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A new stilbene from *Agonis flexuosa* leaves and verification of its histamine release inhibitory activity using *in silico* and *in vitro* studies

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A B S T R A C T

This study aimed to explore the phytoconstituents of *Agonis flexuosa*, F. Myrtaceae and its biological activity. A thorough phytochemical investigation of its leaves led to the isolation of one new stilbene glycoside; (*Z*)-2,3-dihydroxystilbene-5-O-β-D-glucoside (**1**), and fifteen known compounds identified as two stilbenes: (*Z*)-pinosylvin mono methyl ether (**2**) and (*Z*)-pinosylvin-3-O-β-D-glucoside (**3**); six flavanones: (2*S*)-pinostrobin (**4**), (2*S*)-strobopinin (**5**), (2*S*)-cryptostobin (**6**), (2*S*)-pinocembrin (**7**), (2*S*)-dimethylpinocembrin (**8**) and (2*S*)-dimethylstrobopinin (**9**); four flavonoids: quercetin (**10**), kaempferol-7-O-β-D-glucoside (**11**), quercetin-3-O-α-D-rhamnoside (**12**) and quercetin-3-O-β-D-glucoside (**13**), α-terpineol (**14**), β-sitosterol (**15**) and gallic acid (**16**). The structures of the isolated metabolites were elucidated based upon the interpretation of their 1D and 2D NMR (One Dimensional and Two-Dimensional Nuclear Magnetic Resonance), HR-ESI-MS (High Resolution Electrospray Ionization Mass Spectrometry) and optical rotation. All the isolated compounds were evaluated for their antimicrobial activities. Only compound (**6**) showed a selective activity against *P. aeruginosa* with IC$_{50}$ value of 4.88 μM. *In silico* virtual screening was done for the isolated compounds on Human histamine H1 receptor (3RZE) downloaded from protein data bank. All the compounds showed certain degree of binding to the protein displaying free binding energies ranging between -11 to -31 kcal/mol. (*Z*)-2,3-Dihydroxystilbene-5-O-β-D-glucoside (**1**) showed notable fitting to the active site as evidenced by its free binding energy (ΔG) which is computed as -25.09 kcal/mol comparable to diclofenac that displayed (ΔG) of -15.00 kcal/mol. *In vitro* assessment of histamine release inhibitory activity was performed using U937 human monocytes. Compound (**1**) showed a substantial inhibition to histamine release displaying IC$_{50}$ value of 0.16 μM.

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1. Introduction

Natural products showing therapeutic properties have long been used in the alleviation of human diseases and their utilization is as old as human civilization. However after industrial revolution with the concomitant progress in the organic chemistry, synthetic products were greatly used for the treatment of various diseases owing to the belief that single pure compound can be easily obtained and modified to get drugs with higher safety and activity as well (Rates, 2001). Recently, a growing interest has been aroused towards the usage of natural products obtained from plant origin as an everlasting source of secondary metabolites. These phytoconstituents could act as promising leads in drug discovery with less side effects and higher activity in compare to synthetic ones (Labib et al., 2017;

Youssef et al., 2017). This in turn could be formulated in a suitable dosage by several pharmaceutical companies instead of synthetic drugs which are of lower side effects and more welcomed by several patients in compare to synthetic drugs (Saj and Thoppil, 2011).

Genus *Agonis* belongs to family Myrtaceae and is mainly native to the Southwestern Australia. It comprises about 12 species that grow as medium to large shrubs except *A. flexuosa* which grows as a small tree (Wheeler, 2007;). Although, genus *Agonis* is rich with phenolic compounds represented by flavanones which had been previously isolated from *A. spathulata* (Cannon and Martin, 1977), few studies had been conducted on other species.

Agonis flexuosa is commonly known as peppermint willow myrtle that exists in the form of little robust tree, possessing fibrous brown bark, dull green leaves which are long and narrow. Its inflorescences are clustered in the axes in the form of little flowers which are white in color. Its genus name *Agonis* comes from the Greek words (agon) that means the cluster meanwhile its species name derived from Latin word *flexuosa* which means full of bends and this refers to the

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stem zig-zag shape which alters its direction at every node. Traditionally, the leaves were employed by the Noongar people as antiseptic in addition to the utilization of its sapling trunk as shafts and sticks for digging. A strong peppermint odor is released upon crushing the leaves (Rippey and Rowland, 2004). The essential oil of *A. flexuosa* was previously determined by GLC (Gas Liquid Chromatography) in which myrecene represents 45% of the essential oil components. In addition, the oil had shown its antibacterial and antifungal activities (Saj and Thoppil, 2011). However, nothing was further reported in literature concerning the biological activities or the phytoconstituents of *A. flexuosa*. Thus, it was important to explore more about the secondary metabolites of the selected plant and to assess its biological activity, as well.

In this study, an in-depth phytochemical investigation of the methanol extract of *Agonis flexuosa* leaves and its isolated compounds were structurally elucidated using 1D and 2D NMR, HR-ESI-MS and optical rotation as well. Also, antimicrobial activity was assessed using agar diffusion method. In addition, in *silico* virtual screening was done for the isolated compounds on Human histamine H1 receptor (3RZE). *In vitro* assessment of the histamine release inhibitory activity using U937 human monocytes were also performed aiming to explore new anti-allergic and anti-inflammatory entities exerting their effects *via* acting as histamine blockers.

2. Results and discussion

In depth phytochemical investigation of the methanol extract of *Agonis flexuosa* leaves resulted in the isolation of one new stilbene glucoside; (Z)-2,3-dihydroxystilbene-5-O-β-D-glucoside (1) in addition to fifteen known compounds. These known compounds had been identified as (Z)-pinosylvinmonomethyl ether (2) (Ngo and Brown, 1998), (Z)-pinosylvin-3-O-β-D-glucoside (3) (Miyaichi Y et al., 1988), (2S)-pinostrobin (4) (Cannon and Martin, 1977; Smolarz et al., 2006; Bertelli D et al., 2012), (2S)-strobopinin (5) (Smolarz et al., 2006), (2S)-cryptostobin (6) (Byrne et al., 1982; Pavan et al., 2009), (2S)-pinocembrin (7) (Byrne et al., 1982; Pavan et al., 2009; Sfeir et al., 2013), (2S)-dimethylpinocembrin (8) (Bick et al., 1972), (2S)-dimethylstrobopinin (9) (Bick et al., 1972; Mayer, 1990; Sfeir et al., 2013), quercetin (10) (Cannon and Martin, 1977; Smolarz et al., 2006; Mohamed et al., 2018; Bertelli D et al., 2012), kaempferol-7-O-β-D-glucoside (11) (Zhang X et al., 2013), quercetin-3-O-α-L-rhamnoside (12), quercetin-3-O-β-D-glucoside (13) (Zhang X et al., 2013), α-terpineol (14) (Jin and Coates, 2006), β-sitosterol (15) and gallic acid (16) (Mohamed et al., 2018). The structural elucidation of the isolates was accomplished by extensive (1D and 2D NMR) spectroscopic data analysis and HR-ESI-MS and by comparing these data with literature values. All the compounds isolated from the methanol extract of *Agonis flexuosa* leaves were displayed in Fig. 1.

Compound (1) was obtained as a yellowish residue showing molecular formula of $C_{20}H_{22}O_8$ with *m/z*: 389.1250 [M-H]$^-$ (calculated 389.1236) as determined by HRESIMS possessing 10 degrees of unsaturation. ^{1}H and ^{13}C NMR spectral data of compound (1) were displayed in Table 1 and showed the pattern of stilbenes that is characterized by the presence of seven aromatic protons of two benzene rings and two olefinic protons. Ring A was suggested to be a tetrasubstituted benzene ring from the presence of signals of two aromatic methines [δ_C 103.5 / δ_H 6.46 (1H, br.s), C-4, and δ_C 105.6 / δ_H 6.82 (1H, br.s), C-6]. The presence of hydroxyl group at position 2 of ring A was suggested from the presence of a signal of quaternary oxygenated carbon at δ_C146.0 ppm (C-2) which showed an HMBC correlation with H-α (δ_H 6.96 ppm) as shown in Fig. 2. In addition to the presence of another signal of two quaternary oxygenated carbons at 160.2 ppm (C-3, 5) which is found in the known stilbenes; (Z)-pinosylvin mono methyl ether (2) and (Z)-pinosylvin-3-O-β-D-glucoside (3). The ^{1}H NMR spectrum showed a doublet signal at δ_H 4.93 (J = 7.2 Hz) corresponding to an anomeric proton of glucose moiety which showed an HMBC correlation with C-5 (δ_C 160.2 ppm). This

confirmed that the structure of ring A is 1,2,3,5-tetrasubstituted benzene ring. Also, ^{1}H NMR spectrum showed three signals characteristic for five aromatic protons at δ_H 7.24 ppm (1H, *d*, J = 7.2 Hz, H-4'), δ_H 7.49 ppm (2H, d, J = 7.6 Hz, H-2', 6') and δ_H 7.34 ppm (2H, t, J = 7.6, 7.6 Hz, H-3', 5') which confirmed the structure of ring B to be a monosubstituted benzene moiety. The configuration of the olefinic group was confirmed to be *cis* configuration based on the small coupling constant of H-α (J = 11.2 Hz). The full assignment of the protons and carbons was supported by the HMBC correlations between H-α (δ_H 6.96 ppm) and C-1` (δ_C 137.8 ppm) and C-2 (δ_C 146.0 ppm), between H-6 (δ_H 6.82 ppm) and C-α (δ_C129.3 ppm) and C-5 (δ_C 160.2 ppm), between H-4 (δ_H 6.46 ppm) and C-3 and C-5 (δ_C 160.2 ppm), and between the anomeric proton H-1`` (δ_H 4.93 ppm) and C-5 (δ_C 160.2 ppm). Consequently, compound (1) was identified as (Z)-2,3-dihydroxystilbene-5-O-β-D-glucoside which is considered to be a new chemical entity.

Additionally, the absolute configurations at C-2 in compounds (4-9) were determined to be (2S) based on their negative optical rotation values (Gaffield, 1970; Slade et al., 2005). All the known isolated compounds were isolated for the first time from *A. flexuosa*. Pinostrobin (4) was previously isolated as a mixture of 2R and 2S, while cryptostrobin (6) was isolated as 2S from *A.spathulata* (Cannon and Martin, 1977). The identified flavanones were previously isolated from members of family Myrtaceae (Bick et al., 1972; Mayer, 1990; Pavan et al., 2009; Massaro et al., 2014) while this is the first report for the isolation of stilbenes 1-3 from family Myrtaceae.

Regarding the antimicrobial activity, all the isolated compounds were tested for their antimicrobial activity, only (2S)-cryptostobin (6) showed a selective activity against *P. aeruginosa* with IC$_{50}$ value equals to 4.88 μM compared to ciprofloxacin that showed IC$_{50}$ of 1.26 μM. Belonging to the flavonoid class, the antimicrobial potential of (2S)-cryptostobin (6) could be interpreted by the virtue of its inhibitory effect on crucial enzymes incorporated in the microbial growth, replication and invasive potential such as type-IV topoisomerase, nucleic acid synthase, gyrase activity in addition to interfering with the energy metabolism and the action of cytoplasmic membrane. Besides, flavanone group of compounds belonging to the flavonoid class was found to possess an additional property which is the inhibition to the quorum sensing mechanisms. Flavanones particularly, naringenin effectively decrease the formation of elastase and pyocyanin in *P. aeruginosa* with no effect on the microbial growth with concomitant reduction in bacterial cell-cell signaling, or quorum sensing (QS)-controlled genes (Vandeputte et al., 2011).

Additionally, molecular modeling study was carried out to test the binding of the isolated compounds to human histamineH1 receptor targeting the discovery of lead entities for combating allergy and inflammation *via* acting as histamine blockers. All the compounds showed certain degree of inhibition to the protein displaying free binding energies ranging between -11 to -31 kcal/mol as displayed in Table 2. (Z)-2,3-Dihydroxystilbene-5-O-β-D-glucoside (1) showed a notable fitting to the active site as evidenced by its free binding energy (ΔG) which is computed as -25.09 kcal/mol comparable to diclofenac that displayed (ΔG) of -15.00 kcal/mol. This was further confirmed by the binding behavior of the compound (1) at the active site in which three conventional hydrogen bonds; three Van der Waals interactions are formed between the moieties of the compound and the amino acid residues at the active sites as represented by 2D and 3D binding mode of compound as shown in Fig. 3. The hydrogen bonds are formed with Thr1054, Val1057 and Asn1053. Meanwhile, the Van der Waals interactions are observed with the amino acid residues Arg1052 and Asn1055. Besides, a π-cationic interaction occurs between the benzene ring of the compound and the Lys1043 that exists at the active site. However, diclofenac forms one hydrogen bond and a Van der Waals interaction with Asn1053 and Ar1052, respectively at the active site. Besides, quercetin-3-O-α-L-rhamnoside (12), kaempferol-7-O-β-D-glucoside (11), (Z)-

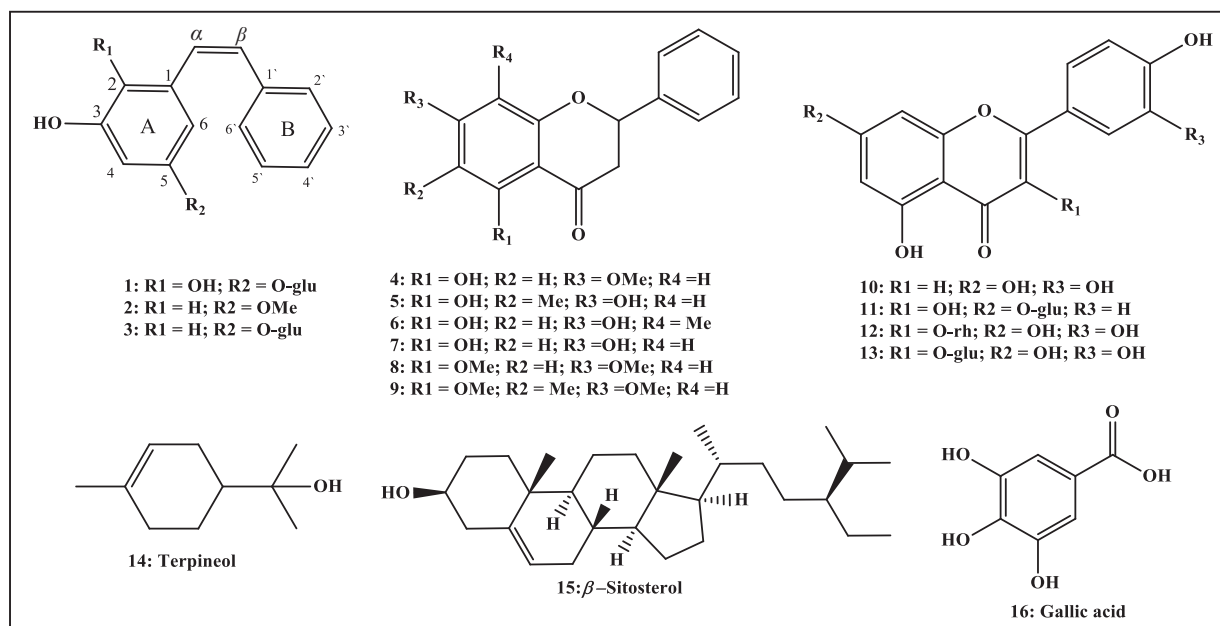**Fig. 1.** Scheme showing all the compounds (**1**−**16**) isolated from the methanol extract of *Agonis flexuosa* leaves.

Table 1
¹H (400 MHz) and ¹³C (100 MHz) data of compound (1) in DMSO-d_6 (δ in ppm, *J* in Hz).

#	δ_C	δ_H (multi, *J* values)	HMBC
1	141.6 (C)		
2	146.0 (C)		
3	160.2 (C)		
4	103.5 (CH)	6.46 (*brs*, 1H)	C-3, C-5
5	160.2 (C)		
6	105.6 (CH)	6.82 (*brs*, 1H)	C-5, C-α
α	129.3 (CH)	6.96 (*d*, 11.2, 1H)	C-2, C-1′
β	130.2 (CH)	7.34, overlap with H-3′, 5′	
1′	137.8 (C)		
2′/6′	129.3 (CH)	7.49 (*d*, 7.6, 2H)	
3′/5′	127.1 (CH)	7.34 (*t*, 7.6, 2H)	
4′	128.3 (CH)	7.24 (*d*, 7.2, 1H)	
Glucose			
1″	100.5 (CH)	4.93 (*d*, 7.2, 1H)	C-5
2″	73.7 (CH)	3.17 − 3.76(*m*)	
3″	77.5 (CH)		
4″	70.3 (CH)		
5″	76.9 (CH)		
6″	61.3 (CH₂)		
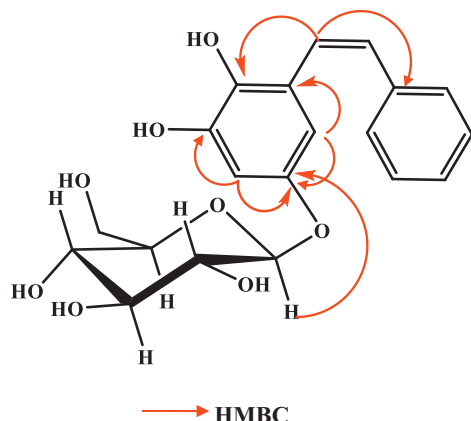
pinosylvin-3-O-β-D-glucoside (**3**) and quercetin-3-O-β-D-glucoside (**13**) showed a strong fitting *via* theformation of multiple bonds within the active sites of the protein displaying Δ G of -31.01, -29.15, -26.05 and -25.71 Kcal/mol, respectively that further ascertain the inhibitory potential of the extract. 2D binding modes of quercetin-3-O-α-L-rhamnoside (**12**), kaempferol-7-O-β-D-glucoside (**11**), (*Z*)-pinosylvin-3-O-β-D-glucoside (**3**) and quercetin-3-O-β-D-glucoside (**13**) at the active site of human histamineH1 receptor are illustrated in Fig. 4

Furthermore, *in vitro* assessment of histamine release inhibitory activity was performed using U937 human monocytes in an effort to confirm the anti-allergic potential of the compound. Compound (**1**) showed a notable inhibition to histamine release displaying an IC₅₀ value of 0.16 μM meanwhile diclofenac showed IC₅₀ of 0.06 μM. Thus it could be concluded that *Agonis flexuosa* could serve as a natural product for many pharmaceutical industries specialized in developing therapeutic agents for the alleviation of inflammation, allergy and their subsequent disorders that is welcomed by a large category of people all over the globe owing to its natural origin.

3. Experimental section

3.1. Plant material

Fresh leaves of *Agonis flexuosa* (Muhl. ex Willd.) Sweet (family Myrtaceae) were collected in April 2018 from private botanical garden, Al-Mariouteya Road, Kirdassah, Giza, Egypt. The plants were identified and authenticated by Eng. Terease Labib, Consultant of Plant Taxonomy at Ministry of Agriculture, El-Orman Botanical Garden and National Gene Bank, Giza, Egypt. The voucher specimen of the plant material was deposited at Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt with the identity (PHG-P- AF-181).

3.2. General experimental procedures

1D and 2D NMR spectra were recorded on a Varian AS 400 MHz spectrometer. Optical rotations were measured by Autopol IV Automatic Polari meter (Rudolph, Hackettstown, NJ, USA). High resolution

**Fig. 2.** HMBC key correlations of compound (**1**).

Table 2
Free binding energies (kcal/mol) of the isolated compounds in the active site of Human histamine receptor using virtual screening programs.

Compound	Human histamine receptor (3RZE)	Number of formed bonds with the amino acid residues
(Z)-2,3- Dihydroxystilbene -5-O-β-D-glucoside (**1**)	−25.09	3; Arg 1052, Asn1053, Asn1055, Lys 1043, Thr1054, Val1057
(Z)-Pinosylvin mono methyl ether (**2**)	−19.17	2; Asn1055, Arg 1052
(Z)-Pinosylvin-3-O-β-D-glucoside (**3**)	−26.05	3; Asn1053, Asn1055, Arg 1052
(2S)-Pinostrobin (**4**)	−17.71	1; Asn1055
(2S)-Strobopinin (**5**)	−19.51	2; Arg 1052, Thr1054
(2S)-Cryptostobin (**6**)	−19.33	2; Arg 1052, Asn1055
(2S)-Pinocembrin (**7**)	−19.10	2; Arg 1052, Val1057
(2S)-Dimethylpinocembrin (**8**)	−20.21	2; Arg 1052
(2S)-Dimethylstrobopinin (**9**)	−19.72	1; Arg 1052
Quercetin (**10**)	−21.55	2; Arg 1052, Asn1053
Kaempferol-7-O-β-D-glucoside (**11**)	−29.15	5; Asn1040, Asn1053, Arg 1052, Lys 1043, Thr1054
Quercetin-3-O-α-L-rhamnoside (**12**)	−31.01	4; Asp 1047, Arg 1052, Asn1053, Thr1054
Quercetin-3-O-β-D-glucoside (**13**)	−25.71	3; Asn1040, Asn1053, Lys 1043
α-Terpineol (**14**)	−11.29	No H-bonds
β-Sitosterol (**15**)	−23.28	No H-bonds
Gallic acid (**16**)	−16.32	3; Thr1054, Val1057, Arg 1052
Diclofenac	−15.00	2; Asn1053, Arg 1052
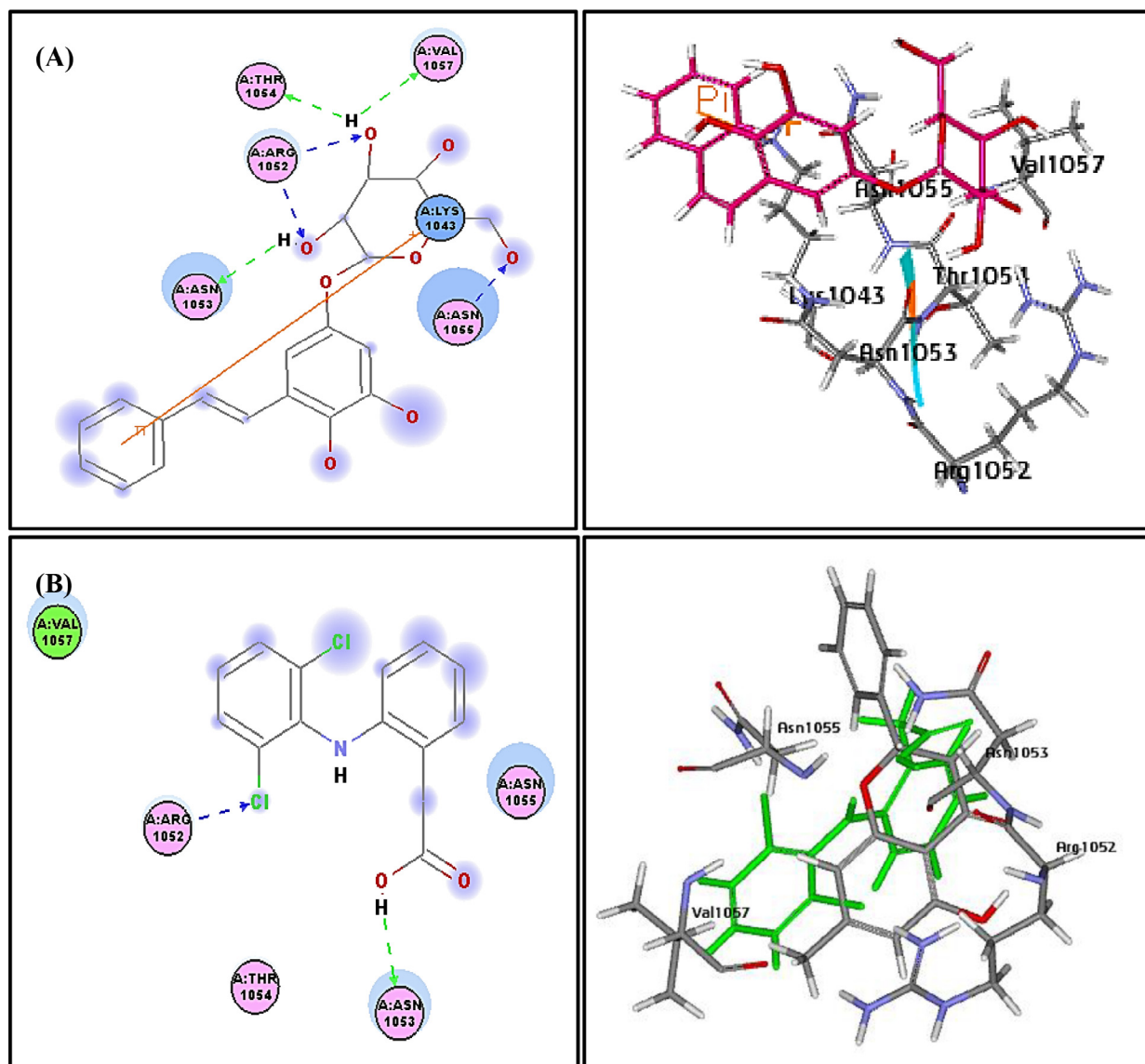
Fig. 3. 2D and 3D binding mode of (Z)-2,3-dihydroxystilbene-5-O-β-D-glucoside (**1**) (A) and diclofenac (B) in the binding site of human histamine receptor (3RZE); Green arrows indicate the formation of conventional hydrogen bonds; Blue arrows indicate the formation Van der Waals interactions. (Z)-2,3-Dihydroxystilbene-5-O-β-D-glucoside (**1**) is drawn in purple color in the 3D mode meanwhile diclofenac takes the green color in the 3D mode. All the amino acid residues are labeled and displayed by element in the 3D mode.
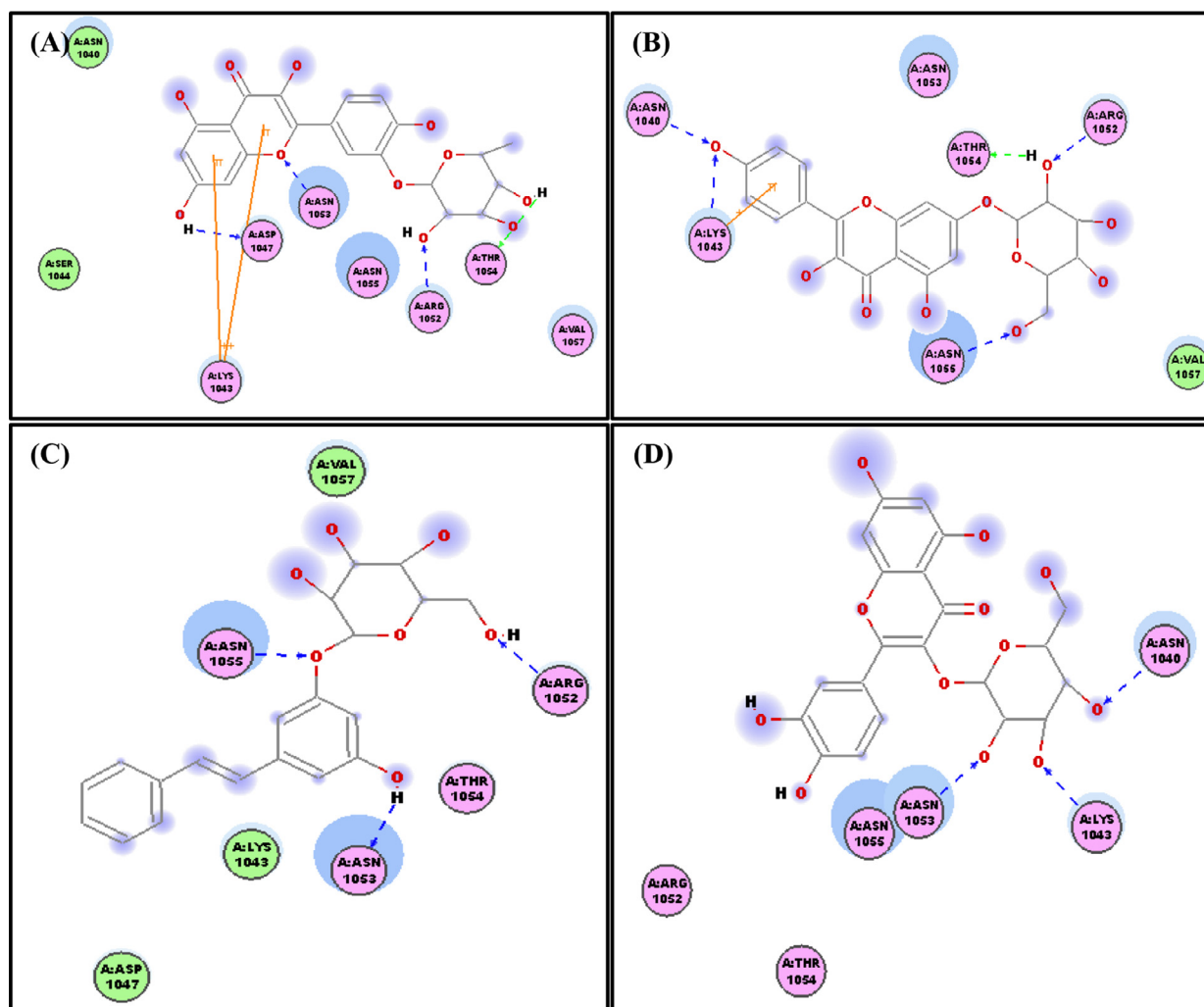
Fig. 4. 2D binding modes of quercetin-3-O-α-L-rhamnoside (**A**), kaempferol-7-O-β-D-glucoside (**B**), (Z)-pinosylvin-3-O-β-D-glucoside (**C**) and quercetin-3-O-β-D-glucoside (**D**) at the active site of human histamineH1 receptor .

mass spectra were measured using a Bruker BioApex-FTMS with electrospray ionization (ESI). Sephadex LH-20 (Mitsubishi Kagaku, Tokyo, Japan) and silica gel (60-120 mesh, Merck) were used for CC. Fractions from CC were monitored using pre-coated aluminum sheets [silica 60 F254, 0.25 mm (Merck, Darmstadt, Germany)], with detection provided by UV light (254 and 366 nm) and by spraying with 1% vanillin-sulfuric acid reagent followed by heating for 5-10 min (105°C).

3.3. Extraction of the plant material

The air-dried and powdered leaves (200 g) were extracted by maceration with MeOH (2L × 3) at room temperature till exhaustion. The combined extracts were evaporated under reduced pressure to afford a dry residue (80 g). Silica gel vacuum liquid chromatography (VLC) was used for the initial fractionation of the MeOH extract (50 g) eluted sequentially with n-hexane, DCM, EtOAc and then with MeOH to afford four fractions: 4 g (n-hexane), 5.2 g (DCM), 28 g (EtOAc) and 6.5 g (MeOH).

3.4. Isolation of secondary metabolites

The DCM fraction (5.2 g) was subjected to silica gel CC (150 g, 30 × 10 cm) using stepwise gradient elution of n-hexane and EtOAc

(100:0−70:30, 1L /fraction) to yield 13 subfractions [D1-D13]. Further purification of subfraction D3 (eluted with n-hexane-EtOAc, 9:1, 300 mg) on Sephadex LH-20 CC (15g, 65 × 1.5 cm) with DCM-MeOH (1:1) as eluent resulted in the isolation of compound (**4**) (10 mg). Subfraction D4 (eluted with n-hexane-EtOAc, 85:15, 60 mg) yielded compound (**14**) (22 mg). Sephadex LH-20 CC (15g, 65 × 1.5 cm) of subfraction D6 (eluted with n-hexane-EtOAc, 85:15, 330 mg) eluted with DCM-MeOH (1:1) afforded compounds (**2**) (8 mg) and (**15**) (18 mg). Subfractions D8 and D9 (eluted with n-hexane-EtOAc, 85:15 and 80:20, 45 &120 mg respectively) yielded compounds (**5**) (12 mg) and (**6**) (12 mg). Further purification of subfraction D10 (eluted with n-hexane-EtOAc, 80:20,510 mg) on silica gel CC (30g, 65 × 1.5 cm), eluted with n-hexane-EtOAc (90:10) then (85:15) yielded compound (**7**) (12 mg). Subfractions D12 and D13 (eluted with n-hexane-EtOAc, 70:30, 65 & 84 mg) afforded compounds (**8**) (6 mg) and (**9**) (8 mg) respectively. A scheme showing the isolation of compounds (**2, 4-9, 14,15**) from the DCM fraction is represented as Fig. S1.

However, the MeOH fraction (6.5 g) was fractionated over silica gel CC (20 g, 30 × 10 cm) using stepwise gradient elution of DCM and MeOH (100:0-75:25, 1L/fraction) to yield 11subfractions [M1-M11]. Subfraction M5 (eluted with DCM-MeOH, 9:1, 500 mg) was subjected to Sephadex LH-20 CC (25 g, 80 × 1.5 cm) with DCM-MeOH (1:1) as eluent yielding compound (**10**) (30 mg). Silica gel CC (35 g, 80 × 3

cm) of subfraction M7 (eluted with DCM-MeOH, 9:1, 1.1 g) using stepwise gradient elution of DCM and MeOH (100:0-75:25, 2L/fraction) yielded 10 subfractions [M-7-A to M-7-J]. Subfraction M-7-F (eluted with DCM-MeOH, 9:1, 510 mg) was chromatographed over Sephadex LH-20 CC (25 g, 80 × 1.5 cm) using DCM-MeOH (1:1) as eluent yielding 11 subfractions [M-7-F-1 to M-7-F-11]. Further purification of subfraction M-7-F-10 (200 mg) on Sephadex LH-20 CC (10 g, 50 × 2 cm) with MeOH-DCM (1:1) as eluent yielded compounds **(3)** (22 mg), **(11)** (17 mg) and **(12)** (31 mg). Subfraction M-7-F-11 (90 mg) was further chromatographed over Sephadex LH-20 CC (5 g, 50 × 2 cm) with DCM-MeOH (1:1) as eluent yielding compound **(16)** (19 mg). Further purification of subfraction M-7-I (eluted with DCM-MeOH, 75:25, 110 mg) on Sephadex LH-20 CC (5g, 65 × 1 cm) using MeOH 100% as eluent afforded compounds **(1)** (15 mg) and **(13)** (35 mg). **A** scheme showing the isolation of compounds **(1, 3, 10-13, 16)** from the MeOH fraction is represented as Fig. S2.

3.5. Antimicrobial bioassay

Pure compounds were tested for antimicrobial activity against a great panel of bacteria and fungi using the method previously described (Bharate et al., 2007; Malak LG, 2013; Labib et al., 2016; Mohamed et al., 2018). The tested fungi were *Candida albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, and *Aspergillus fumigates* ATCC 90906, meanwhile the tested bacteria were methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA), *Cryptococcus neoformans* ATTC 90113, *Staphylococcus aureus* ATTC 29213, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. Amphotericin B (ICN Biomedicals, Ohio) for fungal and ciprofloxacin (ICN Biomedicals, Ohio) for bacterial bioassays were used as positive controls, respectively (Bharate et al., 2007).

3.6. In silico virtual screening study

In an effort to search for new entities which can act as anti-allergic and anti-inflammatory agents which are derived from nature *via* acting as histamine release blockers, molecular modeling study was carried out. This was done to test the binding of the isolated compounds to human histamine H1 receptor (PDB ID 3RZE; 3.10 A°) that was downloaded from the protein data bank (www.pdb.org) using Drug Discovery Studio Software 2.5 (Accelrys Inc., San Diego, USA). Preparation of the structure of the protein was achieved employing the default protocol of Discovery Studio 2.5 for protein preparation. This was performed by elimination of water molecules, addition of hydrogen atoms which was followed by cleaning the protein structure from unwanted interactions. CHARMm was selected as the forcefield meanwhile MMFF94 was chosen for the calculation of partial charge. The active site was selected depending upon the collected data approaching the enzyme catalytic domain. The structures of the compounds were prepared employing ligand preparation protocol that is default in Discovery Studio 2.5. The compounds after being prepared were docked within the active sites of the energy-minimized protein applying C-Docker protocol. Calculation of the binding energies for the chosen docking poses was done using distance dependent dielectric implicit solvation model.The free binding energies were determined in Kcal/mol using the following equation: (Labib et al., 2018; Talaat et al., 2018)

$$\Delta G_{binding} = E_{complex} - (E_{protein} + E_{ligand}) \text{ Where;}$$
$\Delta G_{binding}$: The ligand−protein interaction binding energy,
$E_{complex}$: The potential energy for the complex of protein bound with the ligand,
$E_{protein}$: The potential energy of protein alone and
E_{ligand}: The potential energy for the ligand alone

3.7. In vitro histamine-release inhibition assay

This was determined using U937 human monocytes adopting the method previously described (Venkata M et al., 2012). Briefly, a 96-well cell culture plate containing about 50,000 cells was occupied with a serial dilution of the tested samples covering the range of (1000−7.81 μg/mL) together with 20 nM phorbol myristate acetate for a period of 1 h. Centrifugation at a rate of 10,000 rpm was done for the culture supernatants for either untreated or treated cultures for 5 min at 4°C to determine the percentage of inhibition of histamine release using ELISA kit that is commercially available. Diclofenac was used as a standard reference drug.

3.8. Spectral data

(Z)-2,3-dihydroxystilbene-5-O-β-D-glucoside (1): Yellowish residue (MeOH); UV (MeOH) λ_{max}(log ε) 286 (0.80) nm; IR (KBr) ν_{max}2999, 1588, 1456, 1203 cm^{-1}; ^{1}H (400 MHz) and ^{13}C NMR (400 MHz) data are displayed in Table 1. HR-ESI-MS [M-H]$^-$ *m/z* 389.1250 (calculated for *m/z* C$_{20}$H$_{21}$O$_8$, 389.1236). 1D and 2D NMR spectral data as well as HRESIMS spectra for the new compound and for all the isolated compounds are illustrated in the supplementary data.

4. Conclusions

Phytochemical investigation of the leaves of *Agonis flexuosa* led to the isolation of one new stilbene glucoside; (Z)-2,3-dihydroxystilbene-5-O-β-D-glucoside (**1**), together with fifteen known compounds belonging to flavanones, stilbenes, flavanols, terpenes and phenolic acids. (2S)-cryptostobin (**6**) showed a selective activity against *Pseudomonas aeruginosa* meanwhile the new stilbene (Z)-2,3-dihydroxystilbene-5-O-β-D-glucoside (**1**) showed a notable inhibition to histamine release displaying an IC$_{50}$ value of 0.16 μM. Thus, *Agonis flexuosa* could serve as a natural product for the alleviation of microbial infection, inflammation, allergy and their subsequent disorders that is welcomed by a large category of people all over the globe owing to its natural origin. However, this should be supported by further clinical studies. Additionally, the exact mechanism by which (2S)-cryptostobin (**6**) exerted its antimicrobial effect as well as its structure activity relationship are recommended to be studied.

Declaration of Competing Interest

The authors declare no conflict of interest.

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