瓦山锥化学成分及其酪氨酸酶抑制活性研究

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摘　要:研究瓦山锥(*Castanopsis ceratacantha*)叶子中的化学成分及其酪氨酸酶抑制活性。综合采用 Sephadex LH-20、Toyopearl HW-40F、Diaion HP20SS 等多种柱色谱和高效液相色谱等分离手段对瓦山锥叶子 80% 乙醇提取物进行化学成分的分离纯化,通过波谱数据分析并结合文献对照鉴定化合物结构,并对瓦山锥叶子中的特征化化合物进行酪氨酸酶抑制活性筛选。在前期研究中,本课题组已从瓦山锥叶子 80% 乙醇提取物分离得到 11 个化合物,在此研究基础上继续从瓦山锥叶子 80% 乙醇提取物中分离得到 17 个化合物,分别鉴定为原儿茶酸(**1**)、6-*O*-没食子酰基熊果苷(**2**)、3-甲氧基-4-羟基苯酚 1-*O*-*β*-*D*-(6′-*O*-没食子酰基)吡喃葡萄糖苷(**3**)、瓦隆酸双内酯(**4**)、1,2,3,4,6-五-*O*-没食子酰基-*β*-*D*-吡喃葡萄糖(**5**)、长梗马兜铃素(**6**)、木麻黄鞣亭(**7**)、praecoxin A(**8**)、栗木素(**9**)、栎木素(**10**)、chinquapinic acid(**11**)、栎木鞣花素(**12**)、栗木鞣花素(**13**)、castacrenin D(**14**)、山柰酚(**15**)、异槲皮素(**16**)、aviculin(**17**)。所有化合物皆为首次从瓦山锥植物中分离得到,实验结果显示化合物 **14** 具有较强的酪氨酸酶抑制活性,其他化合物也具有较好的抑制活性。

关键词:瓦山锥;化学成分;单宁;结构鉴定;酪氨酸酶抑制剂
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Study on the chemical composition and tyrosinase inhibitory activity of *Castanopsis ceratacantha*

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Abstract: Study the chemical constituents and their tyrosinase inhibitory activity in the leaves of *Castanopsis ceratacantha*. The compounds were isolated and purified by Sephadex LH-20, Toyopearl HW-40F, Diaion HP20SS and other column chromatography and HPLC. The structures of compounds were elucidated by spectroscopic data and comparison with literatures. The characteristic compounds in the leaves of *C. ceratacantha* were screened for tyrosinase inhibitory activity. In the previous research, our group had isolated 11 compounds from the 80% ethanol extract of *C. ceratacantha*. On the basis of this research, 17 compounds were obtained and their structures were identified as protocatechuic acid (**1**), 6-*O*-galloylarbutin (**2**), 3-methoxy-4-hydroxyphenol 1-*O*-*β*-*D*-(6′-*O*-galloyl) glucopyranoside (**3**), valoneic acid dilactone (**4**), 1,2,3,4,6-penta-*O*-galloyl-*β*-*D*-glucopyranose (**5**), pedunculagin (**6**), casuarictin (**7**), praecoxin A (**8**), castalin (**9**), vescalin (**10**), chinquapinic acid (**11**), vescalagin (**12**), castalagin (**13**), castacrenin D (**14**), kaempferol (**15**), isoquercitrin (**16**), aviculin (**17**). All the compounds were isolated from this plant for the first time. Experimental results show that compound **14** has strong tyrosinase inhibitory activity, and other compounds also have better inhibitory activity.

Key words: *Castanopsis ceratacantha*; chemical constituents; tannins; structural identification; tyrosinase inhibitor

瓦山锥(*Castanopsis ceratacantha*)为壳斗科锥属(*Castanopsis*)植物,又名瓦山栲、黄山栲等,主要分布于云南、贵州、四川西南部,民间广泛用于止血、止渴、止泻(坚果)、慢性溃疡等疾病的治疗[1,2]。目

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前,国内外并没有关于瓦山锥的化学成分和生物活性研究的相关文献,本课题组对瓦山锥叶子乙醇提取物进行了初步化学成分研究[3],研究发现瓦山锥叶子中含有结构新颖的三萜鞣花单宁类化合物,此类化合物是目前发现不多的一类化合物,由五环三萜和独特多元酚通过酯化反应生成,目前只在锥属植物中分离得到。具有较好的抗氧化、抗癌、降脂活性。为了阐明瓦山锥的物质基础,发现新的三萜鞣花单宁类化合物,本实验对瓦山锥 80% 乙醇提取物进行了系统的分离纯化。酪氨酸酶(又称为多酚氧化酶)与生物体内合成色素相关,直接影响黑色素的合成,是黑色素合成的限速酶,在生物体内具有多种重要的生理功能,特别是在皮肤美白、抗氧化作用等方面表现尤为突出,还与色素障碍疾病及恶性黑色素肿瘤的发生和治疗有关。酪氨酸酶在黑色素形成过程中作用位点主要为羟基,瓦山锥乙醇提取物中主要为单宁类化合物,其中以含有多个独特多元酚结构的化合物为主,具有很好的抗氧化、抗癌活性,是否具有酪氨酸酶抑制活性还未见报道。本实验对分离得到的多个特征性单宁类化合物进行了酪氨酸酶抑制活性筛选,以期找到活性较好的天然化合物。

1 材料与方法

1.1 仪器与材料

Brucker Avance 500 MHz 超导核磁共振波谱仪(瑞典 Bruker);N-1100 旋转蒸发仪(东京理化);CA-1111 冷却水循环(东京理化);自动接收仪(日本 Advantec);F254 硅胶薄层板(德国默克);Sephadex LH-20(25 ~ 100 μm;GE Healthcare Bio-Science AB);Toyopearl HW-40F(日本 TOSOH 公司);Diaion HP20SS(75 ~ 150 μm;Mistubishi Chemical);MCI gel CHP 20P(75 ~ 150 μm;Mistubishi Chemical);Toyopearl Butyl-650C(日本 TOSOH 公司);酪氨酸酶(上海源叶);L-多巴(上海源叶);磷酸盐缓冲盐水(北京 Solarbio);提取、分离所用试剂均为分析纯。

瓦山锥于 2017 年 8 月采自云南省景洪市,经广西壮族自治区中国科学院广西植物研究所丁涛副研究员鉴定为壳斗科锥属植物瓦山锥(*Castanopsis ceratacantha*)的树叶,凭证标本(20170826)保存于广西壮族自治区中国科学院广西植物研究所广西植物功能物质研究与利用重点实验室。

1.2 实验方法

1.2.1 提取分离

干燥瓦山锥叶 3.5 kg,切成碎片后用 80% 乙醇室温浸提 2 次,每次 32 L,每次 7 天,合并提取液并过滤,滤液经减压浓缩后得浸膏 481.3 g。浸膏水溶解后经 Sephadex LH-20(8.5 cm × 40 cm)柱层析分离,甲醇-水(0%→100%,每 20% 为 1 梯度,每 1 梯度 2 L)和 60% 丙酮-水(3 L)溶液洗脱,经薄层层析分析划段,得到 8 个馏分:Fr. 1 ~ 8。Fr. 3 经 MCI gel CHP 20P、Diaion HP20SS、Toyopearl HW-40F 等色谱柱反复层析分离纯化,得到化合物 **2**(34 mg)、**11**(9 mg)、**12**(0.3 g)、**13**(0.7 g)、**16**(68 mg)、**17**(0.5 g)。Fr. 4 经 Diaion HP20SS、Toyopearl HW-40F、Sephadex LH-20 等色谱柱反复层析分离纯化得化合物 **1**(86 mg)、**3**(61 mg)、**9**(72 mg)、**10**(38 mg)、**14**(94 mg)、**15**(34 mg)。Fr. 6 经 Diaion HP20SS、Toyopearl HW-40F、Toyopearl Butyl-650C、ODS C_{18} 等色谱柱层析分离纯化,得到化合物 **4**(100 mg)、**15**(74 mg)。Fr. 7 经 MCI gel CHP 20P、Toyopearl HW-40F、Toyopearl Butyl-650C 等色谱柱层析分离纯化,得到化合物 **6**(0.6 g)。Fr. 8 经 MCI gel CHP 20P、Sephadex LH-20、Diaion HP20SS 等色谱柱层析分离纯化,得到化合物 **5**(72 mg)、**7**(62 mg)、**8**(78 mg)。

1.2.2 抑酶活性筛选

以 1 mg/mL 的 L-多巴为底物,现配现用,将购买的蘑菇酪氨酸酶配制成酶活力为 100 U/mL 的酶溶液备用,以曲酸为阳性对照。在酶反应组和空白组分别加入 25 μL 不同浓度的样品和磷酸缓冲液,加入 25 μL 酪氨酸酶在 37 ℃孵育 5 min 后再加入 100 μL 1 mg/mL 的 L-多巴溶液,在 37 ℃保温箱反应 5 min 后,在波长 475 nm 下测定吸光度,反应组对照和空白对照以失活酶代替活性酶[4,5]。按照下述公式计算酶活抑制率,酪氨酸酶抑制率 = [1-($A_{反应组}$-$A_{反应对照}$)/($A_{空白组}$-$A_{空白对照}$)] × 100%。重复上述实验 3 次。

2 结果与讨论

2.1 结构鉴定

化合物 1 白色针状结晶;^{1}H NMR(500 MHz,$(CD_3)_2CO$) δ:7.45(1H,d,J = 2.0 Hz,H-2),7.38(1H,dd,J = 8.3,2.0 Hz,H-6),6.85(1H,d,J = 8.3 Hz,H-5);^{13}C NMR(125 MHz,$(CD_3)_2CO$) δ:115.5(C-5),117.3(C-2),122.8(C-1),123.3(C-6),145.5(C-3),150.7(C-4),168.6(-COOH)。上述波谱数据与文献[6]报道一致,故鉴定化合物 **1** 为

原儿茶酸。

化合物 2　淡黄色粉末;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:7.14(2H,s,H-2″,6″),6.91(2H,d,J = 8.9 Hz,H-2,6),6.66(2H,d,J = 8.9 Hz,H-3,5),4.78(1H,d,J = 7.7 Hz,H-1′),4.57(1H,dd,J = 11.8,2.0 Hz,H-6a′),4.33(1H,dd,J = 11.8,6.9 Hz,H-6b′),3.75 ~ 3.78(1H,m,H-5′),3.45 ~ 3.57(3H,m,H-2′,3′,4′);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:64.6(C-6),71.0(C-4),74.2(C-2),74.7(C-5),77.2(C-3′),102.9(C-1),109.9(C-2″,6″),116.3(C-2′,6′),118.8(C-3′,5′),121.1(C-1″),139.0(C-4″),145.9(C-3″,5″),151.6(C-1′),153.2(C-4′),167.2(C-7″)。上述波谱数据与文献[7]报道一致,故鉴定化合物 **2** 为 6-*O*-没食子酰基熊果苷。

化合物 3　白色粉末;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:7.13(2H,s,H-2″,6″),6.67(1H,d,J = 8.2 Hz,H-5),6.66(1H,d,J = 2.9 Hz,H-2),6.57(1H,dd,J = 8.2,2.9 Hz,H-6),4.81(1H,d,J = 7.8 Hz,H-1′),4.59(1H,dd,J = 11.9,2.0 Hz,H-6a′),4.35(1H,dd,J = 11.9,6.6 Hz,H-6b′),3.70(3H,s,-OCH_3),3.77 ~ 3.80(1H,m,H-5′),3.45 ~ 3.58(3H,m,H-2′,3′,4′);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:56.2(-OCH_3),64.6(C-6′),71.0(C-4′),74.3(C-2′),74.8(C-5′),77.3(C-3′),103.0(C-2),103.4(C-1′),109.4(C-6),109.8(C-2″,6″),115.7(C-5),121.2(C-1″),139.0(C-4″),142.5(C-4),146.0(C-3″,5″),148.5(C-3),152.0(C-1),167.2(C-7″)。上述波谱数据与参考文献[8]报道一致,故鉴定化合物 **3** 为 3-甲氧基-4-羟基苯酚 1-*O*-*β*-*D*-(6′-*O*-没食子酰基)吡喃葡萄糖苷。

化合物 4　白色粉末;^{1}H NMR(500 MHz,DMSO-d_6)δ:7.49(1H,s,H-5),6.99(1H,s,H-6″),6.94(1H,s,H-5′);^{13}C NMR(125 MHz,DMSO-d_6)δ:106.7(C-6″),108.2(C-6),108.4(C-6′),108.6(C-1″),110.5(C-1),112.1(C-1′),114.0(C-5),114.8(C-5′),135.3(C-4″),136.3(C-2′),136.7(C-2),139.2(C-2″),139.6(C-3″),139.7(C-3),141.1(C-3′),143.0(C-5″),148.7(C-4′),149.6(C-4),159.2(C-7′),159.3(C-7),166.0(C-7″)。上述波谱数据与参考文献[9]报道一致,故鉴定化合物 **4** 为瓦隆酸双内酯。

化合物 5　淡黄色粉末;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:7.16,7.10,7.06,7.01,6.99(each 2H,s,galloyl-H-2,6),6.31(1H,d,J = 8.3 Hz,H-1),6.03(1H,t,J = 9.7 Hz,H-3),5.68(1H,t,J = 9.7 Hz,H-4),5.64(1H,dd,J = 9.7,8.3 Hz,H-2),4.55 ~ 4.61(2H,m,H-5,6a),4.33(1H,dd,J = 12.7,5.0 Hz,H-6b);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:62.9(C-6),69.4(C-4),71.7(C-2),73.3(C-5),73.9(C-3),93.3(C-1),109.9,110.0,110.0,110.1,110.2(C-2′,6′),119.5,120.0,120.1,120.2,121.0(C-1′),139.1,139.3,139.4,139.5,139.9(C-4′),145.9,145.9,146.0,146.0,146.1(C-3′,5′),165.2,166.0,166.1,166.3,166.6(C-7′)。上述波谱数据与参考文献[10]报道一致,故鉴定化合物 **5** 为 1,2,3,4,6-五-*O*-没食子酰基-*β*-*D*-吡喃葡萄糖。

化合物 6　淡棕色粉末;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:6.69,6.68,6.62,6.61,6.58,6.53,6.35,6.34(s,HHDP H-3,3′),5.50 ~ 5.46(2H,m,*α*-glu-H-1,*α*-glu-H-3),5.34 ~ 5.22(3H,m,*α*-glu-H-6a,*β*-glu-H-3,*β*-glu-H-6a),5.12 ~ 5.06(4H,m,*α*-glu-H-2,*α*-glu-H-4,*β*-glu-H-1,*β*-glu-H-4),4.86(1H,dd,J = 9.0,8.3 Hz,*β*-glu-H-2),4.62(1H,ddd,J = 9.8,6.9,1.5 Hz,*α*-glu-H-5),4.23(1H,ddd,J = 9.7,6.7,0.9 Hz,*β*-glu-H-5),3.86(1H,dd,J = 13.4,1.0 Hz,*β*-glu-H-6b),3.79(1H,dd,J = 12.9,1.5 Hz,*α*-glu-H-6b);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:63.5(*α*-glu-C-6),63.5(*β*-glu-C-6),67.3(*α*-glu-C-5),69.5(*β*-glu-C-4),69.8(*α*-glu-C-4),72.3(*β*-glu-C-5),75.5(*α*-glu-C-2),75.7(*α*-glu-C-3),77.5(*β*-glu-C-3),78.3(*β*-glu-C-2),91.7(*α*-glu-C-1),95.3(*β*-glu-C-1),107.2,107.3,107.4,107.5,107.6,107.7,108.2,108.3(HHDP-C-3,3′),114.0,114.1,114.8,114.9,115.6,115.7,115.8,115.9(HHDP-C-1,1′),125.9,125.9,126.4,126.4,126.5,126.5,126.5,126.7(HHDP-C-2,2′),136.0,136.1,136.3,136.4,136.4,136.5,136.5,136.5(HHDP-C-5,5′),144.1,144.2,144.3,144.4,144.4,144.4,144.5,144.5(HHDP-C-6,6′),145.0,145.0,145.1,145.1,145.1,145.2,145.2,145.2(HHDP-C-4,4′),167.7,167.8,168.1,168.2,168.8,168.9,169.3,169.3(HHDP-C-7,7′)。上述波谱数据与参考文献[11]报道一致,故鉴定化合物 **6** 为长梗马兜铃素。

化合物 7　深褐色粉末;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:7.17(2H,s,galloyl-2,6),6.66,6.55,6.46,6.36(each 1H,s,HHDP-3′,3″,3‴,3⁗),

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6.20(1H,d,J = 8.5 Hz,H-1),5.43(1H,dd,J = 10.2,8.5 Hz,H-2),5.33(1H,dd,J = 13.3,6.6 Hz,H-6a),5.17(1H,t,J = 9.2 Hz,H-3),5.14(1H,t,J = 9.2 Hz,H-4),4.48(1H,dd,J = 9.8,6.6 Hz,H-5),3.86(1H,d,J = 13.3 Hz,H-6b);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:63.0(C-6),69.1(C-4),73.4(C-5),76.0(C-2),77.2(C-3),92.2(C-1),107.1,107.2,107.5,108.1(HHDP C-1′,1″,1‴,1⁗),110.3(galloyl C-2,6),114.4,115.0,115.8,116.1(HHDP C-3′,3″,3‴,3⁗),119.6(galloyl C-1),125.6,125.9,126.2,126.2(HHDP C-2′,2″,2‴,2⁗),136.2,136.4,136.5,136.6(HHDP C-5′,5″,5‴,5⁗),140.0(galloyl C-4′),144.4,144.5,144.5,145.1,145.1,145.1,145.3(HHDP C-4′,4″,4‴,4⁗,6′,6″,6‴,6⁗),146.3(galloyl C-3,5),165.1(galloyl C-7),168.0,168.2,168.7,169.4(HHDP C-7′,7″,7‴,7⁗)。上述波谱数据与参考文献[12]报道一致,故鉴定化合物**7**为木麻黄鞣亭。

化合物 8 浅棕色粉末;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:7.12(1H,s,α-valoneyl-H-6″),7.12(1H,β-valoneyl-H-6″),6.60(1H,s,α-valoneyl-H-3),6.59(1H,s,β-valoneyl-H-3),6.55(1H,s,α-HHDP-H-3),6.50(1H,s,β-HHDP-H-3),6.35(1H,s,α-HHDP-H-3′),6.34(1H,s,β-HHDP-H-3′),6.24(1H,s,α-valoneyl-H-3′),6.22(1H,s,β-valoneyl-H-3′),5.42(1H,t,J = 9.7 Hz,α-H-3),5.35(1H,d,J = 3.6 Hz,α-H-1),5.21 ~ 5.12(3H,m,α-H-6a,β-H-3,β-H-6a),5.03 ~ 4.92(4H,m,α-H-2,α-H-4,β-H-1,β-H-4),4.79(1H,dd,J = 9.0,8.3 Hz,β-H-2),4.55 ~ 4.50(1H,m,α-H-5),4.14(1H,dd,J = 8.9,6.9 Hz,β-H-5),3.72(1H,d,J = 12.8 Hz,β-H-6b),3.66(1H,d,J = 2.7 Hz,α-H-6b);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:63.5(α-C-6),63.6(β-C-6),67.2(α-C-5),69.6(β-C-4),69.9(α-C-4),72.2(β-C-5),75.5(α-C-2),75.7(α-C-3),77.5(β-C-3),78.2(β-C-2),91.5(α-C-1),95.2(β-C-1),105.4,105.5(α-valoneyl-C-3′,β-valoneyl-C-3′),107.1,107.3,107.3,107.4,107.5,107.6(α-valoneyl-C-3,β-valoneyl-C-3,α-HHDP-C-3,β-HHDP-C-3,α-HHDP-C-3′,β-HHDP-C-3′),109.8,109.9(α-valoneyl-C-6″,β-valoneyl-C-6″),114.3,114.4(α-HHDP-C-1′,β-HHDP-C-1′),114.8,114.9(α-HHDP-C-1,β-HHDP-C-1),115.4,115.4(α-valoneyl-C-1″,β-valoneyl-C-1″),116.0,116.1(α-valoneyl-C-1,β-valoneyl-C-1),117.7,117.7(α-valoneyl-C-1′,β-valoneyl-C-1′),125.5,125.5,125.9,126.0(α-valoneyl-C-2,β-valoneyl-C-2,α-valoneyl-C-2′,β-valoneyl-C-2′),126.4,126.4,126.5,126.6(α-HHDP-C-2,β-HHDP-C-2,α-HHDP-C-2′,β-HHDP-C-2′),136.1,136.1(α-HHDP-C-5′,β-HHDP-C-5′),136.4,136.5(α-HHDP-C-5,β-HHDP-C-5),136.6,136.7(α-valoneyl-C-5,β-valoneyl-C-5),137.0,137.0(α-valoneyl-C-5′,β-valoneyl-C-5′),137.1,137.2(α-valoneyl-C-2″,β-valoneyl-C-2″),139.9,140.0(α-valoneyl-C-4″,β-valoneyl-C-4″),140.1,141.1(α-valoneyl-C-3″,β-valoneyl-C-3″),143.2,143.2(α-valoneyl-C-5″,β-valoneyl-C-5″),144.3,144.3,144.7,144.7(α-HHDP-C-6,β-HHDP-C-6,α-HHDP-C-6′,β-HHDP-C-6′),149.9,149.9(α-valoneyl-C-6′,β-valoneyl-C-6′),145.0,145.0,145.1,145.2(α-valoneyl-C-6,β-valoneyl-C-6,α-HHDP-C-4,β-HHDP-C-4,α-HHDP-C-4′,β-HHDP-C-4′),145.3(α-valoneyl-C-4,β-valoneyl-C-4),147.0,147.1(α-valoneyl-C-4′,β-valoneyl-C-4′),166.9,167.0(α-valoneyl-C-7″,β-valoneyl-C-7″),168.1,168.1,168.1,168.2(α-valoneyl-C-7,β-valoneyl-C-7,α-valoneyl-C-7′,β-valoneyl-C-7′),169.0,169.1(α-HHDP-C-β-7,β-HHDP-C-7),169.5,169.5(α-HHDP-C-β-7′,β-HHDP-C-7′)。上述波谱数据与参考文献[13]报道一致,故鉴定化合物**8**为 praecoxin A。

化合物 9 白色粉末;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:6.74(1H,s,H-III-6),5.63(1H,d,J = 4.7 Hz,H-1),5.18(1H,m,H-5),5.14(1H,dd,J = 4.7,1.4 Hz,H-2),4.97(1H,dd,J = 6.9,1.4 Hz,H-3),4.04(1H,t,J = 6.9 Hz,H-4),3.91(1H,dd,J = 12.2,3.6 Hz,H-6),3.84(1H,dd,J = 12.2,5.7 Hz,H-6);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:62.8(C-6),68.2(C-3),68.8(C-1),69.9(C-4),72.9(C-5),74.4(C-2),109.0(C-III-2),112.9(C-II-2),114.1(C-I-6),114.4(C-III-6),115.2(C-II-6),116.3(C-I-2),123.1(C-I-1),126.8(C-III-1),128.6(C-II-1),132.1(C-II-4),136.1(C-III-4),137.3(C-I-4),143.7(C-I-3),143.9(C-I-5),144.1(C-III-5),144.5(C-II-5),145.4(C-II-3),146.9(C-III-3),163.9(C-I-7),166.0(C-II-7),167.1(C-III-7)。上述波谱数据与参考文献[14]报道一致,故鉴定化合物**9**为栗木素。

化合物 10 白色粉末;^{1}H NMR(500 MHz,

(CD_3)$_2$CO) δ:6.76(1H,s,H-III-6),5.35(1H,t,J = 1.6 Hz,H-2),5.15(1H,m,H-5),4.65(1H,d,J = 2.1 Hz,H-1),4.36(1H,dd,J = 6.9,1.6 Hz,H-3),3.95(1H,t,J = 6.9 Hz,H-4),3.90(1H,dd,J = 12.4,3.4 Hz,H-6),3.82(1H,dd,J = 12.4,6.3 Hz,H-6);^{13}C NMR(125 MHz,(CD_3)$_2$CO) δ:62.1(C-6),65.6(C-3),69.2(C-1),71.1(C-4),74.3(C-5),76.5(C-2),108.7(C-III-2),113.4(C-II-2),114.0(C-I-6),114.8(C-III-6),116.0(C-II-6),117.6(C-I-2),125.0(C-I-1),126.2(C-III-1),128.3(C-II-1),135.0(C-II-4),136.0(C-III-4),137.3(C-I-4),143.8(C-I-3),144.1(C-I-5),144.4(C-III-5),144.5(C-II-5),145.4(C-II-3),147.2(C-III-3),165.9(C-I-7),166.2(C-II-7),168.0(C-III-7)。上述波谱数据与参考文献[15]报道一致,故鉴定化合物**10**为栎木素。

化合物 11 白色粉末;^{1}H NMR(500 MHz,CD_3OD) δ:6.81,6.71,6.61(each 1H,s,arom.-H),5.64(1H,dd,J = 7.6,1.2 Hz,H-5),5.27 ~ 5.25(1H,m,H-1),5.19(1H,t,J = 7.2 Hz,H-4),5.04(1H,dd,J = 13.0,2.6 Hz,H-6a),4.78(1H,d,J = 2.1 Hz,H-2),4.56(1H,dd,J = 7.0,1.1 Hz,H-3),4.04(1H,d,J = 12.8 Hz,H-6b);^{13}C NMR(125 MHz,CD_3OD) δ:66.0(C-6),69.0(C-3),70.1(C-4),71.9(C-5),73.8(C-2),78.7(C-1),108.1(C-V-2),108.7(C-III-2),109.5(C-IV-2),114.1(C-II-2),115.2(C-I-6),115.4(C-III-6),116.2(C-II-6),116.8(C-IV-6),116.8(C-V-6),117.7(C-I-2),124.9(C-I-1),125.2(C-III-1),125.3(C-II-1),126.9(C-IV-1),128.1(C-V-1),136.1(C-II-4),137.1(C-III-4),137.7(C-IV-4),137.9(C-V-4),138.9(C-I-4),144.7(C-I-3),144.9(C-I-5),144.9(C-III-5),145.1(C-II-5),145.2(C-IV-5),146.1(C-II-3),146.1(C-III-3),146.1(C-IV-3),146.2(C-V-3),148.8(C-V-5),166.1(C-I-7),166.5(C-II-7),167.4(C-III-7),168.1(C-IV-7),170.4(C-V-7)。上述波谱数据与参考文献[16]报道一致,故鉴定化合物**11**为 chinquapinic acid。

化合物 12 白色粉末;^{1}H NMR(500 MHz,CD_3OD) δ:6.78,6.65,6.59(each 1H,s,arom.-H),5.60(1H,m,H-5),5.25(1H,br s,H-2),5.17(1H,t,J = 7.2 Hz,H-4),4.95(1H,d,J = 12.9,2.8 Hz,H-6b),4.57(1H,d,J = 2.1 Hz,H-1),4.54(1H,d,J = 7.9,2.0 Hz,H-3),4.03(1H,d,J = 12.9 Hz,H-6a);^{13}C NMR(125 MHz,CD_3OD) δ:66.0(C-1),66.0(C-6),68.8(C-3),70.3(C-4),71.7(C-5),78.6(C-2),107.9(C-V-2),108.7(C-III-2),109.4(C-IV-2),113.9(C-II-2),115.1(C-I-6),115.4(C-III-6),115.5(C-II-6),115.8(C-IV-6),116.9(C-V-6),117.6(C-I-2),124.4(C-I-1),124.7(C-III-1),125.1(C-II-1),126.8(C-IV-1),127.9(C-V-1),136.0(C-II-4),136.9(C-III-4),137.5(C-IV-4),138.1(C-V-4),138.6(C-I-4),144.6(C-I-3),144.9(C-I-5),144.9(C-III-5),145.1(C-II-5),145.2(C-IV-5),145.2(C-II-3),145.3(C-III-3),146.0(C-IV-3),146.1(C-V-3),148.8(C-V-5),165.9(C-I-7),166.5(C-II-7),167.4(C-III-7),168.0(C-IV-7),170.4(C-V-7)。上述波谱数据与参考文献[16]报道一致,故鉴定化合物**12**为栎木鞣花素。

化合物 13 白色粉末;^{1}H NMR(500 MHz,CD_3OD) δ:6.80,6.71,6.60(各 1H,s,arom.-H),5.62(1H,d,J = 4.6 Hz,H-1),5.37(1H,d,J = 7.5,1.6 Hz,H-5),5.17(1H,t,J = 7.5 Hz,H-4),5.03(1H,d,J = 12.7,2.2 Hz,H-6b),5.00(1H,d,J = 7.0,2.2 Hz,H-3),4.97(1H,br s,H-2),4.03(1H,d,J = 12.9 Hz,H-6a);^{13}C NMR(125 MHz,CD_3OD) δ:65.9(C-6),68.9(C-3),70.0(C-1),71.8(C-4),73.6(C-5),75.6(C-2),108.0(C-V-2),108.7(C-III-2),109.5(C-IV-2),113.9(C-II-2),115.0(C-I-6),115.3(C-III-6),115.6(C-II-6),116.1(C-IV-6),116.7(C-V-6),117.6(C-I-2),124.4(C-I-1),124.8(C-III-1),125.2(C-II-1),126.8(C-IV-1),128.0(C-V-1),136.0(C-II-4),137.0(C-III-4),137.6(C-IV-4),137.9(C-V-4),138.8(C-I-4),144.4(C-I-3),144.8(C-I-5),144.8(C-III-5),144.9(C-II-5),145.0(C-IV-5),145.2(C-II-3),145.3(C-III-3),146.0(C-IV-3),146.0(C-V-3),148.3(C-V-5),166.1(C-I-7),166.6(C-II-7),167.4(C-III-7),168.1(C-IV-7),170.4(C-V-7)。上述波谱数据与参考文献[17]报道一致,故鉴定化合物**13**为栗木鞣花素。

化合物 14 白色粉末;^{1}H NMR(500 MHz,CD_3OD) δ:7.33,6.73,6.67,6.57(各 1H,s,arom.-H),5.63(1H,d,J = 7.9 Hz,H-5),5.32(1H,s,H-1),5.30(1H,d,J = 7.9 Hz,H-4),5.26(1H,br s,H-2),5.01(1H,d,J = 7.9 Hz,H-3),4.73(1H,dd,J = 12.5,1.8 Hz,H-6a),3.98(1H,d,J = 12.5 Hz,H-6b);^{13}C NMR(125 MHz,CD_3OD) δ:41.2(C-

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1),66.0(C-6),69.8(C-4),71.2(C-5),72.7(C-3),78.4(C-2),107.1,108.7,110.1,110.1,111.7,112.8,113.6,114.2,114.4,115.0,116.5,117.0(HHDP,TP-C-2,6),121.1,125.2,125.8,126.2,127.6,128.4(HHDP,TP-C-1),135.4,135.6,136.4,136.8,136.8,137.6(HHDP,TP-C-4),143.2,143.3,144.1,144.2,144.3,144.6,144.7,144.8,145.1,145.4,145.6,147.2(HHDP,TP-C-3,5),165.7,166.5,166.7,166.9,169.1,170.1(HHDP,TP-C-7)。上述波谱数据与参考文献[18]报道一致,故鉴定化合物**14**为castacrenin D。

化合物15 黄色针状结晶;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:8.09(2H,d,J = 9.0 Hz,H-2′,6′),6.97(2H,d,J = 9.0 Hz,H-3′,5′),6.50(1H,d,J = 2.1 Hz,H-8),6.23(1H,d,J = 2.1 Hz,H-6);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:94.4(C-8),99.0(C-6),103.8(C-10),116.2(C-3′,5′),122.9(C-1′),130.3(C-2′,6′),136.4(C-3),147.2(C-2),157.6(C-9),160.2(C-4′),161.7(C-5),165.1(C-7),176.5(C-4)。上述波谱数据与参考文献[19]报道一致,故鉴定化合物**15**为山柰酚。

化合物16 黄色针状结晶;^{1}H NMR(500 MHz,CD_3OD)δ:7.64(1H,dd,J = 8.6,2.2 Hz,H-6′),7.62(1H,d,J = 2.2 Hz,H-2′),6.85(1H,d,J = 8.6 Hz,H-5′),6.38(1H,d,J = 2.0 Hz,H-8),6.19(1H,d,J = 2.0 Hz,H-6),5.34(1H,d,J = 7.7 Hz,H-1″),3.76(1H,d,J = 9.7 Hz,H-6″a),3.59(1H,t,J = 9.7 Hz,H-6″b),3.56~3.48(4H,m,H-2″,3″,4″,5″);^{13}C NMR(125 MHz,CD_3OD)δ:72.8(C-4″),75.4(C-2″),77.0(C-3″),77.6(C-5″),94.8(C-8),100.0(C-6),104.2(C-1″),105.6(C-10),116.0(C-2′),117.3(C-5′),122.8(C-6′),123.5(C-1′),135.4(C-3),145.9(C-4′),150.0(C-3′),158.4(C-2),159.0(C-9),162.9(C-5),165.9(C-7),179.2(C-4)。上述波谱数据与参考文献[20]报道一致,故鉴定化合物**16**为异槲皮素。

化合物17 白色粉末;^{1}H NMR(500 MHz,CD_3OD)δ:6.77(1H,d,J = 8.0 Hz,H-5′),6.66(1H,s,H-5),6.64(1H,d,J = 1.9 Hz,H-2′),6.59(1H,dd,J = 8.0,1.9 Hz,H-6′),6.18(1H,s,H-8),4.52(1H,d,J = 1.5 Hz,H-1″),3.88~3.84(2H,m,H-1,2″),3.82(1H,d,J = 2.6 Hz,H_b-2α),3.80(3H,s,-OCH_3),3.77(3H,s,-OCH_3),3.74(1H,dd,J = 10.9,3.6 Hz,H_b-3α),3.68~3.60(2H,m,H_a-3α,3″),3.54~3.49(1H,m,H-5″),3.36~3.33(1H,m,H-4″),3.11(1H,dd,J = 9.8,3.7 Hz,H_a-2α),2.85~2.81(1H,m,H-4),2.07~1.98(1H,m,H-3),1.90~1.83(1H,m,H-2),1.19(1H,d,J = 6.2 Hz,H-6″);^{13}C NMR(125 MHz,CD_3OD)δ:17.9(C-6″),33.6(C-4),40.1(C-3),45.5(C-1),48.3(C-2),56.5(2C,-OCH_3),65.4(C-2α),68.1(C-3α),70.1(C-5″),72.3(C-2″),72.5(C-3″),73.9(C-4″),102.2(C-1″),112.6(C-5),113.6(C-2′),116.1(C-5′),117.2(C-8),123.2(C-6′),129.0(C-10),134.0(C-1′),138.1(C-9),145.2(C-4′),146.1(C-7),147.3(C-3′),149.2(C-6)。上述波谱数据与参考文献[21]报道一致,故鉴定化合物**17**为aviculin。

2.2 抑酶活性筛选结果

按照“1.2.2”所述标准方法平行测定3次取平均值,实验结果显示阳性药曲酸的IC_{50}为0.077 mg/mL,化合物**14**的IC_{50}为0.034 mg/mL,显示较强的抑酶活性,其他化合物的活性较弱。实验结果如下表所示。

表1 不同化合物抑制酪氨酸酶活性的IC_{50}值

Table 1 IC_{50} values of different compounds inhibiting tyrosinase activity

化合物 Compound	IC_{50}(mg/mL)
6	1.81 ± 0.18
7	1.42 ± 0.14
8	1.36 ± 0.11
9	6.51 ± 0.09
10	5.93 ± 0.08
11	9.63 ± 0.17
12	5.17 ± 0.12
13	0.63 ± 0.12
14	0.034 ± 0.01
曲酸* Kojic acid*	0.077 ± 0.01

注:* 阳性对照。

Note: * Positive control.

3 结论

从瓦山锥叶子80%乙醇提取物中分离得到17个化合物,主要为含有多个独特多元酚结构的单宁类化合物,根据现有文献可知主要发现于锥属植物。多数锥属植物中含有三萜鞣花单宁类化合物可能与该属植物的生境和次生代谢途径有关。瓦山锥叶子80%乙醇提取物的液相色谱检测显示含有结构新颖

的三萜鞣花单宁类化合物,但是在系统的分离纯化过程中并未得到,可能在分离过程中此类化合物未得到有效富集,分离纯化的手段需要进一步完善。目前关于结构新颖的三萜鞣花单宁类化合物的生物活性研究报道较少,仅在抗癌、抗氧化、降脂等方面有初步研究。本论文没有从瓦山锥叶子 80% 乙醇提取物中分离得到新颖的三萜鞣花单宁类化合物,但是得到大量含有独特多元酚结构的化合物,根据构效关系筛选了其中 9 个含有多个独特多元酚结构的特征化合物的酪氨酸酶的抑制活性,结果显示化合物 **14** 具有较阳性药更强的抑酶活性,这可能与该化合物中含有更多酚羟基有关。单宁类化合物生物活性丰富,此特殊单宁类化合物的生物活性及作用机理需要进一步挖掘和研究。

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