Indian Journal of Natural Products and Resources
Vol. 11(4), December 2020, pp 312-319

Isolation, characterization, and biological evaluation of flavonols and 1,2-diphenylethanes from *Bauhinia vahlii*

Alekhya Ketha* and Girija Sastry Vedula

Pharmaceutical Chemistry Department, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam 530003, Andhra Pradesh, India

Received 31 July 2020; Revised 02 October 2020

In this study, three known flavonols, namely kaempferol **(1)**, ombuin **(2)**, and quercetin **(3)**, and three known 1,2-diphenylethanes, namely 5-(2-hydroxyphenethyl)-3-methoxy-2-methylphenol **(4)**, 2-(3,5-dimethoxyphenethyl) phenol **(5)** and 2-(2-hydroxyphenethyl)-4,6-dimethoxyphenol **(6)** from the methanolic extract **(ME)** of *Bauhinia vahlii* were identified and sucessfully isolated. They were also evaluated for *in vitro* antioxidant, anti-inflammatory, anti-gout and anticancer effects. Compound **3** (26.00±2.17 µg/mL) showed an almost equivalent IC_{50} value of standard drug (25.55±2.80 µg/mL) against superoxide free radicals. Moreover, compound **3** showed significant inhibition of COXs and 5-LOX enzymes, while compounds **1, 2, 4** and **5** exhibited good inhibition of XO enzymes. Except for compound **5**, all compounds showed a significant reduction of cell growth lysis of MCF-7, DLD-1, HeLa, and A549. Besides, all the metabolites and **ME** showed a very weak degree of specificity against NHME, indicates less toxicity to normal cells. The results suggest that *B. vahlii* can be a favourable natural source for the treatment of oxidative stress, inflammation, gout and cancer, and these actions are linked to the natural active compounds **1, 3, 4** and **6**.

Keywords: Anticancer, Anti-gout, Anti-inflammatory, Antioxidant, *Bauhinia vahlii*, Sulforhodamine B assay.

IPC code; Int. cl. (2015.01)- A61K 36/00, A61K 36/48, A61P, A61P 29/00, A61P 39/06

Introduction

The genus *Bauhinia* belonging to the family Fabaceae is well recorded in the flora of India, Nepal, and Pakistan[1]. *Bauhinia vahlii* Wight & Arn. is a strong climbing shrub, usually called Camel's foot creeper in English, *Asamantaka* in Sanskrit, and *Adattige* in Telugu[2,3]. In the folklore, *Bauhinia* species have wide applications in treating microbial infections, oxidative stress, inflammation, diabetes, and tumours. Mainly, the Indian tribes used *B. vahlii* in the treatment of microbial infections, oxidative stress, chronic inflammation and cancer[1,3]. Biologically, *Bauhinia* has been reported to have antifungal[4,5], anti-mycobacterial[4], anti-inflammatory[4,6], anticancer[4,7], anti-malarial[4,8], antioxidant[9–11], and anti-diabetic[9] activities.

Particularly, *B. vahlii* is reported to have antibacterial[2,12], antioxidant[3,13], anti-inflammation[14], tyrosinase inhibitory[3] and anti-diabetic[14] activities. Besides, a chemical examination of the leaves of *B. vahlii* reported having triterpenes, flavonoids, phenolic acids, and sterols[15]. To date, no proper chemical investigation has been attempted to evaluate the chemical constituents of the whole plant of *B. vahlii*. So, the current investigation aimed to evaluate the chemical composition of the whole plant *B. vahlii* using chromatography and to screen its methanolic extract and isolated metabolites for antioxidant, anti-inflammatory, anti-gout, and anticancer properties.

Material and Methods

Plant collection

The whole plant of *B. vahlii* was collected at Seshachalam hills, Tirupati, Andhra Pradesh, India, in February 2019, and a voucher specimen (DB-SVU-2019-3478) was deposited in the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Extraction and isolation

The whole plant (1 kg) was dried and powdered and extracted using maceration method[16] with methanol (90%, 3 × 1 L × 7 days) at 25 ˚C. All combined and evaporated under low pressure using rotavapor (Shimadzu Rotation evaporator QR 2005-S, Japan) to obtain a methanolic extract of *B. vahlii*

*Correspondent author
Email: alekhya.illa92@gmail.com
Mobile: +91-7032757847
Supplementary figures are available online only.

(**ME,** 34 g, 34%w/w) as a darkish brown solid. By using column chromatography (CC, sintered disc column, 600 mm × 45 mm; Product code: 6101067, Borosil, India) and silica gel (100 g, mesh size 100-200, Merck), **ME** extract (20 g) was fractionated using a hexane/ethyl acetate solvent system (step gradient flow from 100:0, 95:5, 90:10, 5:95, 0:100), which yielded six main fractions, namely F1-6. Similarly, F1 (2.0 g) subjected to CC (sintered disc column, 300 mm × 18 mm; Product code: 6101062, Borosil, India) using the above parameters yielded **1** (450 mg) as a yellow solid. By using step gradient flow (from 100:0, 95:5, 90:10, …, 5:95, 0:100) dichloromethane/ethyl acetate solvent system, F2 (2.5 g) gave **2** (250 mg) as a yellow solid. Similarly, with the dichloromethane/ethyl acetate solvent system, F3 (1.5 g) yielded **3** (800 mg) as a yellow solid, F4 (1.2 g) yielded **4** (300 mg) as a white solid, F5 (1.4 g) yielded **5** (620 mg) as a pale yellow solid, and F5 (2.1 g) yielded **6** (240 mg) as a creamy solid.

Antioxidant activity

DPPH assay

By employing the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay[17,18], the metabolites and extract were exposed to antioxidant activity in triplicate. To 0.004% DPPH, known concentrations of the test samples are added and incubated for 30 minutes at 37 ˚C and then measured absorbance at 517 nm against the blank. Ascorbic acid was used as a reference drug.

Superoxide radical scavenging assay

By employing superoxide radicals[19,20], the secondary metabolites and extract were exposed to antioxidant activity in triplicate. To prepared NADH, PMS and NBT added know concentrations of the test sample and incubated for 30 minutes at 37 ˚C. Absorbance was measured at 562 nm against the blank. Ascorbic acid was used as a reference drug.

Anti-inflammatory assays

Cyclooxygenase (COX1/2) inhibitory assay

The abilities of compounds **(1-6)** and **ME** to inhibit isoenzymes COX-1/2 were performed using COX (ovine/human) inhibitor assay[21] kit (Cayman, No.: 560131). To 10 µL of either COX1 or COX2 added 960 µL of 0.1 M Tris-HCl buffer and different concentrations of test samples and incubated for 10 minutes at 37 ºC. Later, 10 µL of 100 µM arachidonic acid, after 2 minutes 1 M HCl of 50 µL and Ellman's reagent, were added. The absorbance was noted spectrophotometrically at 410 nm against the blank. The percentage of inhibition was calculated with the OD values by which IC_{50} values were determined by linear regression. Indomethacin and diclofenac were used as reference drugs for COX1 and COX2, respectively.

5-lipoxygenase (5-LOX) inhibitory assay

The compounds **(1-6)** and **ME** were tested against 5-LOX (human recombinant) using 5-LOX assay[21] kit (No. 437996, Sigma Aldrich). To 90 µL of 5- LOX enzyme solution added different test sample concentrations, 100 µL of de chromogen, and finally added 10 µL of the substrate (arachidonic acid) and gently shake or 10 min and absorbance was recorded at 490 nm against the blank. The percentage of inhibition was calculated with the OD values by which IC_{50} values were determined by linear regression. Zileuton was used as a reference drug.

Anti-gout assay

Xanthine oxidase (XO) inhibitory assay

All the isolated compounds **(1-6)** and **ME** were subject to XO inhibitory assay[21] using Sigma Aldrich assay kit. To 10 µL of the substrate (xanthine, 5 mM), added of sodium phosphate buffer (470 µL), different test sample concentrations, and 10 µL of XO enzyme and incubated for 5 minutes at room temperature and absorbance was recorded at 295 nm against the blank. The percentage of inhibition was calculated with the OD values by which IC_{50} values were determined by linear regression. Allopurinol was used as a reference drug.

Cytotoxicity assay

Utilizing the SRB assay[22,23], the secondary metabolites **(1-6)** and **ME** were implemented for their *in vitro* anticancer activity in triplicate (n = 3) using four cancer cells: MCF-7 (Breast), DLD-1 (Colon), HeLa (Cervical), A549 (Lung), and one normal cell line: normal human mammary epithelial (NHME). All the cells are purchased in good order from National Centre for Cell Science, Pune, and preserved according to Haritha *et al*[24], and performed as per the established protocol[22,23]. Doxorubicin was used as a reference drug.

Results and Discussion

Chemistry

Three known flavonols **(1-3)** and three known 1,2-diphenylethanes **(4-6)** were successfully identified

from the **ME** by utilizing chromatographic and analyses of their spectral NMR data and elemental composition. The obtained data were interrelated with those reported in the previous literature (Fig 1).

Compound 1 [Kaempferol][25] (Fig. 1): M.p.: 275-276; R_f: 0.6 (hexane:ethyl acetate, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6): 2.27 (s, 1H, OH), 2.98 (s, 1H, OH), 3.93 (s, 1H, OH), 5.03 (s, 1H, OH), 6.35 (s, 1H, Ar-H), 6.45 (s, 1H, Ar-H), 7.12-7.13 (d, 2H, *J*= 4 Hz, Ar-H), 7.61-7.63 (d, 2H, *J*= 8 Hz, Ar-H) (Fig. S1). ^{13}C NMR (400 MHz, DMSO-d_6): 94.96 (C-8), 100.15 (C-6), 104.82 (C-4), 116.49 (C-12/14), 122.48 (C-10), 131.48 (C-11/15), 136.89 (C-2), 146.42 (C-1), 158.13 (C-9), 160.36 (C-13), 160.49 (C-5), 164.68 (C-7), 175.78 (C-3) (Fig. S2). CHNS analysis for $C_{15}H_{10}O_6$: calcd. C-62.94%, H-3.52%, found C-62.96%, H-3.54(%). ESI-MS: calcd. *m/z* for $C_{15}H_{10}O_6$: 286.24 [M], found 285.14 [(M+H$^+$), positive mode], 287.24 [(M-H$^+$), negative mode] (Fig. S3).

Compound 2 [Ombuin][26] (Fig. 1): M.p.: 202-203; R_f: 0.5 (hexane:ethyl acetate, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6): 2.97 (s, 1H, OH), 3.55 (s, 1H, OH), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 5.03 (s, 1H, OH), 6.23 (d, 1H, *J*= 1 Hz, Ar-H), 6.24-6.25 (d, 1H, *J*= 4 Hz, Ar-H), 6.85-6.87 (d, 1H, *J*= 8 Hz, Ar-H), 7.02-7.06 (m, 2H, Ar-H) (Fig. S4). ^{13}C NMR (400 MHz, DMSO-d_6): 57.07 (C-10), 57.81 (C-17), 93.54 (C-8), 98.70 (C-6), 106.03 (C-4), 113.24 (C-15), 116.39 (C-12), 121.00 (C-16), 124.86 (C-11), 138.13 (C-2), 146.92 (C-13), 147.87 (C-1), 150.82 (C-14), 159.09 (C-9), 161.63 (C-5), 166.42 (C-7), 176.27 (C-3) (Fig. S5). CHNS analysis for $C_{17}H_{14}O_7$: calcd. C-61.82%, H-4.27%, found C-61.76%, H-4.24(%). ESI-MS: calcd. *m/z* for $C_{17}H_{14}O_7$: 330.29 [M], found 331.63 [(M+H$^+$), positive mode], 329.20 [(M-H$^+$), negative mode] (Fig. S6).

Compound 3 [Quercetin][25] (Fig. 1): M.p.: 316-317; R_f: 0.4 (hexane:ethyl acetate, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6): 2.77 (s, 1H, OH), 3.01 (s, 1H, OH), 3.50 (s, 1H, OH), 3.87 (s, 1H, OH), 5.01 (s, 1H, OH), 6.19 (d, 1H, *J*= 1 Hz, Ar-H), 6.19-6.20 (d, 1H, *J*= 4 Hz, Ar-H), 6.77-6.78 (d, 1H, *J*= 4 Hz, Ar-H), 6.98-7.01 (m, 2H, Ar-H) (Fig. S7). ^{13}C NMR (400 MHz, DMSO-d_6): 94.81 (C-8), 99.99 (C-6), 104.66 (C-4), 116.36 (C-11), 116.67 (C-14), 122.01 (C-15), 122.19 (C-10), 137.48 (C-2), 145.68 (C-12), 147.21 (C-1), 148.73 (C-13), 157.97 (C-9), 160.34 (C-5), 164.52 (C-7), 175.62 (C-3) (Fig. S8). CHNS analysis for $C_{15}H_{10}O_7$: calcd. C-59.61%, H-3.34%, found C-59.62%, H-3.34(%). ESI-MS: calcd. *m/z* for $C_{15}H_{10}O_7$: 302.24 [M], found 303.66 [(M+H$^+$), positive mode], 301.25 [(M-H$^+$), negative mode] (Fig. S9).

Compound 4 [5-(2-hydroxyphenethyl)-3-methoxy-2-methylphenol][4] (Fig. 1): M.p.: 320-321; R_f: 0.6 (hexane: CH_2Cl_2, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6): 3.62 (s, 1H, OH), 6.35 (s, 2H, Ar-H), 3.00 (s, 4H, CH_2), 3.86 (s, 3H, OCH_3), 2.32 (s, 3H, CH_3), 2.90 (s, 1H, OH), 6.82-6.92 (m, 2H, Ar-H), 7.04-7.10 (m, 2H, Ar-H) (Fig. S10). ^{13}C NMR (400 MHz, DMSO-d_6): 8.26 (C-9), 30.25 (C-7), 37.79 (C-8), 57.60 (C-10), 104.62 (C-4), 109.86 (C-6), 112.14 (C-2), 117.46 (C-3'), 121.05 (C-5'), 128.41 (C-1'), 128.61 (C-4'), 130.69 (C-6'), 141.63 (C-5), 155.68 (C-2'), 159.06

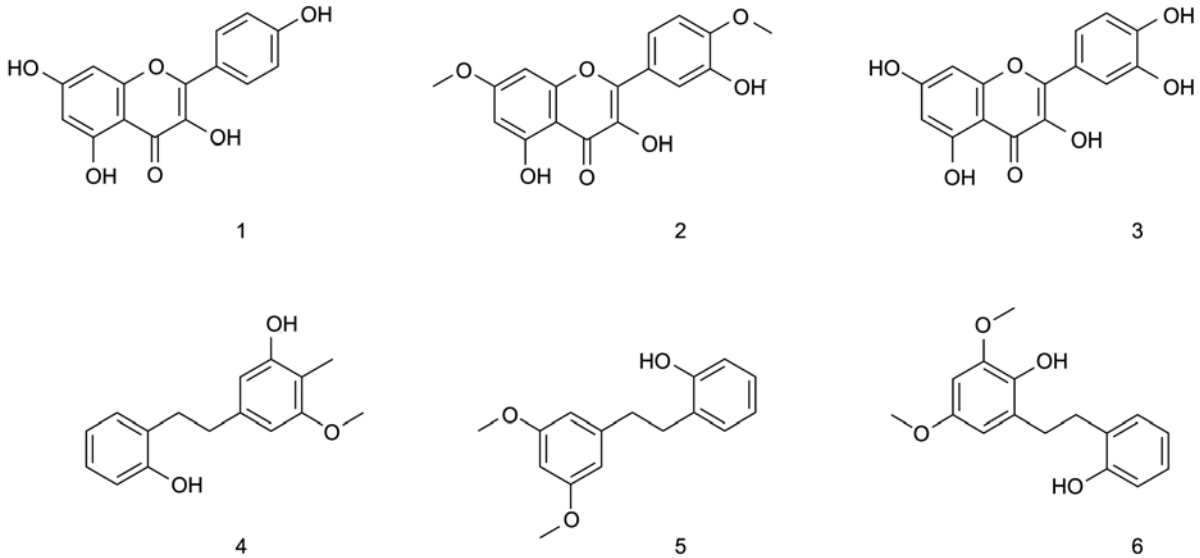

Fig. 1 — Chemical representation of isolated flavonols **(1-3)** and 1,2-diphenylethanes **(4-6)** from *Bauhinia vahlii*.

(C-1), 159.70 (C-3) (Fig. S11). CHNS analysis for $C_{16}H_{18}O_3$: calcd. C-74.40%, H-7.02%, found C-74.46%, H-7.04(%). ESI-MS: calcd. *m/z* for $C_{16}H_{18}O_3$: 258.32 [M], found 259.19 [$(M+H^+)$, positive mode], 257.15 [$(M-H^+)$, negative mode] (Fig. S12).

Compound 5 [2-(3,5-dimethoxyphenethyl)phenol][4] (Fig. 1): M.p.: 230-23; R_f: 0.5 (hexane: CH_2Cl_2, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6): 2.39 (s, 1H, OH), 2.99 (s, 4H, CH_2), 3.86 (s, 6H, OCH_3), 6.48 (s, 1H, Ar-H), 6.53 (d, 2H, *J*= 0.8 Hz, Ar-H), 6.83-6.84 (d, 1H, *J*= 4 Hz, Ar-H), 6.90-6.92 (d, 1H, *J*= 8 Hz, Ar-H), 7.04-7.09 (m, 2H, Ar-H) (Fig. S13). ^{13}C NMR (400 MHz, DMSO-d_6): 30.20 (C-7), 37.74 (C-8), 56.80 (C-10/9), 97.95 (C-4), 108.00 (C-2/6), 117.41 (C-5'), 121.00 (C-13), 128.36 (C-4'), 128.56 (C-1'), 130.64 (C-2'), 145.32 (C-1), 155.63 (C-6'), 162.57 (C-3/5) (Fig. S14). CHNS analysis for $C_{16}H_{18}O_3$: calcd. C-74.40%, H-7.02%, found C-74.49%, H-7.05(%). ESI-MS: calcd. *m/z* for $C_{16}H_{18}O_3$: 258.32 [M], found 259.16 [$(M+H^+)$, positive mode], 257.30 [$(M-H^+)$, negative mode] (Fig. S15).

Compound 6 [2-(2-hydroxyphenethyl)-4,6-dimethoxyphenol][4] (Fig. 1): M.p.: 342-343; R_f: 0.4 (hexane:CH_2Cl_2, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6): 2.83 (s, 1H, OH), 2.97 (s, 4H, CH_2), 3.68 (s, 1H, OH), 3.83 (s, 6H, OCH_3), 6.26-6.27 (d, 1H, *J*= 4 Hz, Ar-H), 6.33-6.34 (d, 1H, *J*= 4 Hz, Ar-H), 6.79-6.81 (d, 1H, *J*= 8 Hz, Ar-H), 6.87-6.89 (d, 1H, *J*= 8 Hz, Ar-H), 7.01-7.07 (m, 2H, Ar-H) (Fig. S16). ^{13}C NMR (400 MHz, DMSO-d_6): 29.63 (C-7), 30.07 (C-8), 56.40 (C-9), 57.14 (C-10), 97.02 (C-5), 107.22 (C-3), 117.00 (C-3'), 120.60 (C-5'), 127.95 (C-4'), 128.15 (C-1'), 129.84 (C-2), 130.23 (C-6'), 140.02 (C-1), 150.83 (C-6), 153.38 (C-4), 155.22 (C-2') (Fig. S17). CHNS analysis for $C_{16}H_{18}O_4$: calcd. C-70.06%, H-6.61%, found C-70.07%, and H-6.64(%). ESI-MS: calcd. *m/z* for $C_{16}H_{18}O_4$: 274.32 [M], found 275.97 [$(M+H^+)$, positive mode], 273.93 [$(M-H^+)$, negative mode] (Fig. S18).

Antioxidant activity

Initially, **ME** examined against DPPH and superoxide assays and its IC_{50} values were found to be 75.07±5.50 and 79.75±4.50 µg/mL, respectively, compared to standard (ascorbic acid) with 27.05±2.50 and 25.55±2.80 µg/mL, respectively (Fig. 2). Based on the preliminary antioxidant analysis of **ME**, we subjected its metabolites **(1-6)** for antioxidant activity. Among all the tested compounds, only compound **3** showed an almost equivalent IC_{50} value of superoxide radicals with 26.00±2.17 µg/mL (Fig. 2). For DPPH free radical, the IC_{50} values for **1, 2, 3, 4, 5,** and **6** remained to be 56.25±3.34, 60.0±2.75, 40.00±1.45, 53.00±2.02, 50.50±2.50, and 53.50±2.33 µg/mL, respectively. The concentration of **1, 2, 4, 5** and **6** needed for 50% inhibition of superoxide radicals was determined to be 62.50±5.13, 80.00±7.41, 37.25±2.00, 36.00±2.50, and 48.00±2.57 µg/mL, respectively (Fig. 2).

Anti-inflammatory and anti-gout activity

The *in vitro* anti-inflammatory and anti-gout effects of isolated compounds **(1-6)** were performed using COX-1 & 2, 5-LOX, and XO enzymes and the results were reported in IC_{50} values (Table 1). The concentration required for 50% inhibition of COX1 enzyme for **3** and **ME** were found to be 49.58±0.21 and 32.11±1.13 µg/mL, respectively, whereas the

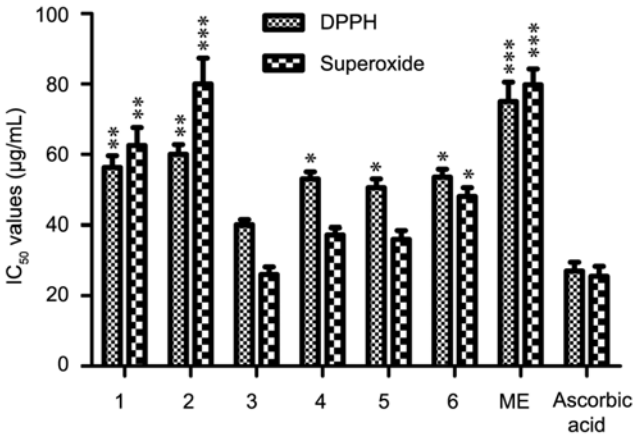

Fig. 2 — IC_{50} values of **1-6** and **ME** against DPPH and superoxide free radicals. Values were provided in mean±SD (n=3); **p* <0.05, ***p* <0.01 and ****p* <0.01 as compared with the standard group using one-way ANOVA with Student-Newman-Keuls *post hoc test*.

Table 1 — Effects of 1-6 and ME on COX1/2, 5-LOX, and XO enzymes

Sample	IC_{50} values (µg/mL)*			
	COX1	COX2	5-LOX	XO
1	>100	42.18±2.96^a	>100	66.17±2.17^b
2	>100	>100	49.38±0.28^a	52.30±0.12^b
3	49.58±0.21^a	55.25±0.54^b	68.56±0.11^b	>100
4	>100	66.75±0.42^b	75.18±0.97^b	80.17±0.66^c
5	>100	>100	71.14±2.17^b	82.17±2.24^c
6	>100	>100	>100	>100
ME	32.11±1.13^a	50.15±1.15^b	69.18±1.87^b	82.16±2.18^c
Standard	5.74±0.68	6.17±0.71	7.00±0.74	9.10±0.64

**Mean±SD values (n=3); ^{a}p <0.05, ^{b}p <0.01 and ^{c}p <0.01 as compared with the standard group using one-way ANOVA with Student-Newman-Keuls* post hoc test.

reference drug, indomethacin with 5.74±0.68 µg/mL (Table 1). From the results of COX2 enzyme inhibitory assay, it was noticed that the **1, 3, 4** and **ME** showed significant inhibition efficiency on COX2 enzyme with the IC_{50} of 42.18±2.96, 55.25±0.54, 66.75±0.42 and 50.15±1.15 µg/mL, respectively, compared to diclofenac with 6.17±0.71 µg/mL (Table 1). Moreover, the IC_{50} values of compounds **1, 2, 4, 5** and **6** on COX1 and compounds **2, 5** and **6** on COX2 were above 100 µg/mL (Table 1).

The concentration of **2, 3, 4, 5** and **ME** needed to inhibit 5-LOX activity at 50% was found to be 49.38±0.28, 68.56±0.11, 75.18±0.97, 71.14±2.17 and 69.18±1.87 µg/mL, respectively, while that of zileuton was 7.00±0.74 µg/mL (Table 1). The isolated compounds **1, 2, 4, 5** and **ME** exhibited significant inhibition of XO enzyme with IC_{50} values of 66.17±2.71, 52.30±0.12, 80.17±0.66, 82.17±2.24 and 82.16±2.18 µg/mL, respectively, whereas allopurinol with 9.10±0.64 µg/mL (Table 1). The concentration of compounds **3** and **6** required for 50% reticence of the XO enzyme was found to be above 100 µg/mL (Table 1).

Cytotoxicity studies

Firstly, **ME** were tested against MCF-7, DLD-1, HeLa, and A549 cancer cell lines at 100 µg/mL concentration. From the primary screening of SRB assay, it noticed that **ME** showed a prominent specificity against the series of cancer cells. Later, all the isolated metabolites **(1-6)** were subjected to cytotoxicity studies on the same cancer cell lines. From the SRB assay, except **5**, all metabolites showed significant specificity against all the tested cancer cells.

The IC_{50} values of **1, 2, 3, 4, 6** and **ME** on MCF-7 were determined to be 46.52±1.89, 83.84±0.23, 26.40±1.11, 80.60±1.47, 70.91±1.24 and 44.95±1.19 µg/mL, respectively, whereas standard, doxorubicin with 4.40±0.80 µg/mL (Table 2). Moreover, from the results of SRB assay on DLD-1, the IC_{50} values of **1, 2, 3, 4, 6** and **ME** found to be 52.57±3.80, 74.91±0.70, 14.00±1.12, 85.40±1.54, 67.80±1.15 and 72.23±3.58 µg/mL, respectively, whereas doxorubicin with 5.57±0.97 µg/mL (Table 2). Furthermore, the concentration of **1, 2, 3, 4, 6** and **ME** needed for 50% cell death of HeLa found to be 48.75±1.25, 89.17±1.78, 27.90±1.16, 56.80±2.17, 92.55±0.79 and 70.17±4.61 µg/mL, respectively, whereas doxorubicin with 5.55±0.24 µg/mL (Table 2). Similarly, the concentration needed for 50% cell death of A549 of **1, 2, 3, 4, 6** and **ME** determined to be 44.14±1.67, 82.33±2.82, 36.12±2.00, 60.80±2.45, 87.01±0.93 and 74.83±2.47 µg/mL, respectively, while doxorubicin with 8.50±0.77 µg/mL (Table 2). Besides, all the isolated compounds (**1-6**) and **ME** showed a very mild degree of specificity against NHME indicates that the samples are non-toxic to normal human cells.

Discussion

In the present study, six known secondary metabolites (**1-6**) were identified from the methanolic extract of *B. vahlii* (Fig. 1). This identification of flavonols **(1-3)** and 1,2-diphenylethanes **(4-6)** provides new information on the phytochemical profile of *B. vahlii*. Also, the biological profile of *B. vahlii* justified its natural ability to fight against free radicals, inflammation and cancer. From the DPPH and superoxide free radical assay, it observed that all the metabolites **(1-6)** from **ME** inhibited them prominently (Fig. 2). Also, the *in vitro* enzymatic screening of **ME** proved its aptitude to treat inflammation and gout. It was even justifying that compounds **3** and **4** have potent inhibition of COXs, 5-LOX and XO enzymes (Table 1).

Inflammation is regulated by higher levels of eicosanoids, namely prostaglandins, thromboxanes,

Table 2 — Cytotoxicity studies of 1-6 and ME on four different cancer cell lines

Sample	IC_{50} values (µg/mL)*			
	MCF-7	DLD-1	HeLa	A549
1	46.52±1.89^b	52.57±3.80^b	48.75±1.25^b	44.14±1.67^b
2	83.84±0.23^c	74.91±0.70^c	89.17±1.78^c	82.33±2.82^c
3	26.40±1.11^a	14.00±1.12^a	27.90±1.16^a	36.12±2.00^b
4	80.60±1.47^c	85.40±1.54^c	56.80±2.17^b	60.80±2.45^b
5	>100	>100	>100	>100
6	70.91±1.24^c	67.80±1.15^b	92.55±0.79^c	87.01±0.93^c
ME	44.95±1.19^b	72.23±3.58^c	70.17±4.61^c	74.83±2.47^c
Doxorubicin	4.40±0.80	5.57±0.97	5.55±0.24	8.50±0.77

*Mean±SEM values (n=3); $^a p$ <0.05, $^b p$ <0.01 and $^c p$ <0.01 as compared with the standard group using one-way ANOVA with Student-Newman-Keuls *post hoc test*.

and leukotrienes in the human body[27,28]. COXs and 5-LOX are key enzymes that catalyze the production of prostaglandins, thromboxanes and leukotrienes, and hydroperoxy fatty acids from arachidonic acid[29,30]. Significantly, inhibition of any one of the eicosanoids will activate the other pathway and prolongs inflammation[31]. For instance, inhibition of only prostaglandins will lead to elevated levels of leukotrienes by activation of the alternative path, i.e., 5-LOX pathway. Thus, routes of COXs and 5-LOX are chosen for the rate-limiting steps to reduce pain and inflammation[32]. Therefore, COX and 5-LOX (dual inhibitors) drugs inhibit the production of eicosanoids (prostaglandins, thromboxanes, and leukotrienes) and entirely prevent inflammation by lesser adverse effects[31–33]. Generally, NSAIDs are the drugs of choice to control the production of eicosanoids, and eventually relief from inflammation[34]. The outcomes of our study exhibited that isolated compound **3** act as both COX and 5-LOX (dual) inhibitors, whereas compounds **1** and **4** prominently inhibit COX2 enzyme (Table 1).

On the other hand, XO is an enzyme that catalyzed purines xanthine/hypoxanthine to form uric acid. To some extent, the formation of uric acid does not cause any biological effects in human body[35]. Beyond the limits, the higher deposition of uric acid, especially in the joints of the human body, leads to painful inflammation in joints, termed as gout[35,36]. Also, XO is an excellent source for free radicals (containing oxygen) that cause inflammatory-related diseases such as atherosclerosis and cancer[36]. Hence, inhibition of XO results in controlling gout, as well as its related conditions. This study suggests that isolated compounds **1, 2, 4,** and **5** possess prominent XO inhibitory effects (Table 1) that might be supportive in the treatment of gout and its complications. Taken together, *B. vahlii* scientifically proved as a potential source for the management of oxidative stress, inflammation and gout. This observation helps to investigate the anticancer ability of *B. vahlii,* and the outcomes of SRB assay showed that compounds **1, 2, 3, 4,** and **6** have a significant degree of specificity against MCF-7, DLD-1, HeLa, and A549 (Table 2).

Earlier research studies have reported that oxidative stress is the key causative factor in chronic inflammation and various types of cancers[37,38]. Usually, the longer the existence of free radicals, the greater is the risk of cancer. In general, chronic exposure to free radical mediators leads to a sharp increase in mutagenesis, cell propagation, and activation of the oncogene, eventually causes cell proliferation that lost control over normal growth[39,40]. So, as metabolites and **ME** showed good antioxidative properties, we further investigated their anticancer abilities using the SRB assay. From the outcomes, it is justified that compound **4, 5,** and **ME** has an ability to lysis the cells of breast, colon, lung, and cervical cancer cell lines. Also, all the metabolites and **ME** are less toxic towards the human cell lines.

Conclusion

To conclude, the results of the present study indicated that the flavonols **(1-3)** and 1,2-diphenylethanes **(4-6)** from methanolic extract of *B. vahlii* displayed antioxidant activity by inhibiting DPPH and superoxide free radicals, anti-inflammatory activity by inhibiting COXs and 5-LOX, anti-gout activity by XO inhibition, and anticancer activity by inhibiting the growth of MCF-7, DLD-1, HeLa, and A549. The key metabolite responsible for *in vitro* activities claimed to be compounds **1, 3, 4,** and **6**. The results provide evidence that supports the traditional uses of *B. vahlii*. Also, these findings suggest that the plant *B. vahlii* can take an account as a good natural source of remedial medicine for oxidative stress, inflammation, gout and cancer. Hence, the results of the current study remain useful for further research to identify the potential bioactive molecules from *Bauhinia* genus. The future scope is to identify the binding affinity of compounds **1, 3, 4,** and **6** against tested cancer cell lines using *in silico* studies, which eventually helps in selective derivatization of the parent moieties.

Acknowledgement

All the authors are thankful to Maratha Mandal Dental College, Belgaum, India, for providing necessary facilities in the completion of anticancer activities.

Conflict of interest

No conflict of interest between any of the authors.

References

1 Burlakoti C and Kunwar R M, Folk herbal medicines of Mahakali watershed area, Nepal, *Med Plants Nepal An Anthol Contemp Res*, 2008, 187-193.

2 Dugasani S, Balijepalli M, Tandra S and Pichika M, Antimicrobial activity of *Bauhinia tomentosa* and *Bauhinia vahlii* roots, *Pharmacogn Mag*, 2010, **6**(23), 204-207.

3 Panda P, Dash P and Ghosh G, Chemometric profile, antioxidant and tyrosinase inhibitory activity of Camel's foot creeper leaves (*Bauhinia vahlii*), *Nat Prod Res*, 2018, **32**(5), 596-599.

4 Boonphong S, Puangsombat P, Baramee A, Mahidol C, Ruchirawat S, *et al.*, Bioactive compounds from *Bauhinia purpurea* possessing antimalarial, antimycobacterial, antifungal, anti-inflammatory, and cytotoxic activities, *J Nat Prod*, 2007, **70**(5), 795-801.

5 Maillard M P, Recio-Iglesias M C, Saadou M, Stoeckli-Evans H and Hostettmann K, Novel antifungal tetracyclic compounds from *Bauhinia rufescens* LAM, *Helv Chim Acta*, 1991, **74**(4), 791-799.

6 Rao Y K, Fang S H and Tzeng Y M, Anti-inflammatory activities of flavonoids and a triterpene caffeate isolated from *Bauhinia variegata*, *Phyther Res*, 2008, **22**(7), 957-962.

7 Yuenyongsawad S, Bunluepuech K, Wattanapiromsakul C and Tewtrakul S, Anticancer activity of compounds from *Bauhinia strychnifolia* stem, *J Ethnopharmacol*, 2013, **150**(2), 765-769.

8 Kittakoop P, Kirtikara K, Tanticharoen M and Thebtaranonth Y, Antimalarial preracemosols A and B, possible biogenetic precursors of racemosol from *Bauhinia malabarica* Roxb, *Phytochem*, 2000, **55**(4), 349-52.

9 Farag M A, Sakna S T, El-fiky N M, Shabana M M and Wessjohann L A, Phytochemical, antioxidant and antidiabetic evaluation of eight *Bauhinia* L. species from Egypt using UHPLC–PDA–qTOF-MS and chemometrics, *Phytochem*, 2015, **119**, 41-50.

10 Sowndhararajan K and Kang S C, Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight & Arn, *Saudi J Biol Sci*, 2013, **20**(4), 319-325.

11 Aderogba M A, Ogundaini A O and Eloff J N, Isolation of two flavonoids from *Bauhinia monandra* (Kurz) leaves and their antioxidative effects, *African J Tradit Complement Altern Med*, 2006, **3**(4), 59-65.

12 Singh M and Singh P, Phytochemical characterization and antibacterial activity of leaf extract of *Bauhinia vahlii* in Doon valley, Uttrakhand against human pathogens, *Scitech J*, 2014, **1**(3), 20-23.

13 Sowndhararajan K, Siddhuraju P and Manian S, *In vitro* evaluation of the antioxidant activities in the differentially processed seeds from underutilized legume, *Bauhinia vahlii* Wight & Arn, *Food Sci Biotechnol*, 2010, **19**(2), 503-509.

14 Das S N, Jagannath P V and Dinda S C, Evaluation of anti-inflammatory, anti-diabetic activity of Indian *Bauhinia vahlii* (stembark), *Asian Pac J Trop Biomed*, 2012, **2**(3), 1382-1387.

15 Elbanna A H, Mahrous E A, Khaleel A E and El-Alfy T S, Chemical investigation of *Bauhinia Vahlii* Wight and Arnott leaves grown in egypt, *Int J Pharm Pharm Sci*, 2016, **8**(6), 269-272.

16 Tatipamula V B and Kukavica B, Protective effects of extracts of lichen *Dirinaria consimilis* (Stirton) D. D. Awasthi in bifenthrin- and diazinon-induced oxidative stress in rat erythrocytes *in vitro*, *Drug Chem Toxicol*, 2020, 1-8.

17 Tatipamula V B, Killari K N, Sastry V G and Ketha A, *Taxithelium napalense* acts against free radicals and diabetes mellitus, *Bangladesh J Pharmacol*, 2017, **12**(2), 197-203.

18 Talluri M R, Ketha A, Battu G R, Tadi R S and Tatipamula V B, Protective effect of *Aurelia aurita* against free radicals and streptozotocin-induced diabetes, *Bangladesh J Pharmacol*, 2018, **13**(3), 287-295.

19 Tatipamula V B, Vedula G S and Sastry A V S, Chemical and pharmacological evaluation of manglicolous lichen *Roccella montagnei* Bel em. D. D. Awasthi, *Futur J Pharm Sci*, 2019, **5**(8), 1-9.

20 Tatipamula V B, Kolli M K, Lagu S B, Paidi K R, Reddy P R, *et al.*, Novel indolizine derivatives lowers blood glucose levels in streptozotocin-induced diabetic rats: A histopathological approach, *Pharmacol Rep*, 2019, **71**(2), 233-242.

21 Ketha A, Vedula G S and Sastry A V S, *In vitro* antioxidant, anti-inflammatory, and anticancer activities of methanolic extract and its metabolites of whole plant *Cardiospermum canescens* Wall, *Futur J Pharm Sci*, 2020, **6**, 11.

22 Tatipamula V B, Vedula G S and Sastry A V S, Antarvedisides A-B from manglicolous lichen *Dirinaria consimilis* (Stirton) and their pharmacological profile, *Asian J Chem*, 2019, **31**(4), 805-812.

23 Tatipamula V B and Ketha A, Manglicolous lichen *Parmotrema tinctorum* (Despr . ex Nyl .) Hale□: Isolation , characterization and biological evaluation, *Indian J Chem - Sect B*, 2020, **59B**, 856-861.

24 Haritha P, Patnaik S K and Tatipamula V B, Chemical and pharmacological evaluation of manglicolous lichen *Graphis ajarekarii* Patw. & C. R. Kulk, *Vietnam J Sci Technol*, 2019, **53**(3), 300-308.

25 De Souza L A, Tavares W M G, Lopes A P M, Soeiro M M and De Almeida W B, Structural analysis of flavonoids in solution through DFT 1H NMR chemical shift calculations: Epigallocatechin, kaempferol and quercetin, *Chem Phys Lett*, 2017, **676**, 46-52.

26 Salem M, Hussein S, El-Sharawy R, El-Khateeb A, Ragab E A, *et al.*, Antioxidant and antiviral activities of the aqueous alcoholic leaf extract of *Boscia angustifolia* A. Rich. (Capparaceae) and its major component "ombuin," *Egypt Pharm J*, 2016, **15**(1), 1-5.

27 Abdulkhaleq L A, Assi M A, Abdullah R, Zamri-Saad M, Taufiq-Yap Y H, *et al.*, The crucial roles of inflammatory mediators in inflammation: A review, *Vet World*, 2018, **11**(5), 627-635.

28 Tatipamula V B and Vedula G S, Anti-inflammatory properties of *Dirinaria consimilis* extracts in albino rats, *J Biomed Sci*, 2018, **4**(1), 3-8.

29 Sud'ina G F, Pushkareva M A, Shephard P and Klein T, Cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) selectivity of COX inhibitors, *Prostaglandins, Leukot Essent Fat Acids*, 2008, **78**(2), 99-108.

30 Tatipamula V B and Vedula G S, *In vitro* anti-inflammatory and cytotoxicity studies of two mangrove associated lichens, *Dirinaria consimilis* and *Ramalina leiodea* extracts, *Hygeia J drugs Med*, 2018, **10**(1), 16-26.

31 Nguyen H T, Vu T, Chandi V, Polimati H and Tatipamula V B, Dual COX and 5-LOX inhibition by clerodane diterpenes from seeds of *Polyalthia longifolia* (Sonn.) Thwaites, *Sci Rep*, 2020, **10**, 1-10.

32 Martel-Pelletier J, Lajeunesse D, Reboul P and Pelletier J P, Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs, *Ann Rheum Dis*, 2003, **62**(6), 501-509.

33 Chandrasekaran C V, Deepak H B, Thiyagarajan P, Kathiresan S, Sangli G K, *et al.*, Dual inhibitory effect of *Glycyrrhiza glabra* (GutGard™) on COX and LOX products, *Phytomedicine*, 2011, **18**(4), 278-284.

34 Fosslien E, Adverse effects of nonsteroidal anti-inflammatory drugs on the gastrointestinal system, *Ann Clin Lab Sci*, 1998, **28**(2), 67-81.

35 Nagao A, Seki M and Kobayashi H, Inhibition of xanthine oxidase by flavonoids, *Biosci Biotechnol Biochem*, 1999, **63**(10), 1787-1790.

36 Hellsten Y, Frandsen U, Orthenblad N, Sjødin B and Richter E A, Xanthine oxidase in human skeletal muscle following eccentric exercise: A role in inflammation, *J Physiol*, 1997, **498**(1), 239-248.

37 Federico A, Morgillo F, Tuccillo C, Ciardiello F and Loguercio C, Chronic inflammation and oxidative stress in human carcinogenesis, *Int J Cancer*, 2007, **121**(11), 2381-2386.

38 Sosa V, Moliné T, Somoza R, Paciucci R, Kondoh H, *et al.*, Oxidative stress and cancer: An overview, *Ageing Res Rev*, 2013, **12**(1), 376-390.

39 Rao C B, Babu D C, Bharadwaj T V, Srikanth D, Vardhan K S, *et al.*, Isolation, structural assignment and synthesis of (SE)-2-methyloctyl 3-(4-methoxyphenyl) propenoate from the marine soft coral *Sarcophyton ehrenbergi*, *Nat Prod Res*, 2015, **29**(1), 70-76.

40 Klaunig J E, Kamendulis L M and Hocevar B A, Oxidative stress and oxidative damage in carcinogenesis, *Toxicol Pathol*, 2010, **38**(1), 96-109.