ABSTRACT
An aliphatic alkene namely pentapentacontene (4) was isolated for the first time from a natural source, Gardenia aqualla, along with fourteen other compounds including nonacosanol (1), tetra-triacontanol (2), octatriacontanol (3), β-sitosterol (5) and stigmasterol (6), daucosanol (7), ursolic acid (8), uvaol (9), 3β,19α,23β,24α-tetrahydroxyurs-12-en-28-oic acid (10), lupenone (11), oleanolic acid (12), vanillin (13), vanillic acid (14) and D-mannitol (15). α-glucosidase inhibitory assay revealed that MeOH and EtOAc extracts of leaves had the best activity with IC50 of 9.65 and 20.03 μg/ml respectively. All the tested compounds showed dose dependent inhibition of α-glucosidase and some of them were found to be comparable to acarbose. Compound 10 was the most potent with IC50 = 1.72 μM. It also showed the most interesting antibacterial activity, against the isolate strain of S. typhi and P. aeruginosa and also exhibited the most significant antifungal activities against all the tested yeasts.

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1. Introduction

Diabetes Mellitus (DM), one of the most alarming health problems of the 21st century, is a chronic degenerative disease associated with hyperglycemia, originated by a resistance to insulin. The number of people suffering from diabetes around the world was estimated at 245 million in 2017, and 3.1% of them living in Africa (IDF 2017). DM accounts for roughly 90–95% of the total diabetes cases worldwide (IDF 2017), and the risk of developing this disease is strongly associated with genetics, obesity and physical inactivity (Broholm et al. 2019). Current DM therapies constituted of sulfonylureas, biguanides, thiazolidinediones and dipeptidyl peptidase-4 (DPP-4) inhibitors, aiming to reach normal glycemia and prevent later complications have already been related to side effects (Ruiz-Vargas et al. 2019) due to the constant use of these pharmaceuticals. One of the promising therapeutic approaches for reducing post-prandial hyperglycemia in DM patients is the inhibition of α-amylase and α-glucosidases enzymes. Moreover, α-glucosidase inhibitors presented just minor side effects, and therefore constitute a serious option for the control of DM (Ruiz-Vargas et al. 2019). However, DM patients are more susceptible to infections because of higher sugar levels and weakened immune systems. They are therefore more exposed to microbial infections. Infections are a source of DM imbalance, and DM patients are more likely to develop an overgrowth of Candida albicans (Casqueiro et al. 2012). These findings led to an increased interest in the discovery of new and more effective antimicrobial agents and α-glucosidase inhibitors.

Screening of α-glucosidases inhibitors and antimicrobial agents from plants sources is increasing. Such compounds have been recently developed from natural sources (Broholm et al. 2019; Ruiz-Vargas et al. 2019; Benameur et al. 2021). Gardenia aqualla Stapf & Hutch (Rubiaceae), is commonly used for the treatment of microbial diseases, breast cancer and DM (Nyemb et al. 2018). This work reports the first isolation of an aliphatic alkene from a natural source, and other secondary metabolites from aerial parts of G. aqualla together with their antimicrobial and enzyme inhibitory activities.
2. Results and discussion

The MeOH extract of seeds, the EtOAc and the MeOH extracts of the leaves of *G. aqualla* were separated by column chromatography of silica gel yielding thirteen pure compounds and one mixture (1–15). Among them, compound 4 was reported for the first time from natural source.

Compound 4 was obtained as a white shiny powder with a molecular formula C\textsubscript{55}H\textsubscript{110} deduced from its TOF-MS-ESI\textsuperscript{+} spectra which exhibited a pseudo molecular ion peak [M + Na]\textsuperscript{+} at m/z 792.6. The \textsuperscript{1}H-NMR spectrum displayed a signal of an olefinic methine proton at \(\delta_H 5.74\) (1H; ddt; \(J = 16.9, 10.2, 6.7\) Hz) and signals of terminal olefinic methylene protons at \(\delta_H 4.86\) (1H; ddt; \(J = 10.2, 2.3, 1.2\) Hz) and 4.92 (1H; ddd; \(J = 17.1, 3.6, 1.6\) Hz). These signals were respectively assigned to H-2, H-1a and H-1b. The signal at \(\delta_H 1.97\) (2H, m) was attributed to the methylene protons adjacent to H-2. The broad signal at \(\delta_H 1.21–1.30\) (m) was attributed to the hydrocarbon chain (CH\(_2\))n.

The last signal of three protons at \(\delta_H 0.81\) (3H, t, \(J = 7.0\) Hz) was attributed to the lone terminal CH\(_3\). \textsuperscript{13}C NMR spectra exhibited an olefinic methine carbon at \(\delta_C 139.2\) (C-2), an olefinic terminal methylene carbon at \(\delta_C 114.0\) (C-1), a set of methylene carbons at \(\delta_C 22.8–33.9\) (C-3–C-54) and a terminal methyl carbon \(\delta_C 14.2\) (C-55). The COSY spectrum of compound 4 showed correlations between the two terminal methylene proton H-1a and H-1b that showed also correlation with the methine proton H-2 which in turn correlated with the methylene at \(\delta_H 1.97\) (2H, m). The correlation of the methylene protons at \(\delta_H 1.97\) (2H, m) with the broad methylene peak at \(\delta_H 1.21–1.30\) (m), as well as the one of this broad methylene peak with the terminal methyl protons were also visible. The length of the aliphatic chain was deduced from the extensive analysis of the TOF-MS-ESI\textsuperscript{+} of compound 4, that shows a characteristic ion fragment [(M + Na)\textsubscript{C}2\textsubscript{2}H\textsubscript{4}]\textsuperscript{+} at m/z 764.1 resulting from the cleavage of the bond C-2–C-3 (Figure S1). Thus, the structure of compound 4 was deduced as pentapentacontene (4) (Figure 1; Table S1).

The other compounds were identified as nonacosanol (1) (Masoodi et al. 2010), tettratriacontanol (2) (Parmar et al. 1992), octatriacontanol (3) (Li et al. 2016) \(\beta\)-sitosterol (5) and stigmasterol (6) (Nyemb et al. 2018), daucosanol (7) (Nyemb et al. 2018), ursolic acid (8) (Venditti et al. 2016a), uvaol (9) (El-Shiekh et al. 2017), 3\(\beta\),19\(\alpha\),23\(\beta\),24\(\alpha\)-tetrahydroxyurs-12-en-28-oic acid (10) (Tao et al. 2012), lupenone (11) (Xu et al. 2018), oleanolic acid (12) (Venditti et al. 2016b), vanillin (13) (Xiang et al. 2011), vanillic acid (14) (Skrzypczak-Pietraszek and Pietraszek 2012) and D-mannitol (15) (Venditti et al. 2015).

The antimicrobial activity of the isolated compounds was evaluated against four bacterial strains and four yeasts using the microdilution method. Tested compounds displayed important antibacterial activities (Table S2) with minimum inhibitory concentrations (MICs) ranging from 16\(\mu\)g/ml to 128\(\mu\)g/ml, depending of compounds and bacteria strains. The most interesting activity was recorded for uvaol (9) against *Pseudomonas aeruginosa* (MIC 16\(\mu\)g/ml) and 3\(\beta\),19\(\alpha\),23\(\beta\),24\(\alpha\)-tetrahydroxyurs-12-en-28-oic acid (10) against *Salmonella typhi* and *P. aeruginosa* (MIC 32\(\mu\)g/ml for each). The results of antifungal activities (Table S3) reveal that only 10 had moderate activities (MIC 32\(\mu\)g/ml) against all the tested yeasts.
From the results of α-glucosidase inhibition (Table S4), MeOH leave extract showed the highest inhibition (IC₅₀ 9.65 μg/ml). Amongst all the tested compounds, ursolic acid (8), uvaol (9) and 3β,19α,23β,24α-tetrahydroxyurs-12-en-28-oic acid (10) exhibited significant activities, the strongest inhibition being recorded for the last one with an IC₅₀ of 1.72 μM. However, all these compounds demonstrated stronger activities than acarbose (IC₅₀ 234.6 μM).

3. Conclusion

In this study, three potent α-glucosidase inhibitors, 8, 9 and 10 were isolated along with twelve other compounds from G. aqualla. Among these, compound 4, pentapentactone, has been isolated for the first time from natural source. In addition, compound 10 exhibited notable antibacterial and antifungal activities against all the tested bacterial and yeasts strains. Moreover, this is to the best of our knowledge the first time that G. aqualla is evaluated for its α-glucosidase inhibitory activities with results showing that the isolated compounds resulted more effective than the standard drug used as positive control. The results obtained confirm the potential of this plant in the treatment of microbial diseases and DM and provide evidences for some of the traditional uses of this plant species.

Disclosure statement

No potential conflict of interest was reported by the authors.

Figure 1. Structures of isolated compounds (1–15) from seeds and leaves of G. aqualla.
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References
Benameur Q, Gervasi T, Pellizzeri V, Platcová M, Grulová D, Cicero N, Meriem-Hind b. 2021. Comparison of sensitivity to a commercial Origanum vulgare essential oil between extended-spectrum β-lactamases (ESBL-) and non-ESBL-producing Enterobacteriaceae isolates, Nat. Prod. Res., DOI: 10.1080/14786419.2021.1933969
Broholm SL, Gramsbergen SM, Nyberg NT, Jäger AK, Staerk D. 2019. Potential of Sorbus berry extracts for management of type 2 diabetes: metabolomics investigation of 1H NMR spectra, α-amylase and α-glucosidase inhibitory activities, and in vivo anti-hyperglycaemic activity of S. norvegica. J Ethnopharmacol. 242:112061.
Casqueiro J, Casqueiro J, Alves C. 2012. Infections in patients with diabetes mellitus: a review of pathogenesis. Indian J Endocr Metab. 16(7):27–36.
El-Shiekh RA, Al-Mahdy DA, Hifnawy MS, Tzanova T, Evain-Bana E, Philippot S, Bagrel D, Abdelsattar EA. 2017. Chemical and biological investigation of Ochrosia elliptica Labill. cultivated in Egypt. RecNatProd. 11(6):552–557.
International Diabetes Federation. 2017. IDF Diabetes Atlas, 8th ed. [accessed 2019 July 27]. http://www.diabetesatlas.org/.
Li XJ, Liu ZZ, Kim K-W, Wang X, Li Z, Kim Y-C, Yook CS, Liu XQ. 2016. Chemical constituents from leaves of Pileostegia viburnoides Hook.f.et Thomps. Nat Prod Sci. 22(3):154–161.
Masoodi MH, Ahmed B, Khan SA, Shah MY. 2010. Alcohols from whole plant of Lychnis coronaria L. Inter Res J Pharm. 1:337–341.
Nyemb JN, Magnibou ML, Tallé E, Tchinda AT, Tchuenguem RT, Henoumont C, Laurent S, Mbafor JT. 2018. Lipids constituents from Gardenia aqualla Stapf & Hutch. Open Chem. 16: 371–376.
Parmar VS, Jha HN, Gupta AK, Prasad AK, Gupta S, Boll PM, Tyagi OD. 1992. New antibacterial tetraatriacanortol derivatives from Agave Americana L. Tetrahedron. 48:1281–1284.
Ruiz-Vargas JA, Morales-Ferra DL, Ramírez-Ávila G, Zamilpa A, Negrete-León E, Acevedo-Fernández JJ, PeñaRodríguez LM. 2019. α-Glucosidase inhibitory activity and in vivo antihyperglycemic effect of secondary metabolites from the leaf infusion of Ocimum campechianum mill. J Ethnopharmacol. 243:112081.
Skrzypczak-Pietraszek E, Pietraszek J. 2012. Chemical profile and seasonal variation of phenolic acid content in bastard balm (Melittis melissophyllum L., Lamiaceae). J Pharm Biomed Anal. 66: 154–161.
Tao J-Y, Dai S-J, Zhao F, Liu J-F, Fang W-S, Liu K. 2012. New ursane-type triterpene with NO production suppressing activity from Nauclea officinalis. J Asian Nat Prod Res. 14(2):97–104.
Venditti A, Ballero M, Serafini M, Bianco A. 2015. Polar compounds from Parentucellia viscosa (L.) Caruel from Sardinia. Nat Prod Res. 29(7):602–606.
Venditti A, Frezza C, Riccardelli M, Foddai S, Nicoletti M, Serafini M, Bianco A. 2016b. Unusual molecular pattern in Ajugoideae subfamily: the case of Ajuga genevensis L. from Dolomites. Nat Prod Res. 30(9):1098–1102.
Venditti A, Lattanzi C, Ornano L, Maggi F, Sanna C, Ballero M, Alvino A, Serafini M, Bianco A. 2016a. A new glucosidic phthalide from Helichrysum microphyllum subsp. tyrrenicum from La Maddalena Island (Sardinia, Italy). Nat Prod Res. 30(7):789–795.

Xiang M, Su H, Hu J, Yan Y. 2011. Isolation, identification and determination of methyl caffeate, ethyl caffeate and other phenolic compounds from Polygonum amplexicaule var. sinense. J Med Plants Res. 5:1685–1691.

Xu F, Huang X, Wu H, Wang X. 2018. Beneficial health effects of lupenone triterpene: A review. Biomed Pharmacother. 103:198–203.