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# Anti-proliferative Active Flavonoids from the Methanol Extract of Indian Herb *Artimisia reticulata*

**Ajoy Kumar Bauri[1, *], Peggy Jane Brodie[2], Sabine Foro[3], Nhu Quynh Nguyen Do[4]**

[1]Bio-Organic Division, Bhabha Atomic Research Centre, Mumbai, India
[2]Department of Chemistry, Virginia Tech, Blacksburg, USA
[3]Institute of Materials Science, Darmstadt University of Technology, Darmstadt, Germany
[4]Accident and Emergency Department, Franco Vietnamese Hospital, Ho Chi Minh City, Vietnam

## Email address

abauri@rediffmail.com (A. K. Bauri), nguyendonhuquynh@yahoo.com (N. Q. N. Do)

[*]Corresponding author

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**Abstract:** Fractionations of defatted methanol extract of herb *Artemisia reticulata* have led to isolation of a series of flavonoids (1-5). Anti-proliferative assay of these flavonoids was carried out against A2780 human ovarian cancer cell lines to compare with the prescribed drug taxol. Amongst these five flavonoids, compound (4) exhibited maximum anti-proliferative activity against the above cell lines. The structure of the compounds was established by combination of the $^{1}H$ and $^{13}C$ NMR spectrum, EIMS analysis and was confirmed by X-ray diffraction study.

**Keywords:** Flavonoids, *Artemisia reticulata*, Anti-proliferative Activity

## 1. Introduction

*Artemisia* species (family*: Asteraceae*) are globally distributed across the Europe and temperate Asian countries including Pakistan, India, Nepal, Tibet and China. In India, around seventeen *Artemisia* species with different varieties have been reported [1, 2]. These species are distributed in the Himalayan region across Jammu and Kashmir (Gurez Kistwar valleys), Himachal Pradesh (Kulu velley and Lahul & Kangara district) and Uttar Pradesh over an altitude range of 2100-4200 m. This herb is rich source of a number of potently bioactive compounds [1, 2] and belongs to class of sesquiterpenoids such as artemisin, artemisinin and flavonoids [3, 4]. Liu *et al*. reported [3] the first occurrence of sesqueterpenoids from *Artemisia annua* [5]. Recently, artemisin has been derived from herb *Artemisia annu* which belongs to sesquiterpenoid and its final clinical trial against the treatment of malaria [6]. Many sesquiterpenoids and penta cyclic triterpenoids have been also reported from *A. annua* [5]. In this connection, an alternative abundant source for artemisin and artemisinin from other varieties of *Artemisia* species has been investigated. Unfortunately, phytochemical investigation of the selected species, *A. reticulata* did not possess aforesaid terpenoids. Instead of that, a series of flavonoids was segregated by column chromatographic separation and identified by chemometric, spectrometric and spectroscopic methods in comparison with data available in literature. Herein isolation, structure elucidation and biological evaluation of constituents from methanolic extract of *A. reticulata* are described.

Flavonoids have antioxidant activity and becoming very popular because they possess many health promoting effects [7, 8]. Recent research indicates that flavonoids can be nutritionally helpful for triggering enzymes that reduce the risk of certain cancers, heart disease and age related degenerative diseases [7, 8]. Some of the biological activities attributed to flavonoids are anti-cancer [9], anti-allergic [10], anti-inflammatory [10] and anti-viral [11]. The flavonoid quercertin is known for its ability to relieve hay fever [9], eczema [11], sinusitis [10] and asthama [12]. Recently, epidemiological studies have illustrated that heart diseases [7, 8] are inversely related to flavonoid intake and also prevent the oxidation of low density lipoprotein [7, 8] thereby

reducing the risk for development of atherosclerosis. The flavone, salviginen was isolated in year 1971 by Brieskorn *et al*. from the plant *Salvia officinalis* / sage [13]. It was shown to display diverse bioactivities including aromatase, estrogen inhibitory [14] and cardiovascular activity [15], but its most notable trait is its anti-ulcer [16], anti-tumor [16] and anti-cancer [4].

In the present study, attempts have been made to isolate flavonoids from the methanol extract of *A. reticulata* (locally known as Kirmani aajowan, used as a major ingredient for preparation of herbal product Indian traditional medicine) by using column chromatography over silica gel with a gradient solvent elution with help of a binary mixture of solvent *n*-hexane and ethyl acetate. The bioassay of pure isolates (1-5) was conducted against human ovarian cell line A2780 in compare to prescribed drug taxol as control. Amongst them, compound (4) exhibited maximum anti-proliferative activity against the above cell line.

## 2. Results and Discussion

The methanol extract of *A. reticulata* fractionated by column chromatography over silica gel with gradient solvent system by using a mixture of *n*-hexane-ethyl acetate and several fractions have been collected. The individual fraction was monitored by TLC. The fractions with same TLC profile were combined together and further subjected to purification by column chromatography over silica gel followed by preparative TLC afforded compounds (1-5) (Figure 1). The chromatographic behavior of these compounds sprayed with $H_2SO_4$ on micro silica plate followed by heating at 110°C for 5 min displayed yellow spot [17] and sprayed with neutral $FeCl_3$ solution on micro silica plate visualized as dark green spot [17]. The positive Shinoda test [18] of these compounds suggested that these compounds belonging to flavonoid class. In addition, the individual component was obtained by final purification yielding substance with typical UV spectrum having absorptions at around 277 and 334 nm. The UV absorption was also been measured upon addition of different kind of UV shift reagents [19].

Compound (1) was isolated as a faint yellow bar shaped crystalline solid with molecular formula $C_{17}H_{14}O_5$ as deduced by EIMS at m/z 298 in combination with the $^1H$ and $^{13}C$ NMR spectrum. It showed absorption at around 3393.10 and 1654.60 $cm^{-1}$ due to the presence of phenolic-OH and >C=O groups in the compound. The chromatographic behavior of this compound sprayed with $H_2SO_4$, neutral $FeCl_3$ solution displayed yellow [17] and dark green colorations [18], typical UV absorptions [19] and positive Shinoda test [18] indicated that compound (1) belongs to flavonone. In UV spectrum, it showed absorptions at 241, 272 and 324 nm due to the presence of benzoyl (band I) and cinnamoyl (band II) part respectively [19] therefore, it was a flavones with free phenolic-OH groups. The exact location of phenolic-OH at C-5 in ring A of flavones (1) was confirmed by UV absorption in presence of anhydrous $AlCl_3$ in acidic pH red shift [19]. $^1H$ NMR spectrum of compound (1) showed $A_2B_2$ spin system at $\delta_H$ 7.84 & 7.02 ppm integrating for two protons each with coupling constants 2.2 & 8.8 Hz assigned as H-1′, H-6′ and H-3′, H-5′ of B ring protons. A sharp singlet at $\delta_H$ 12.80 ppm was ascribed as $C_5$-OH proton chelated with adjacent carbonyl group. In addition, a pair of doublets at $\delta_H$ 6.48, 6.36 ppm with coupling constants 2.2 Hz integrating for one proton each represented H-6 and H-8 of A ring and two singlets at $\delta_H$ 6.58, 6.55 ppm integrating for one proton each indicated H-3, H-8. In addition, other two sharp singlets at $\delta_H$ 3.88 and 3.78 ppm integrating for three protons due to each methoxyl groups located at C-7 and C-4′ of A and B ring respectively. $^{13}C$ NMR spectrum showed seventeen carbon peaks (Table 1) indicating two methoxylpeaks at $\delta_H$ 56.4, 55.6 ppm six methine and nine quaternary carbon signals [20]. Based on above physical, chemical and spectral data, the structure of compound (1) was assigned as 4′, 7-dimethoxy 5-hydroxyflavone.

Compound (2) was isolated as a yellow crystalline solid with molecular formula $C_{18}H_{16}O_6$ as established by EIMS at m/z 328 in combination with the $^1H$ and $^{13}C$ NMR spectrum. In IR spectrum, it showed absorptions at around 3583.10 and 1658.50 $cm^{-1}$ indicating the presence of the phenolic-OH and >C=O groups in compound (2). In UV spectrum, it showed absorptions at 242, 276 and 334 nm due to benzoyl (band I) and cinnamoyl (band II) part [19] of flavones respectively. The compound performed positive Shinoda test [18], thus it was deduced as a flavone. The assignment of phenolic-OH at C-5 in ring A of flavone (1) was confirmed by UV absorption by showing red shift in presence of anhydrous $AlCl_3$ in acidic pH [9]. $^1H$ NMR spectrum of compound 2 showed a sharp singlet at $\delta_H$ 12.81 ppm was ascribed as $C_5$-OH proton chelated with adjacent carbonyl group [19]. One $A_2B_2$ spin system at $\delta_H$ 7.84 & 7.02 ppm integrating for two protons each with coupling constants 2.2 & 8.8 Hz assigned as H-1′, H-6′ and H-3′, H-5′ of B ring protons. In addition, three sharp singlets at $\delta_H$ 3.97, 3.92, and 3.98 ppm integrating for three protons each due to three methoxyls located at C-6, C-7 in A ring and C-4′ in B ring of compound (2), two singlets at 6.59 and 6.55 ppm integrating for one proton each was determined salvigenin skeleton [20].

$^{13}C$NMR spectrum showed eighteen carbon peaks comprising of three methoxyl peaks resonanatee at $\delta_H$ 55.80, 56.20 and 59.80 ppm, six methine and nine quaternary carbon signals (Table 1). On comparison of spectroscopic data available in literature [20], compound (2) was identified as 5-hydroxy 4′, 6, 7-trimethoxy flavone named as salvigenin. Finally, the structure of it was confirmed by X-ray diffraction study.

Compound (3) was isolated as a yellow crystalline solid with molecular formula $C_{18}H_{15}O_7$ as established by EIMS at m/z 344 in combination with the $^1H$ and $^{13}C$ NMR spectrum. In IR spectrum, it showed absorptions at around 3583.10 and 1645.30 $cm^{-1}$ due to the presence of phenolic-OH and >C=O groups into it. Typical UV absorptions of this compound at 243, 277 and 334 nm was due to the presence of benzoyl (band I) and cinnamoyl (band II) parts, thus it was assumed as flavone [19]. The UV absorption of the compound in the

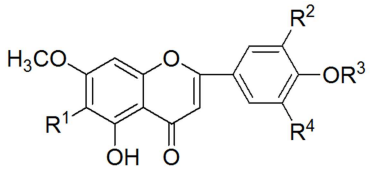
presence of anhydrous $AlCl_3$ in acidic pH having red shift [16] evidenced the location of phenolic-OH at C-5 in ring A of flavones (3). In its $^1H$ NMR spectrum, it displayed an ortho, a meta and a double doublet at $\delta_H$ 6.96, 7.32 and 7.50 ppm integrating for one proton each with coupling constants 8.2, 2.0 Hz and 8.2, 2.0 Hz due to AMX spin system in ring B. An additional singlet displayed at $\delta_H$ 3.85 integrating for three protons due to extra methoxyl peak in compound (3) instead of one aromatic proton in compound (2). The presence of the single phenolic-OH functionality in compound (3) was consistent with the difference from molecular weight of 30 amu in compare to the compound (2) indicated the presence of an additional methoxyl group in compound (3). The position of the $-OCH_3$ group could be assigned in B-ring either at C-3′ or C-5′ position. The exact location of $-OCH_3$ group in B ring of the compound (3) was assigned at C-3′ in ring B by measurement of the special interaction between H'-2 and $C'_3$-$OCH_3$ and was confirmed by XRD study.

In $^{13}C$ NMR spectrum (Table 1), one aryl methine resonance of compound (2) was replaced by an oxy-aryl carbon peak in compound (3) with slight variation of chemical shifts of the remaining carbon signals. The presence of the single phenolic-OH functionality in compound (3) was consistent with the difference in molecular weight of 30 amu of the compound (2). On comparison of spectroscopic data available in literature [20] and based on the above evidences, the structure of the compound (3) was assigned as 5-hydroxy 3', 4′, 6, 7-tetramethoxy flavone.

Compound (4) was isolated as a light yellow crystalline solid with molecular formula $C_{18}H_{16}O_7$ as deduced by EIMS at m/z 358 in combination with the $^1H$ and $^{13}C$ NMR spectrum. In IR spectrum, it showed absorptions at 3583.09 and 1650.66 $cm^{-1}$ revealing the presence of phenolic-OH and >C=O groups into it. In UV spectrum, it displayed absorptions at 243, 277 and 335 nm due to the presence of benzoyl and cinnamoyl moiety respectively, and thus it was assigned as a flavone [16]. It showed red shift in UV absorption upon addition of $AlCl_3$ in methanol solution revealing the presence-OH group positioned at C-5 [16]. In its $^1H$ NMR spectrum, it exhibited three pairs doublets viz. ortho & meta coupled proton at $\delta_H$ 7.03 ppm with coupling constants 8.4 & 2.2 Hz, meta coupled proton at $\delta_H$ 7.41 ppm with coupling constant 2.2 Hz and ortho coupled proton at 7.39 ppm with coupling constant 8.4 Hz due to AMX spin system of the B-ring of flavone (4). Two aromatic sharp singlets integrating for each one proton at $\delta_H$ 6.58 and 6.64 ppm were ascribed as H-8 and H-3 respectively in ring A and C of compound (4). In addition, three sharp singlets at $\delta_H$ 4.00, 3.98, 3.92 ppm integrating for each three protons were represented three methoxyl peaks and one sharp singlet at $\delta_H$ 12.82 ppm integrating for one protondue to $C_5$-OH chelated with adjacent carbonyl group was evidenced by UV absorption by displaying 35 nm bath chromic or red shift in presence of anhydrous $AlCl_3$ in acidic pH [16].

Compound (4) implied very similar signals in its $^1H$ NMR spectrum as on compare to compound (3) except the mass difference 14 amu due to the presence of phenolic-OH group instead of $-OCH_3$ in compound (3). Based on all the spectral evidence, the structure of the compound was assigned as 3′, 5-dihydroxy 4′, 6, 7-trimethoxy flavones. Finally, it was confirmed by single crystal X-ray diffraction study.

Compound (5) was isolated as a yellow crystalline solid with molecular formula $C_{19}H_{18}O_8$ as deduced by EIMS at m/z 378 in combination of the $^1H$ and $^{13}C$ NMR spectrum. In IR, it showed absorptions at around 3417.20 and 1659.45 $cm^{-1}$ to reveal the presence of phenolic-OH and >C=O groups in compound (5). In its UV spectrum, it displayed absorptions at 243, 277 and 334 nm due to benzoyl and cinnamoyl part and substantiated positive Shinoda test thus it was assigned as a flavone. The placement of phenolic-OH at C-5 in ring A of flavones (5) was established by UV absorption in presence of anhydrous $AlCl_3$ in acidic pH [19]. In $^1H$ NMR spectrum of the compound (5), a broad singlet at $\delta_H$ 7.10 ppm integrating for two aromatic protons, two separate singlets at $\delta_H$ 6.59 and 6.56 ppm integrating for one proton each were assigned as H-1′, H-6′ and H-8, H-3 respectively. In addition, there were three sharp singlets at $\delta_H$ 4.00, 3.92 and 3.89 ppm integrating for six and three protons each represented four methoxyl peaks located at C-6, C-7, C-3′ and C-5′ respectively, a sharp singlet was appeared at $\delta_H$ 12.57 ppm due to $C_5$-OH proton chelated with adjacent carbonyl group, also supported by red shift in UV absorption in presence of anhydrous $AlCl_3$ in acidic pH value [19]. $^1H$ NMR spectrum of the compound (5) was observed very similar signals as compare to compound (4), except an additional $-OCH_3$ signal in place one aromatic proton signal in compound (4) and was positioned at C-4′ of B ring in the compound (5). It was also evidenced with additional mass 30 amu in mass spectrum in compare to compound (4). Based on the above evidences, the structure of compound (5) was determined as 4′, 5-dihydroxy-3', 5′, 6, 7-tetramethoxy flavone. Finally, the structure of the compound was confirmed by single crystal X-ray diffraction study.




1. $R^1 = R^2 = R^4 = H$, $R^3 = CH_3$; Compound **1**
2. $R^2 = R^3 = R^4 = H$, $R^1 = OCH_3$; Compound **2**
3. $R^1 = R^2 = OCH_3$, $R^3 = CH_3$, $R^4 = H$; Compound **3**
4. $R^1 = OCH_3$, $R^2 = OH$, $R^3 = CH_3$, $R^4 = H$; Compound **4**
5. $R^1 = R^2 = R^4 = OCH_3$, $R^3 = H$; Compound **5**

***Figure 1.*** *Structure of the flavonoids (1-5) from the methanol extract of Artemisia reticulata.*

The compounds (1-5) from *A. reticulata* were subjected to evaluate on anti-proliferative activity against human ovarian cancer cell lines A2780 as described in literature [21]. The results revealed that only compound (4) possessed inhibitory activity against cell lines A2780 (Table 1) and others compounds are inactive. The activities against A2780 cell

lines of compound (4) suggests that the structural features are very important for their activities, probably due to presence number of free phenolic hydroxyl group available in B ring of flavones at its meta position. Therefore, the present study confirmed that compound (4) is lead structure to exhibit inhibitory activities against cell lines A2780.

***Table 1.*** *$^1H$ and 13 C NMR ($CD_3OD$, 200 MHz) Data for Compounds (1-5).*

| Position | Comp 1 | | Comp 2 | | Comp 3 | | Comp 4 | | Comp 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta(J_{H-H})$ Hz | $\delta_C$ | $\delta$ ($J_{H-H}$ Hz) | $\delta_C$ | $\delta$ ($J_{H-H}$) Hz | $\delta_C$ | $\delta(J_{H-H})$ Hz | $\delta_C$ | $\delta(J_{H-H})$ Hz | $\delta_C$ |
| 1 | - | | - | - | - | | - | | - | |
| 2 | - | 132.28 | - | 132.28 | - | 123.73 | - | 124.12 | | 147.22 |
| 3 | 6.58, s | 104.67 | 6.55, s | 103.75 | 6.59, s | 104.43 | 6.58, s | 104.47 | 6.59, s | 138.25 |
| 4 | - | 182.56 | - | 182.37 | - | 182.56 | - | 182.70 | | 182.45 |
| 4a | - | 105.58 | - | 108.28 | - | 104.50 | - | 104.47 | | 104.45 |
| 5 | 12.80, s | 162.52 | 12.81, s | 162.32 | 12.80, s | 158.73 | 12.82, s | 158.80 | 12.87, s | 158.62 |
| 6 | 6.36, d (2.2) | 92.66 | - | 152.92 | - | 153.30 | - | 150.50 | | 153.05 |
| 7 | - | 146.3 | - | 163.72 | - | 163.97 | - | 164.50 | | 163.76 |
| 8 | 6.48, d (2.2) | 98.08 | 6.59, s | 103.75 | 6.54, s | 90.58 | 6.54, s | 90.62 | 6.56, s | 90.47 |
| 8a | - | 146.24 | - | 158.43 | - | 153.02 | - | 154.33 | | 158.62 |
| 1' | - | 123.36 | - | 123.16 | - | 120.06 | - | 119.16 | | 122.09 |
| 2' | 7.84, dd (8.8, 2.2) | 114.47 | 7.85, d (8.68) | 127.67 | 7.32, d (2.2) | 111.13 | 7.46, d (2.0) | 110.73 | 7.10, s | 132.25 |
| 3' | 7.02, dd (8.8, 2.2) | 128.05 | 7.02, d (8.68) | 114.20 | - | 153.20 | - | 154.33 | - | 153.05 |
| 4' | - | 162.32 | - | 152.72 | - | 152.28 | - | 146.06 | - | 163.76 |
| 5' | 7.02, dd, (8.8, 2.2) | 128.05 | 7.02, d (8.68) | 114.20 | 6.96, d (8.2) | 108.74 | 6.96, d (8.2) | 112.36 | - | 153.05 |
| 6' | 7.84, dd, (8.8, 2.2) | 114.47 | 7.84, d (8.68) | 127.67 | 7.51, dd (8.2, 2) | 111.13 | 7.32, dd (2.2, 8.2) | 110.73 | 7.10, s | 132.25 |
| $C_6OCH_3$ | - | - | 3.92, s | 55.80 | 3.91, s | 56.10 | 4.00, s | 56.36 | 3.92, s | 56.21 |
| $C_7OCH_3$ | 3.88, s | 55.45 | 3.97, s | 59.80 | 3.96, s | 60.84 | 3.98, s | 60.90 | 3.98, s | 60.21 |
| $C_3'OCH_3$ | - | - | - | - | 3.96, s | 56.32 | 3.92, s | - | 4.0, s | 56.42 |
| $C_4'OCH_3$ | 3.89, s | 55.60 | 3.98, s | 56.20 | 3.96, s | 56.32 | - | 56.18 | | - |
| $C_5'OCH_3$ | - | | - | | - | | - | - | 4.0, s | 56.42 |

# 3. Experimental Section

## 3.1. General Experimental Procedure

Melting points were determined using a Fischer melting point apparatus. UV spectra were measured on Shimadzu UV-2100 UV-Vis spectrophotometer. NMR spectra were recorded in $CDCl_3$ or $CD_3OD$ on a Bruker Avance 200 spectrometer using residual $CHCl_3$/HDO as an internal standard. Chemical shift are given in ppm ($\delta_H$ & $\delta_C$), relative to residue $CHCl_3$/HDO (7.25 & 77.00 / 4.78 or 3.30 & 49.00 ppm). Mass spectra were registered using Fision 8000 (8000 series, UK) and Shimadzu QP5050A mass spectrometer (Japan). Silica gel 60 (230-400 mesh, Merck) was used for analytical TLC. Silica gel 60 (70-230 mesh, Merck) was used for column chromatography. All compounds were visualized by TLC using 10% $H_2SO_4$ in MeOH followed by heating at 110° C for 5 min, DNPh in EtOH and neutral $FeCl_3$ in MeOH.

## 3.2. Anti-proliferative Activity Bioassay/Biological Evaluation

Bioassay was performed at Virginia Polytechnic Institute and State University against A2780 human ovarian cancer cell lines using standard methodology as described previously [21]. Flavones (1-5) isolated from *A. reticulata* were tested with different concentrations against human ovarian cancer cell lines A2780 in compare to prescribed drug taxol as control. The inhibition activities of these compounds were estimated after 48 h. The percentage of inhibition and $IC_{50}$ were calculated with different concentrations and concentration in µg/mL was estimated on molecular weights of the compounds (Table 2).

***Table 2.*** *Anti-proliferative activity of compounds (1-5) tested against human ovarian cancer cell line A2780.*

| Compound | Mole Wt | Linear slope | $IC_{50}$µg/mL |
|---|---|---|---|
| 1 | 298 | - | NA |
| 2 | 328 | - | >20 |
| 3 | 358 | - | >20 |
| 4 | 344 | - | 2.6 |
| 5 | 374 | - | >20 |

NA = Not tested

## 3.3. Plant Material

The herb *A. reticulata* (in local dialect known as Kirmani aajowan, used as herbal medicine in Indian traditional system of medicine, Ayurdev) was purchased from local market Mumbai in 2007. The plant was authenticated by Dr. Salungke, Landslide & Cosmetic Division, BARC. A voucher specimen has been deposited in Herbarium of Landslide & Cosmetic Division, BARC.

## 3.4. Chemical Profiling

Chemical profiling was carried out by fractionation of the methanol soluble extract of the plant employing column chromatography over silica gel followed by preparative TLC to isolate the active ingredients. The chemical constituents were characterized by spectrometric and spectroscopic analysis and compared with data reported in literature. These flavones are reported in literature but isolated from new abundant source, *A. reticulata* and their biological evaluation has been carried out against A2780 cell lines.

### 3.5. Extraction and Isolation

Fresh dried plant (~250 g) was ground and extracted with methanol (500 ml x 3 x 5 x days) at room temperature. The filtrates were combined and solvent were removed in *vacuo* to yield crude methanol extract (amt~20 g). This extract was column chromatographed over silica gel (500 g, particle size 150-200 mesh) and eluted with increasing concentration of ethyl acetate in hexane followed by methanol in chloroform with increasing gradient of polarity of eluting solvent system. The volume of each aliquots collected approximately 250 ml. Each and every fraction was monitored by TLC; fractions with similar TLC profiles were combined to yield fractions F1-F10. Fraction F-1 was further subjected to preparative TLC eluting with ethyl acetate-hexane (10:90) to yield compound (1) ($R_f$ ~ 0.57, 12 mg) along with two sesquiterpenes. Fraction F-3 was subjected to further column chromatograph over silica eluting with ethyl acetate-hexane (15:85) followed by crystallization in acetone to obtain compound (2) as major product ($R_f$ ~ 0.66, ~ 1.0 g). Fraction F-4 was separated on a silica gel column eluted with a gradient solvent system using ethyl acetate-hexane to furnish four sub-fractions designated as F-4/1-F4/4. Sub-fraction F-4/1 gave additional compound (2) (30 mg). Sub-fraction F-4/2 was separated by preparative TLC, eluting with ethyl acetate-dichloromethane (20:80) to yield compound (3) ($R_f$ ~ 0.58, 120 mg) along with sesquiterpene almost in 1:1 ratio. Sub-fraction F-4/3 has subjected to preparative TLC developing with ethyl acetate-hexane (15:85) gave compound (4) (~50 mg). Fraction F-6 has been subjected to PTLC and eluting with ethyl acetate-hexane (20:80) gave compound (5) (30 mg).

### 3.6. Characterization of the Isolates

Compound (1): Faint yellow crystalline solid substance, m. p. 190°C; $^1H$ and $^{13}C$ NMR ($CDCl_3$, 200 MHz): see Table 1; $\upsilon_{max}$ (KBr): 3393.10, 2976.64, 2885.95, 2927.43, 2905.20, 2885.9, 2849.3, 1701.9, 1670.0, 1654.6, 1607.4, 1444.4, 1384.7, 1354.7, 1271.8, 1164.8, 1038.5, 890.9, 831.2, 756.9. $\lambda_{max}$ (MeOH): 241 (0.135), 272 (0.168), 324 (0.184) nm; EIMS: m/z (%) 298 [$M^+$] (67.11), 268 (100), 253 (19.26), 241 (91.40), 226 (25.51), 212 (32.07), 199 (13.11), 184 (44.45), 169 (45.45), 155 (65.24), 128 (62.97), 123 (89.17), 115 (52.15), 104 (22.46), 82 (44.27), 78 (99.00), 66 (28.00), 55 (26.70).

Compound (2): Yellow crystal, m.p. 198°C, molecular formula $C_{18}H_{16}O_6$ dedueced from EIMS. $^1H$ ($CDCl_3$, 200 MHz) and $^{13}C$ NMR ($CDCl_3$, 50 MHz) see Table 1; $\upsilon_{max}$ (KBr): 3583.1, 2924.5, 1588.1, 1453.1, 1350.9, 1294.0, 1205.3, 119.16, 112.36, 110.73, 104.47, 90.62. $\lambda_{max}$ (MeOH): 241 (0.135), 272 (0.168), 324 (0.184) nm.

EIMS: m/z (%) 328 [$M^+$] (72.0), 314 (77.9), 300 (60.49), 282 (100), 268 (40.72), 254 (13.01), 214 (14.58), 182 (19.24), 153 (30.15), 136 (56.75), 126 (44.98), 117 (33.73), 90 (18.89), 70 (15.00), 64 (14.06).

Crystal data: $C_{18}H_{16}O_6$, $M_r$ = 328.31, crystal system triclinic, space group p-1, a = 7.0979 (8) A, b = 7.5025 (8) A, c = 14.964 (1) A, α = 76.64 (1) deg, β = 85.74 (1) deg, γ = 81.37 (1) deg, Z = 2, $D_c$ = 1.424 mg/ $m^{-3}$, F(000) 344, crystal dimenssion 0.55 x 0.35 x 0.08 mm.

Compound (3): Yellow crystal, m.p. 180°C, $M_r$358, molecular formula $C_{19}H_{18}O_7$ dedueced by EIMS. $^1H$ NMR data see Table 1; $^{13}C$ NMR data see Table 1; $\upsilon_{max}$(KBr): 3583.1, 2924.5, 1588.1, 1453.1, 1350.9, 1294.0, 1205.3, 119.16, 112.36, 110.73, 104.47, 90.62. $\lambda_{max}$ (MeOH): 241 (0.135), 272 (0.168) and 324 (0.184) nm. EIMS: m/z (%) 358 [$M^+$] (100), 341 (28.7), 313 (43.9), 298 (30.12), 270 (15.2), 245 (15.2), 214 (10.5), 198 (12.4), 180 (12.8), 163 (29.5), 148 (72.5), 137 (23.9), 108 (16.8), 91 (18.6), 91 (18.6), 71 (15.6).

Crystal data: $C_{19}H_{18}O_7$, $M_r$ = 358.33, crystal system monoclinic, space group P 21/c, a = 27.929 (8) °A, b = 8.6045 (8) °A, c = 14.382 (1) °A, $\alpha$ = 90 deg, β = 104.95 (1) deg, γ = 14.382 (1) deg, Z = 8, $D_c$ = 1.426 mg/ $m^{-3}$, F (000) 1504, crystal dimenssion 0.10 x 0.08 x 0.03 mm.

Compound (4): Yellow crystal, m.p. 195°C, $M_r$ 344, molecular formula $C_{18}H_{16}O_7$ dedueced by ESIMS. $^1H$ and $^{13}C$ NMR data see Table 1; $\upsilon_{max}$ (KBr): 3583.09, 3397.97, 2925.48, 2375.87, 2341.16, 1653.66, 1605.45, 1512.88, 1495.56, 1455.99, 1362.46, 1270.86, 1203.36, 1122.37, 665.32. $\lambda_{max}$ (MeOH): 241 (0.135), 272 (0.168) and 324 (0.184) nm. EIMS: m/z (%) 345 (53.01) 330 (43.5), 298 (16.25), 182 (42.03), 154 (70.8), 135 (29.2), 125 (18.4), 106 (26.3), 86 (24.74), 70 (100), 58 (64.94).

Crystal data: $C_{18}H_{16}O_7$, $M_r$ 344.31, space group monoclinic P 2l/c, a = 5.5495 (4) °A, b = 37.686 (6) °A, c = 7.4795 (4) °A, α = 90 deg, β = 96.489 deg, γ = 90 deg, Z = 4, Dc = 1.471 mg/$m^{-3}$, F (000) 720, crystal dimenssion 0.40 x 0.15 x 0.15 mm.

Compound (5): Yellow crystal, m.p. 246°C, molecular formula $C_{19}H_{18}O_8$ dedueced by ESIMS. $^1H$ and $^{13}C$ NMR data see Table 1; $\upsilon_{max}$ (KBr): 3417.24, 3019.01, 1659.45, 1607.38, 1510.95, 1492.63, 1456.96, 1428.03, 1337.39, 1301.72, 1266.04, 1214.93, 1182.15, 1122.37, 836.96, 757.89. $\lambda_{max}$ (MeOH): 241 (0.135), 272 (0.168) and 324 (0.184) nm; EIMS: *m/z* (%) 374 [$M^+$] (5.0), 359 (100), 344 (21.0), 332 (18.5), 298 (24.5), 188 (11.0), 178 (15.5), 178 (15.5), 164 (27.3), 153 (56.7), 148 (11.1), 126 (13.9), 119 (11.0), 99 (11.4), 84 (25.3), 69 (51.5), 58 (55.9).

Crystal data: $C_{19}H_{18}O_8$, $M_r$ = 374.33, space group p $2_l$/c monoclinic, a = 16.120 (3) °A, b = 15.416 (3) °A, c = 13.674 (3) °A, α = 90 deg, β = 94.35 deg, γ = 90 deg, Z = 8, $D_c$ = 1.468 mg/$m^{-3}$, F(000) 720, crystal dimenssion 0.18 x 0.10 x 0.08 mm.

## 4. Conclusions

Phytochemical profiling and dereplication by employing column chromatography, spectrometric and spectroscopic analyses have been successful in identifying five (1-5) flavonones. These flavones are previously reported in literature [19, 20, 22], but isolated from different resource, *A. reticulata*. In this study, we report that among the series of above flavones, compound (4) has been identified as

potential anti-cancer lead compound against cell lines A2780.

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## Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

## Appendix

*Supplementary Information*

Supporting Information (Spectroscopic data consisting $^{1}H$ NMR, $^{13}C$ NMR, MS and X-ray diffraction data of compounds (1-5) are enclosed in attached file (SI.pdf). Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to Director, CCDC, 12 Union Road, Cambridge CB2, 1EZ, UK.

## References

[1] Khare CP (2008). Indian Medicinal Plants, page 64-65; Springer-Verlag Heidelberg Publishers

[2] Bora KS, Sharma A (2010). Phytochemical and Pharmacological potential of *Artemisa absinthium* & *Artemisia asiatica* Nakai. J. Pharm. Res. 3 (2), 325-28.

[3] Liu JM, Ni MY, Fan JF, Tu YY, Wu ZH, Qu YL, Chou MS (1979*).* Acta Chim. Sinica, 37, 129-143.

[4] Androutsopoulous VP, Li N, Arroo RRJ (2009). J. Nat. Prod. 72, 1390-1394.

[5] Dhingra V, Rao V, Narasu ML (2000). Life Sci., 66 (4), 279-300.

[6] WHO report on implementation of global malaria control strategy (1993-2000). Technical Report Series No. 839, World Health Organization, ISBN 13: 9789241208390, order no. 11000839, WHO. Press Editions de I'MOS-Edicones de la OMS.

[7] Flavonoids: Chemistry, Biochemistry and applications Edited by Oyvind Anderses, University of Bergen, Norway; Kenneth R Markham, industrial Research Ltd Lower Hutt, New Zealand.

[8] Flavonoids and Heart Health: Proceedings of the ILSI North America Flavonoid Work shop, May 31-June 1, (2005), Washington (USA).

[9] Teillet F, Boumendjel A, Boutonnat J, Ronot X (2007). Med. Res. Rev. 28 (5), 715-7.

[10] Yanling L, Hao F, Wenfang X (2007). Recent advance in the research of flavonoids anticancer agents. Mini Review in Medicinal Chemistry 7, 663-678.

[11] Kaul TN, Middleton Jr MD, Ogra PL (2005). Flavonoid as Anti-viral agent: J. Med. Viro. 15, 71-79.

[12] Lopez LM (2002). Curr. Med. Chem. 2 (6), 691-714.

[13] Brieskorn CH, Biechele W (1971). Deut Apoth Ztg 111, 141; Anal Abstra. 214404.

[14] US Patent No. 06506792 (2003). Inventor: Reed, Michel John. London, United Kingdom Pott; Barry Victor Lloyd, Bath, United Kingdom, 2003/1/14/2000/8/14.

[15] Sonmez UDB, Selcuk T, Osman O, Ufuk K, Gulacti T, Ayhan U (2005). Vascular Pharmacology 43, 220-226.

[16] Ren W, Qiao Z, Wang H, Zhu L, Zhang L (2003). Med. Res. Rev. 23519-534.

[17] Waldi D (1965). Spray Reagents for Thin Layer Chromatography Academic Press, New York, page 501.

[18] Dean FM (1963). Naturally Occurring Oxygen Ring Compounds. Butterworth & Co Ltd., London, pp. 288-89.

[19] Mabry TJ, Markham KR, Thomas MB (1970). The Systematic Identification of Flavonoids. Springer-Verlag, New York, pp. 36-38.

[20] Horie T, Ohtsuru Y, Shibatat K, Yamashita K, Tsukamaya M, Kawamukat Y (1998). Phytochemistry 41, 865-874

[21] Cao S, Brodie PJ, Randrianaivo R, Ratovoson F, Callmander M, Andriantsiferana R, Rasamison VE, Kingston DGI (2007). J. Nat. Prod. 70, 679-681.

[22] Tarawneh A, Leon F, Pettaway S, Elokely KM, Klein ML, Lambert J, Mansoor A, Cutler SJ (2015). J. Nat. Prod. 78, 14611465.