordamida 44 ta fraksiyalarga ajratildi (1-rasm).



1-rasm. Flash qurilmasi yordamida fraksiyalarga ajratish

Ajratib olingan 44 ta fraksiyaning har biri alohida rotorli bug'latgich yordamida tarkibidagi erituvchisi haydalib qolgan na'munalar pensilin idishlarga joylanib BioBase BK-FD50S Pilot Freeze Dryer qurilmasida muzlatib, quritib paroshok xoldagi na'munalar olindi (2-rasm).






2-rasm. *Karelinia caspia L.* o'simligidan ajratilgan fraksiyalar

Karelinia caspia L. o'simligi polifenollari tarkibidan ajratib olingan 44 ta fraksiyaning xar biri YuQX usulida R_f qiymatlari hamda YuSSX da tozalik darajasi o'rganilib o'xshash fraksiyalar qo'shib yuborildi (1-jadval). Natijada 3, 6, 10-fraksiyalar 95-97 % tozalik darajasida ekanligi aniqlandi. 13-16, 19-20, 27-28- fraksiyalar bir xil qiymatlarni ko'rsatganligi uchun qo'shib yuborildi. Shuningdek ushbu fraksiyalarning tozalik darajasi YuSSX da o'rganilganda 95-98% oralig'ida ekanligi va miqdori ko'pligi sababli 5 ta fraksiyaning UB, IQ, ^{1}H va ^{13}C YaMR spektr ma'lumotlari o'rganildi.

1-jadval

Karelinia caspia L. o'simligidan ajratilgan fraksiyalarning tozalik darajasi va miqdori

№	Fraksiya	Tozalik darajasi %	Miqdori, mg	Summaga nisbatan unimi	O'simlikka nisbatan unimi
1	2-fraksiya	31	12	1.29%	0.03%
2	3-fraksiya	96,5	25	1.625	0.04%
3	4-fraksiya	84,4	6	0.65%	0.01%
4	5-fraksiya	46	9	0.97%	0.02%
5	6-fraksiya	95,9	29	2.05%	0.05%
7	7-fraksiya	65	13	1.40%	0.03%
8	8-fraksiya	78,2	4	0.43%	0.01%
10	9-fraksiya	36	11	1.19%	0.03%
11	10-fraksiya	97	51,5	5.65%	0.13%
12	11-fraksiya	87	13	1.40%	0.03%
13	12-fraksiya	66.5	7	0.75%	0.02%
14	13-16-fraksiya	96,5	51	5.50%	0.12%
15	17-fraksiya	55,1	29	3.12%	0.07%
16	18-fraksiya	51.3	17	1.83%	0.04%
17	19-20-fraksiya	96.2	34	3.66%	0.08%
18	21-fraksiya	83	8	0.86%	0.02%
19	22-fraksiya	79	12	1.29%	0.03%
20	23-fraksiya	81.1	14	1.51%	0.03%
21	24-fraksiya	44	5	0.54%	0.01%
22	25-fraksiya	63	7	0.75%	0.02%
23	26-fraksiya	86	18	1.94%	0.04%
24	27-28-fraksiya	95,1	44	4.74%	0.11%
25	29-fraksiya	71	31	3.34%	0.08%
26	30-32-fraksiya	95,3	14,5	7.00%	0.16%
27	33-34-fraksiya	96.2	13	7.87%	0.18%
28	35-44-fraksiya	75,7	389	41.92%	0.95%

Natijalar va ularning tahlili.

Karelinia caspia L. o'simligi yer ustki qismidan ajratib olingan 5 ta moddaning xar biri QX va YQX usullari yordamida R_f qiymatlari, UB, IQ spektroskopiya, YuSSX xromatografiyalari, ^{1}H va ^{13}C YMR spektr ma'lumotlari o'rganib tahlil qilinganda ushbu moddalar quyudagilar ekanligi aniqlandi.

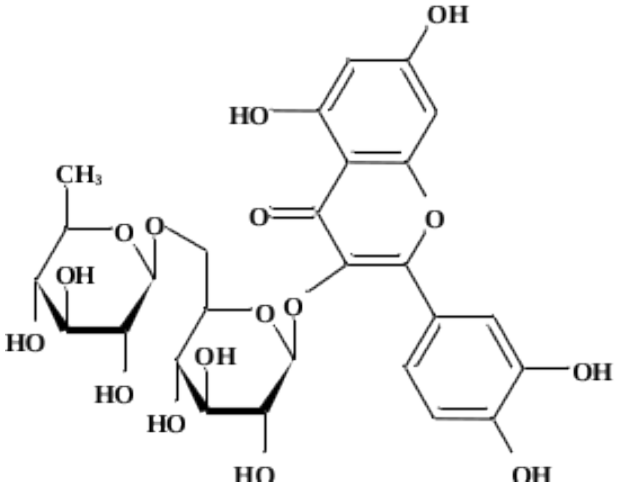
1-modda. $C_7H_6O_5$, Мм 170, oq rangli amorf kukun, R_f 0.50, 0.28 (QX, ЮҚХ, 7,10-sistema), S.h. 184-185^{0}C, UB-spektr (EtOH, λ_{max}, nm): 320, 256, IQ (ν, см$^{-1}$) spektr: 3425, 3180 (OH), 1665 (>C=O), 1610, 1575, 1510, 1455 (Ar), ^{13}C YAMR spektr (δ, 150 MGrs, CD3OD + CCl_4, m.u.): 149.09 (2C, gall C-3,5), 146.04 (gall C-4), 115.4 (2C, gall C-2,6), 126.9 (C, gall C-1), 168.6 (C gall C-7). Olingan natijalar adabiyotlarda keltirilgan ma'lumotlar bilan solishtirildi va taxlil qilindi. Natijada ushbu modda 3,4,5-trioksibenzoat kislota ekanligi aniqlandi.

1-moddaning kimyoviy tuzilishi.

2-modda. $C_9H_8O_4$, Mm 180, Och sariq rangli amorf kukun, Rf 0.42, 0.30 (QX, YQX, 7,10-sistema), S.h. 223–225 °C, UB-spektr (EtOH, λmax, nm): 325, 298, 245, IQ spektr: (v, sm^{-1}): 3430, 3350 (OH), 1680 (>C=O), 1620, 1600 (C=C, aromatik), 1515, 1460 (Ar), 1270, 1160 (C–O). ^1H va ^{13}C YAMR spektr (δ, 150 MGrs, $CD_3OD + CCl_4$, m.u.): 7.02, (d (2.1), kof k-t, H-2), 6.76 (d (8.1), kof k-t, H-5), 6.90 (dd, (8.1, 2.1) kof k-t, H-6), 7.52 (d (15.8), kof k-t, H-7), 6.19 (d (15.8), kof k-t, H-8) ^{13}C YAMR spektr: 127.53 (C, Kof k-t C-1), 114.94 (C, 7.02, d (2.1) C-2), 146.44 (C, kof k-t, C-3), 149.09 (C, kof k-t, C-4), 116.25 (C, kof k-t, 6.76, d (8.1), C-5), 122.68 (C, kof k-t, 6.90,dd (8.1, 2.1), C-6), 146.96 (C, kof k-t, 7.52, d (15.8), C-7), 115.34 (C, kof k-t, 6.19, d (15.8), C-8), 170.81 (C, kof k-t, C-9). Olingan natijalar adabiyotlarda keltirilgan ma'lumotlar bilan solishtirildi va taxlil qilindi. Natijada ushbu modda 3,4-digidroksisinnamik kislota ekanligi aniqlandi.

2-moddaning kimyoviy tuzilishi.

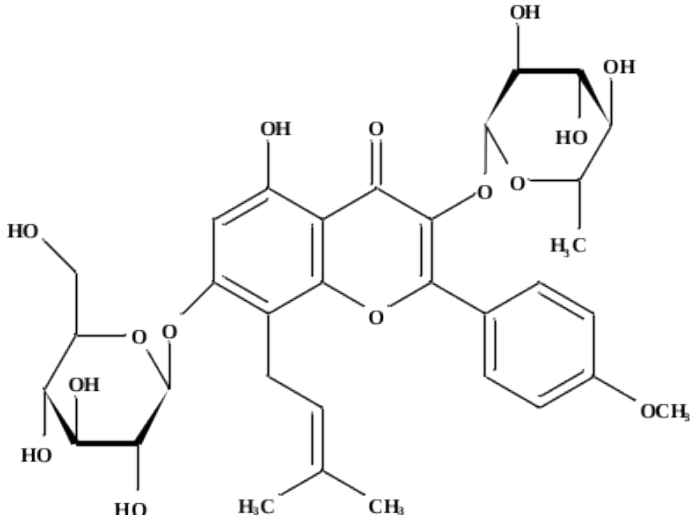
3-modda. $C_{27}H_{30}O_{16}$, Mm 610, Och sariq rangli amorf kukun, R_f 0.35, 0.18 (QX, YQX, 7,10-sistema), S.h 190–195 °C, UB-spektr (EtOH, λmax, nm): 358, 256, IQ spektr(v, sm^1): 3420–3300 (OH), 1660 (>C=O), 1610, 1585, 1515 (Ar), 1450, 1375, 1275, 1205 (C–O), 1070, 1025 (C–O–C, glikozid bog'lari). ^1H va ^{13}C YAMR spektr (δ, 150 MGrs, $CD_3OD + CCl_4$, m.u.): 6.20, (d (2.1), H-6), 6.39 (d (2.1), H-8), 7.67 (d (2.1), 'H-2), 6.87 (d (8.5), 'H-5), 7.63 (dd (8.5, 2.1),'H-6), 5.11 (d (7.7),''H-1), 3.48 (dd, (9.2, 7.7),''H-2), 3.42 (m,''H-3), 3.27 (m,''H-4), 3.33 (m,''H-5), 3.39 (dd, (11.1, 6.2),''H-6), 4.52 (d, (1.6),'''H-1), 3.64 (dd, (3.5, 1.6),'''H-2), 3.54 (dd, (9.5, 3.5),'''H-3), 3.28 (m, '''H-4), 3.45(m, '''H-5), 1.12 (d, (6.2), '''H-6 ^{13}C YAMR spektr: 159.34 (C-2), 135.64 (C-3), 179.42 (C-4), 162.98 (C-5), 99.94 (C-6), 166.00 (C-7), 94.87 (C-8), 158.51 (C-9), 105.63 (C-10), 123.13 (C-1'), 117.70 (C-2'), 145.83 (C-3'), 149.80 (C-4'), 116.06 (C-5'), 123.57(C-6'), 104.73 (C-1''), 75.73 (C-2''), 78.18 (C-3''), 71.39 (C-4''), 77.21 (C-5''), 68.55(C-6''), 102.42 (C-1'''), 72.00 (C-2'''), 72.24 (C-3'''), 73.94 (C-4'''), 69.71 (C-5'''), 17.87(C-6'''). Natijada ushbu modda 3,3',4',5,7-pentagidroksi-flavon-3-O-ramnozilglukozid ekanligi aniqlandi.



3-moddaning kimyoviy tuzilishi.

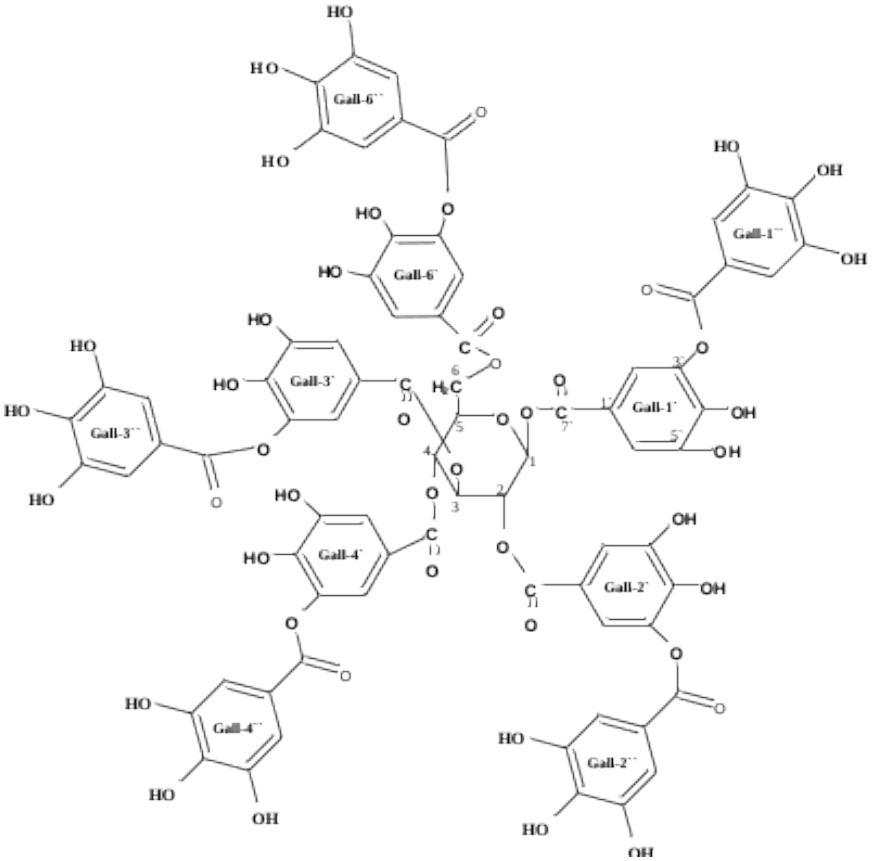
4-modda. $C_{33}H_{40}O_{15}$, Mm 676, Och sariq rangli amorf kukun, R_f 0.34, 0.20 (QX, YQX, 7,10-sistema), S.h 231-233 °C, UB-spektr (EtOH, λmax, nm): 270, 315, IQ (spektr v, sm^1): 3415–3300 (OH), 1655 (>C=O), 1610, 1585, 1510 (Ar), 1460, 1380, 1275, 1210 (C–O), 1075–1030 (glikozid bog'lari). ^1H YAMR spektr (Py-d_5, δ, m.u, J/Гц, 600 МГц).): 7.25, (c, H-6), 8.17 (d, (8.9), H-2'), 7.13 (d (8.9), H-3'), 7.13 (d, (8.9), H-5'), 8.17 (d, (8.9), H-6'), 6.29 (d, (1.6), H-1''), 5.10 (dd, (3.4, 1.6), H-2''). ^{13}C YAMR spektr: 158.20 (C-2), 136.69 (C-3), 179.98 (C-4), 161.15 (C-5), 99.78 (C-6), 162.28 (C-7), 109.85 (C-8), 154.45 (C-9), 107.51 (C-10), 124.06 (C-1'), 131.72 (C-2'), 114.93 (C-3'), 162.68 (C-4'), 114.93 (C-5'), 131.72(C-6'), 104.42 (C-1''), 72.43 (C-2''), 73.01 (C-3''), 73.68 (C-4''), 72.62 (C-5''), 18.80 (C-6''), 103.16 (C-1'''), 75.44 (C-2'''), 79.21 (C-3'''), 71.60 (C-4'''), 79.58 (C-5'''), 62.76 (C-6'''), 22.97 (C-1''''), 123.63 (C-2''''), 132.02 (C-3''''), 18.58 (C-4'''), 26.18 (C-5'''), 55.91 (4'-OC).

Natijada ushbu modda 4′-O-metil-8-γ,γ-dimetilallilkaempferol-3-ramnozid-7-glukozid ekanligi aniqlandi.



4-moddaning kimyoviy tuzilishi.

5-modda. $C_{76}H_{52}O_{46}$, Mm 1701, Och jigarrang rangli amorf kukun, Rf 0.28, 035 (QX, YQX, 7,13-sistema), S.h 220–230 °C, UB-spektr (EtOH, λmax, nm): 278, 218, IQ (ν, sm^{-1}) IQ spektr: 3420–3300 (OH guruhlari), 1715–1680 (>C=O, galloil efirlari), 1610, 1585, 1510 (Ar), 1450, 1310, 1245 (C–O, ester), 1080–1020 (polisaxarid va efir bog‘lari). ^{1}H PMR spektr (δ, 600 MGrs, $CD_3OD + CCI_4$, m.u.): 6.28, (d (8.3), H-1), 5.63 (dd (9.7, 8.3), H-2), 5.95 (t (9.7), H-3), 5.67 (t (9.7), H-4), 4.47 (m, H-5), 4.43, 4.57 (m, H-6), 7.10 (c, gall-1′, H-2′,6′), 7.00 (c, gall-2′, H-2′,6′), 6.95 (c, gall-3′, H-2′,6′), 7.03 (c, gall-4′, H-2′,6′), 7.16 (c, gall-6′, H-2′,6′), 7.11 (c, gall-1′′, H-2′,6′), 7.11 (c, gall-2′′, H-2′,6′), 7.11 (c, gall-3′′, H-2′,6′), 7.11 (c, gall-4′′, H-2′,6′), 7.27 (c, gall-6′′, H-2′,6′), ^{13}C YAMR spektr (δ, 150 MGrs, $CD_3OD + CCI_4$, m.u.): Glu: 93.74 (C-1), 72.11 (C-2), 74.26 (C-3), 69.71 (C-4), 74.30 (C-5), 63.03 (C-6), gall-1′: 119.62 (C-1), 110.59 (C-2), 139.8-140.6 (C-3), 140.68 (C-4), 146.34 (C-5), 166.15 (C-7), gall-2′: 120.26 (C-1), 110.36 (C-2), 139.8-140.6 (C-3), 140.26 (C-4), 146.25 (C-5), 166.95 (C-7), gall-3′: 120.38 (C-1), 110.30 (C-2), 139.8-140.6 (C-3), 140.10 (C-4), 146.17 (C-5), 167.23 (C-7), gall-4′: 120.22 (C-1), 110.42 (C-2), 139.8-140.6 (C-3), 140.33 (C-4), 146.25 (C-5), 166.85 (C-7), gall-6′: 121.03 (C-1), 110.35 (C-2), 139.8-140.6 (C-3), 139.91 (C-4), 146.34 (C-5), 167.87 (C-7), gall-1′′: 120.26 (C-1), 110.81 (C-2,6), 146.48 (C-3,5), 140.44 (C-4), 166.15 (C-7), gall-2′′: 120.26 (C-1), 110.30 (C-2,6), 146.46 (C-3,5), 139.46 (C-4), 170.44 (C-7), gall-3′′: 121.98 (C-1), 110.30 (C-2,6), 146.46 (C-3,5), 139.46 (C-4), 170.44 (C-7), gall-4′′: 121.98 (C-1), 110.30 (C-2,6), 146.46 (C-3,5), 140.44 (C-4), 166.57 (C-7), gall-6′′: 121.98 (C-1), 110.30 (C-2,6), 146.43 (C-3,5), 139.46 (C-4), 170.44 (C-7). Natijada ushbu modda 1,2,3,4,6-penta-O-(3,4-digidroksi)-3,4,5-trigidroksibenzoil-β-D-glukopiranoza ekanligi aniqlandi.



5-moddaning kimyoviy tuzilishi.

Karelinia caspia L o'simligi polifenollari tarkibidan ajratib olingan qolgan fraksiyalarning tozalik darajasini oshirish va kimyoviy tuzilishlarini o'rganish ishlari amalga oshirilmoqda.

Xulosa

Karelinia caspia L o'simligidan maqbul usul yordamida polifenollar yig'indisi ajratib olindi. Polifenollar yig'indisi alohida fraksiyalarga ajratilib har bir fraksiyalarning tozalik darajalari organildi. Beshta moddaning kimyoviy tuzilishi aniqlandi. Hozirda kimyoviy tuzilishi aniqlangan moddalarning biologik faolliklarini aniqlash ishlari olib borilmoqda.

Mualliflar hissasi, manfaatlar to'qnashuvi va moliyalashtirish

Mualliflar hissasi: barcha mualliflar tadqiqot konsepsiyasi, ma'lumotlarni tahlil qilish va maqola matnini tayyorlashda teng hissa qo'shgan. Manfaatlar to'qnashuvi: mualliflar manfaatlar to'qnashuvi yo'qligini bildiradi.

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