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Original Article

CHEMICAL INVESTIGATION OF *BAUHINIA VAHLII* WIGHT AND ARNOTT LEAVES GROWN IN EGYPT

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ABSTRACT

Objective: Plants of genus *Bauhinia* are famous for their rich flavonoid content. Several phytochemical and biological investigations affirmed the role of flavonoids in the different biological impacts exerted by *Bauhinia* plants. The present study aims to investigate the major phytoconstituents of the leaves of *B. vahlii* Wight and Arnott.

Methods: Powdered leaves were extracted with *n*-hexane (HE) and the defatted marc was extracted with 70% ethanol. The defatted ethanolic extract (DEE) was further partitioned with solvents of increasing polarities. The HE and polar fractions of DEE were purified using different chromatographic techniques and isolated compounds were identified through their melting points, 1D and 2D NMR, UV and MS spectral data.

Results: A total of nine compounds were isolated and identified. Taraxerol (1), a pentacyclic triterpene, and *β*-sitosterol (2) were isolated from HE. Investigation of polar fractions of DEE yielded six flavonoids and a phenolic acid, namely luteolin (3), quercetin (4), gallic acid (5), avicularin (6), quercitrin (7), hyperoside (8) and quercetin-3-*O*-*β*-sophoroside (9).

Conclusion: Flavonols of the quercetin nucleus were the major detected constituents in *B. vahlii* leaves. Taraxerol, avicularin and quercetin-3-*O*-*β*-sophoroside are isolated for the first time from the genus *Bauhinia*. Results of this study encourage future pharmacological investigation of *B. vahlii* due to the presence of biologically active flavonoids and phytosterols.

Keywords: *Bauhinia vahlii* Wight, Arnott., Polar extractives, Flavonols, Quercetin, Taraxerol

INTRODUCTION

Bauhinia (family Fabaceae) is a genus of trees or shrubs widely distributed in the tropical and subtropical regions of Africa, Asia and South America. Plants of the genus *Bauhinia* are commonly known as “cow’s paw” or “camel’s foot” because of the characteristic shape of their leaves. The leaves and stem barks have been used in folk medicine of some countries as remedies for the treatment of different health conditions including diabetes, pain and some inflammatory disorders [1].

Due to their presumed therapeutic value, several species of the genus *Bauhinia* were chemically investigated with the leaf being the more thoroughly studied organ [1]. Reports regarding the main secondary metabolites in *Bauhinia* species indicated the presence of flavonoids, terpenes, sterols, aromatic acids, saponins, oxepins, tannins, bibenzyls and alkaloids with flavonoids identified as the major class of secondary metabolites [1, 2].

Several investigations of *B. variegata*, one of the famous *Bauhinia* plants, resulted in the isolation of a wide range of flavonoid aglycones and glycosides [3-6]. From *B. malabarica* seven flavonols were identified as kaempferol, quercetin and their glycosides [7]. Also, different quercetin and kaempferol derivatives were isolated from *B. forficata* leaves and flowers [8]. Additionally, Neto *et al.* reported the isolation of quercetin, guaijaverin and quercitrin from the leaves of *B. ungulata* [2].

Bauhinia vahlii Wight and Arnott. is a huge climber native to India with only few reports that can be traced concerning its main secondary metabolites. Sultana *et al.* reported the isolation of agathisflavone, quercetin, kaempferol, isoquercitrin, campesterol, stigmasterol and *β*-sitosterol from the leaves acetone extract [9]. In addition, Kumar RJ *et al.* isolated (+)-catechin, methyl gallate and methyl-4-*O*-methyl gallate from the pericarps of the fruits [10].

In this study, the leaves of *B. vahlii* were subjected to a deeper chemical investigation of their major phytoconstituents that might help in predicting their possible therapeutic impacts.

MATERIALS AND METHODS

Plant material

Samples of *B. vahlii* leaves were collected during the flowering stage in May 2013 from the garden of Mohammed Ali museum, Kasr Al-Aini, El-Manial, Egypt. The identity of the leaves was authenticated by Dr. Mohammed El-Gebaly, senior botanist at National Research Center, Giza, Egypt. A voucher specimen (No. 11-6-2013s-1) was kept at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Preparation of plant extracts and fractions

Air-dried powdered *B. vahlii* leaves (2.9 kg) were extracted exhaustively with *n*-hexane and the extracts were combined and evaporated under reduced pressure yielding the *n*-hexane extract (HE). The defatted leaves were then extracted with 70% ethanol till exhaustion and the combined extracts were evaporated under reduced pressure yielding defatted ethanolic extract (DEE). DEE was suspended in distilled water and partitioned successively with solvents of increasing polarities; dichloromethane, ethyl acetate and *n*-butanol saturated with water yielding their corresponding DCM, EtOAc and BuOH fractions, respectively.

Chemicals

Authentic reference samples used in co-chromatography were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Diaion HP-20 AG for column chromatography was obtained from Mitsubishi Chemical Industries Co. Ltd, Japan. Silica gel H 60 for vacuum liquid chromatography (VLC) was purchased from E-Merck (Darmstadt, Germany). Silica gel 60 and silica gel RP-18 for column chromatography were obtained from Fluka, Sigma-Aldrich Chemicals, Germany. Sephadex LH-20 was purchased from Pharmacia Fine Chemicals AB (Uppsala, Sweden). Precoated TLC plates and silica gel 60 F 254 was obtained from Fluka, Sigma-Aldrich Chemicals, Germany. The chromatograms were visualized under UV light (at 254 and 366 nm) before and after exposure to

ammonia vapor, as well as spraying with *p*-anisaldehyde/sulfuric acid [11] and/or natural products/polyethylene glycol (NP/PEG) spray reagents [12].

Apparatus and equipment

Schimadzu double beam spectrophotometer (UV-1650, Japan) was utilized for determination of UV shifts of flavonoids. Mass spectra were studied using Thermo scientific ISQLT single quadrupole, USA. NMR spectra were recorded at 400 (^{1}H) and 100 MHz (^{13}C) on a Bruker NMR-spectrometer, Japan. The NMR spectra were recorded in deuterated CH_3OH, $CHCl_3$ or CH_3COCH_3 using TMS as an internal standard. Electrothermal 9100 (Labequip, Markham, Ontario, Canada) was used for determination of melting points (m. p.) (uncorrected).

Phytochemical investigation of plant extractives

***n*-Hexane extract**

The *n*-hexane extract (20 g) was chromatographed on a VLC column (Silica gel H 60, 300 g, 16 D x 7 L cm). Elution was performed gradually using *n*-hexane, *n*-hexane/DCM mixtures, and DCM/EtOAc mixtures. Collected fractions (300 ml each) were monitored by TLC and those with the similar chromatographic pattern were pooled together. Fraction eluted with 100% DCM was chromatographed on a silica gel 60 column (2.5 D x 25 L cm) using *n*-hexane/EtOAc mixture (98:2) as an eluent to yield compound 1 (30 mg). Fractions eluted with 20-30% EtOAc in DCM were combined and chromatographed on a silica gel 60 column (3 D x 30 L cm) using *n*-hexane/DCM (10:90) to yield compound 2 (90 mg).

Ethyl acetate fraction

The fraction (10 g) was chromatographed on a VLC column (Silica gel H 60, 190 g, 12.5 D x 7 cm L). Gradient elution was performed starting with DCM, DCM/EtOAc mixtures, and EtOAc/methanol mixtures. Fractions were collected (300 ml each) and monitored by TLC and similar ones were pooled yielding nine fractions designated as fractions I-IX. Fraction II (eluted with 50-70% EtOAc in DCM) was chromatographed on a sephadex LH-20 column using methanol/water (20:80) v/v as an eluent to yield 2 compounds; 3 (15 mg) and 4 (90 mg). Fraction III (eluted with 80% EtOAc in DCM) was chromatographed on a sephadex LH-20 column using methanol/water (50:50) v/v for elution to yield compound 5 (25 mg). Fraction V (eluted with 100% EtOAc) was chromatographed on a sephadex LH-20 column using methanol/water (50:50) v/v as an eluent yielding 2 compounds; 6 (24 mg) and 7 (165 mg). Finally, fraction VII (eluted with 5-15 % methanol in EtOAc) was chromatographed on a VLC silica gel RP-18 column (2 D X 20 L cm) using methanol/water gradient elution with 5% increments of methanol. Subfractions eluted with 15-20% methanol in water were pooled and chromatographed on a sephadex LH-20 column using methanol/water (50:50) v/v for elution to yield compound 8 (12 mg).

***n*-Butanol fraction**

The fraction (15 g) was dissolved in water and chromatographed on a Diaion HP-resin column (4 D x 30 L cm). Successive elution was performed starting with water followed by 25% and 50% methanol in water and finally 100% methanol. Fractions eluted with 50 and 100% methanol in water showed similar pattern on TLC and were pooled together. This collective fraction was further chromatographed on a polyamide column (5 D x 15 L cm) using gradient elution with methanol/water mixtures with 10% increments of methanol. Sub fractions eluted with 30-40% methanol in water were pooled and further purified on a sephadex LH-20 column (1 D x 10 L cm) using methanol/water (50:50) v/v as an eluent to yield compound 9 (10 mg).

Analysis of isolated compounds

Compound 1

White powder, m. p. = 276-278 °C, ^{1}H-NMR (400 MHz, $CDCl_3$, ppm, J/Hz): 0.79 (3H, *s*, CH_3-24), 0.81 (3H, *s*, CH_3-28), 0.9 (6H, *s*, CH_3-26, 30), 0.92 (3H, *s*, CH_3-25), 0.95 (3H, *s*, CH_3-29), 0.97 (3H, *s*, CH_3-23), 1.08 (3H, *s*, CH_3-27), 3.19 (1H, *dd*, *J* = 11.16, 4.7 Hz, H-3), 5.53 (1H, *dd*, *J* = 8.16, 3.2 Hz, H-15).^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 37.3 (C-1), 27 (C-2), 79.13 (C-3), 38.5 (C-4), 55.5 (C-5), 18.4 (C-6), 34.1 (C-7), 36.69 (C-8), 49.11 (C-9), 37.6 (C-10), 16.48 (C-11), 34.77 (C-12), 37.74 (C-13), 158.11 (C-14), 116.84 (C-15), 35.66 (C-16), 38.8 (C-17), 48.57 (C-18), 40.3 (C-19), 28.34 (C-20), 32.68 (C-21), 36.4 (C-22), 28 (C-23), 15.2 (C-24), 15.4 (C-25), 29.8 (C-26), 25.9 (C-27), 29.72 (C-28), 32.9 (C-29), 21.23 (C-30). EI/MS m/z (70 ev): 426 ($M^{+\cdot}$, $C_{30}H_{50}O$, 3%), 302 ($M^{+\cdot}$-C_9H_{16}, 43%), 287 ($M^{+\cdot}$-$C_{10}H_{19}$, 53%), 204 ($M^{+\cdot}$-$C_{15}H_{26}O$, 100%).

Compound 2

White powder, m. p. = 140-140 °C, ^{1}H-NMR (400 MHz, $CDCl_3$, ppm, J/Hz): 0.71 (3H, *d*, *J* = 7.2, CH_3-21), 0.85 (3H, *t*, *J* = 5.6, CH_3-29), 0.87 (3H, *d*, *J* = 5.5, CH_3-26), 0.88 (3H, *s*, CH_3-18), 0.94 (3H, *d*, *J* = 6.4, CH_3-27), 1.03 (3H, *s*, CH_3-19), 3.55 (1H, *m*, H-3), 5.37 (1H, *d*, *J* = 4, H-6). EI/MS m/z (70 ev): 414 ($M^{+\cdot}$, $C_{29}H_{50}O$, 47%), 396 ($M^{+\cdot}$-H_2O, 30%), 273 ($M^{+\cdot}$-side chain-2H, 40%), 255 ($M^{+\cdot}$-side chain-H_2O, 58%).

Compound 3

Yellow powder, m. p. = 329-330 °C, ^{1}H-NMR (400 MHz, CD_3OCD_3, ppm, J/Hz): 6.22 (1H, *d*, *J* = 2 Hz, H-6), 6.48 (1H,, *d*, *J* = 1.96 Hz, H-8), 6.69 (1H, *s*, H-3), 6.92 (1H, *d*, *J* = 8 Hz, H-5'), 7.45 (1H, *dd*, H-2', H-6', partially overlapping).

Compound 4

Yellow powder, m. p. = 315-317 °C, ^{1}H-NMR (400 MHz, CD_3OCD_3, ppm, J/Hz): 6.13 (1H, *d*, *J* = 2 Hz, H-6), 6.39 (1H, *d*, *J* = 2 Hz, H-8), 6.86 (1H, *d*, *J* = 8.5 Hz, H-5'), 7.56 (1H, *dd*, *J* = 2, 8.5 Hz, H-6'), 7.68 (1H, *d*, *J* = 2 Hz, H-2').

Compound 5

White needle crystals that gives dark blue color with $FeCl_3$, m. p. = 256 °C, ^{1}H-NMR (400 MHz, CD_3OCD_3, ppm, J/Hz): 6.92 (2H, *s*, H-2 and 6), 9.16 (3H, *br. s*, OH-3, 4, 5), 12.1 (1H, *br. s*, COOH).

Compound 6

Yellow powder, m. p. = 240-243 °C, ^{1}H-NMR (400 MHz, CD_3OD, ppm, J/Hz): 6.2 (1H, *d*, *J* = 2 Hz, H-6), 6.39 (1H, *d*, *J* = 2 Hz, H-8), 6.89 (1H, *d*, *J* = 8.4 Hz, H-5'), 7.49 (1H, *dd*, *J* = 2.2, 8.2 Hz, H-6'), 7.52 (1H, *d*, *J* = 2.1 Hz, H-2'), 5.46 (1H, *br. s*, H-1''), 3.49-4.33 (sugar protons). ^{13}C-NMR (100 MHz, CD_3OD, ppm): 157.3 (C-2), 133.47 (C-3), 178.59 (C-4), 161.73 (C-5), 98.57 (C-6), 164.96 (C-7), 93.36 (C-8), 158 (C-9), 104.25 (C-10), 121.9 (C-1'), 115.7 (C-2'), 144.94 (C-3'), 148.4 (C-4'), 114.9 (C-5'), 121.61 (C-6'), 108.21 (C-1''), 77.27 (C-2''), 81.98 (C-3''), 86.66 (C-4''), 61.4 (C-5'').

Compound 7

Yellow powder, m. p. = 182-185 °C, ^{1}H-NMR (400 MHz, CD_3OD, ppm, J/Hz): 6.19 (1H, *d*, *J* = 2 Hz, H-6), 6.36 (1H, *d*, *J* = 2 Hz, H-8), 6.9 (1H, *d*, *J* = 8.3 Hz, H-5'), 7.3 (1H, *dd*, *J* = 2, 8.3 Hz, H-6'), 7.33 (1H, *d*, *J* = 2 Hz, H-2'), 5.35 (1H, *d*, *J* =1.2 Hz, H-1''), 0.94 (3H, *d*, *J* = 6 Hz, H-6''), 3.33-4.21 (sugar protons). ^{13}C-NMR (100 MHz, CD_3OD, ppm): 157.1 (C-2), 134.8 (C-3), 178.21 (C-4), 161.78 (C-5), 98.39 (C-6), 164.5 (C-7), 93.39 (C-8), 157.87 (C-9), 104.46 (C-10), 121.54 (C-1'), 115.5 (C-2'), 145 (C-3'), 148.36 (C-4'), 114.93 (C-5'), 121.43 (C-6'), 102.11 (C-1''), 70.6 (C-2''), 70.71(C-3''), 71.84 (C-4''), 70.48 (C-5''), 16.22 (C-6'').

Compound 8

Yellow powder, m. p. = 215-217 °C, ^{1}H-NMR (400 MHz, CD_3OD, ppm, J/Hz): 6.1 (1H, *d*, *J* = 2 Hz, H-6), 6.28 (1H, *d*, *J*= 2 Hz, H-8), 6.76 (1H, *d*, *J* = 8 Hz, H-5'), 7.48 (1H, *dd*, *J* = 2, 8 Hz, H-6'), 7.74 (1H, *d*, *J* = 2 Hz, H-2'), 5.05 (1H, *d*, *J* = 8 Hz, H-1''), 3.3-4.7 (sugar protons).^{13}C-NMR (100 MHz, CD_3OD, ppm): 157.42 (C-2), 134.52 (C-3), 178.11 (C-4), 161.69 (C-5), 98.97 (C-6), 165.65 (C-7), 93.73 (C-8), 157.2 (C-9), 104.14 (C-10), 121.1 (C-1'), 116.37 (C-2'), 144.46 (C-3'), 148.73 (C-4'), 114.86 (C-5'), 121.6 (C-6'), 103.94 (C-1''), 71.77 (C-2''), 73.75 (C-3''), 68.83 (C-4''), 75.78 (C-5''), 60.58 (C-6'').

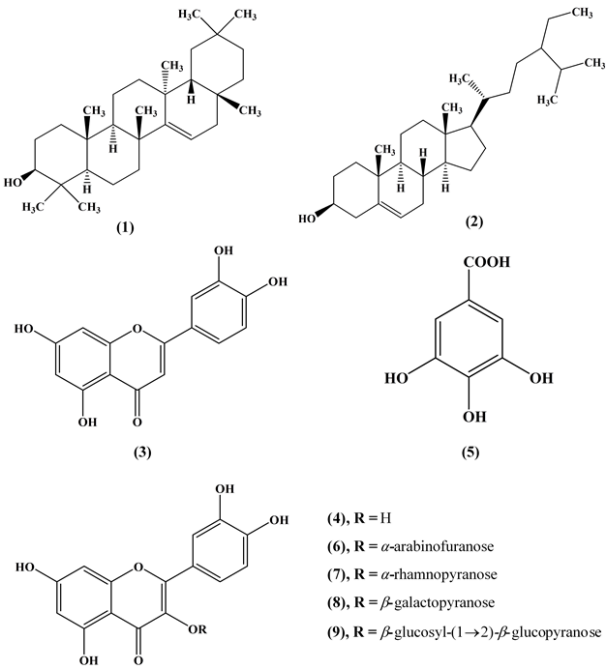
Compound 9

Yellow powder, m. p. = 198-200 °C, ^{1}H-NMR (400 MHz, CD_3OD, ppm, J/Hz): 6.17 (1H, *d*, *J* = 2 Hz, H-6), 6.37 (1H, *d*, *J* = 2 Hz, H-8), 6.87 (1H,

d, *J* = 8.5 Hz, H-5'), 7.55 (1H, *dd*, *J*= 2.2, 8.4 Hz, H-6'), 7.73 (1H, *d*, *J* = 2.2 Hz, H-2'), 5.24 (1H, *d*, *J* = 7.6 Hz, H-1''), 4.75 (1H, *d*, *J* = 7.3 Hz, H-1''') 3.3-4.1 (sugar protons).^{13}C-NMR (100 MHz, CD_3OD, ppm): 156.3 (C-2), 133.9 (C-3), 177.5 (C-4), 161.8 (C-5), 98.2 (C-6), 165 (C-7), 93.1 (C-8), 156 (C-9), 104.3 (C-10), 121.2 (C-1'), 116 (C-2'), 145 (C-3'), 148.6 (C-4'), 116.2 (C-5'), 122 (C-6'), 100 (C-1''), 79.8 (C-2''), 75.7 (C-3''), 69.6 (C-4''), 77 (C-5''), 60.2 (C-6''), 103.9 (C-1'''), 74.5 (C-2'''), 76.8 (C-3'''), 69.6 (C-4''), 76.9 (C-5''), 60.2 (C-6''). HSQC (δ H/C): 5.24, 100 (HC-1''), 4.75, 103.9 (HC-1''') and 4.1, 79.8 (HC-2''). HMBC (δ H/C): 5.24, 133.9 (H-1''→C-3), 4.75, 79.8 (H-1'''→C-2'') and 4.06, 103.9 (H-2''→C-1''').

RESULTS AND DISCUSSION

A total of nine compounds were successfully isolated from *B. vahlii* leaves extractives. Phytochemical study of HE resulted in isolation of 2 compounds; a triterpene (compound 1) and a phytosterol (compound 2), while chemical investigation of both EtOAc and BuOH resulted in isolation of six flavonoids and one phenolic acid designated as 3-9 (fig. 1). The identities of these compounds were deduced from their melting points, spectral data and comparison to published data.



^{1}H-NMR spectrum of compound 1 revealed the presence of eight methyl groups and a single olefinic proton appearing at δ 5.53 ppm. The characteristic signal for H-3 observed at δ 3.19 ppm (*dd*, *J* = 11.16, 4.7 Hz) suggested the presence of a triterpene nucleus with one double bond and a single hydroxyl group at 3-position. HSQC spectrum of compound 1 showed the presence of eight methyl groups at δ H/C observed at (0.79,15.4), (0.81,29.6), (0.90,29.8), (0.90,21.2), (0.92,15.4), (0.95,33.2), (0.97,27.9) and (1.08,25.9) assigned for C-24, C-28, C-26, C-30, C-25, C-29, C-23 and C-27, respectively. Furthermore, analysis of HMBC spectrum of compound 1 identified the resonance of the two oleifinic carbons at δ 158.1 and 116.8 ppm characteristic for C-14 and C-15 double bond of taraxerol. The identification of compound 1 as taraxerol was confirmed through several HMBC correlations including 3H-26→C-14 and 3H-27→C-14. The EI/MS spectrum of compound 1 further confirmed its identification as taraxerol by showing a molecular ion peak ($M^{+\cdot}$) at m/z 426 (3%) calculated for the molecular formula $C_{30}H_{50}O$ in addition to the characteristic base peak at 204 [$M^{+\cdot}$-$C_{15}H_{26}O$] at 100% intensity. These obtained spectral data of compound 1 also comply with the previously reported data of taraxerol [13].

Compounds 2, 3, 4 and 5 were identified through their melting points, ^{1}H-NMR spectra and co-chromatography with authentic samples as *β*-sitosterol, luteolin, quercetin and gallic acid, respectively.

Compounds 6-8 were identified as quercetin-3-*O*-monosaccharides. The quercetin nucleus and the sugar moiety in each compound were concluded from their respective ^{1}H and ^{13}C-NMR spectral data. The 3-*O*-glycosidation was confirmed by their UV spectral behavior in methanol and after addition of different UV shift reagents in addition to the upfield shift of their C-3 resonances [14]. Using co-chromatography with authentic samples and upon a comparison of the spectral data with the available literature [15, 16], compounds 6, 7 and 8 were identified as quercetin-3-*O*-*α*-arabinofuranoside (Avicularin), quercetin-3-*O*-*α*-rhamnopyranoside (Quercitrin) and quercetin-3-*O*-*β*-galactopyranoside (Hyperoside), respectively.

^{1}H and ^{13}C-NMR spectra of compound 9 indicated it is a quercetin-3-*O*-disaccharide. The presence of two sugar residues was inferred from the presence of two anomeric proton signals at 5.24 (1H, *d*, *J* = 7.6 Hz) and 4.75 (1H, *d*, *J* = 7.3 Hz) with their corresponding carbons at δC 100 and 103.9. The 3-OH was confirmed to be the site of glycosidation through analysis of the HMBC spectrum of the compound which showed H-1''→C-3 correlation at (5.24, 133.9). Further analysis of the HMBC spectrum indicated the linkage between the two sugar moieties to be 1'''→2'' through the observation of H-1'''→C-2'' and H-2''→C-1''' correlations at δ H/C observed at (4.75, 79.8) and (4.06, 103.9), respectively. Comparing to published data [17], compound 9 was identified as quercetin-3-*O*-*β*-glucosyl-(1→2)-*β*-glucoside (Quercetin-3-*O*-*β*-sophoroside).

CONCLUSION

Chemical investigation of the leaves of *B. vahlii* Wight and Arnott. grown in Egypt revealed that they are rich in flavonoids mainly of quercetin nucleus. The leaves *n*-hexane extract was also rich in phytosterols and triterpenes. To the best of our knowledge, this is the first report for the isolation of taraxerol, avicularin and quercetin-3-*O*-*β*-sophoroside from genus *Bauhinia,* while luteolin, quercitrin, hyperoside and gallic acid are reported for the first time to be isolated from species *B. vahlii*. These findings come in agreement with previous reports indicated flavonoids, particularly flavonols, to be the dominant secondary metabolites in genus *Bauhinia* [1]. Our findings also encourage pharmacological investigation of different extracts of *B. vahlii* due to the presence of biologically active phytosterols, triterpenes, and flavonoids. Taraxerol and sterols are reported to possess anti-inflammatory action [13, 18], also accumulated reports revealed that biological impacts of many *Bauhinia* species are mainly related to their flavonoid content [1, 19, 20].

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CONFLICT OF INTERESTS

All authors have non to declare

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