Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating 2017: 3.53
JMPS 2017; 5(2): 136-139

Received: 18-01-2017
Accepted: 19-02-2017

Divya Goel
Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India

Vijender Singh
School of Pharmacy, Sharda University, Plot No. 32-34, Knowledge Park – III, Greater Noida, Uttar Pradesh, India

Mohammed Ali
Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India

Shahnaz Sultana
College of Pharmacy, Jajan University, Jajan, Saudi Arabia

Showkat R Mir
Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India.

Correspondence
Mohammed Ali
Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India

New Phytoconstituents from the shoots of *Artemisia annua* L. cultivar *jwarharti*

Divya Goel, Vijender Singh, Mohammed Ali, Shahnaz Sultana and Showkat R Mir

Abstract
Artemisia annua L. cultivar *jwarharti* (Asteraceae) is an aromatic annual herb up to 2 m in height found in temperate Asia, especially China and naturalized in many countries of the world. The plant is prescribed against fever, malaria, skin diseases, jaundice, malignant ulcers and haemorrhoids. Phytochemical investigation of the methanolic extract of the shoots of *A. annua* var. *jwarharti* led to the isolation of three new chemical constituents characterized as 5, 7, 4′- trimethoxy-8,3′-dihydroxyflavone (**2**), 3,4-dihydroxybenzyl 2′,3′,4′-trihydroxybenzoate (**3**) and 3,4-dihydroxybenzyl 2′,3′,4′-trihydroxybenzoate 4,4′-β-D-dixylopyranoside (**4**) along with the known compound stigmasterol. The structures of all these phytoconstituents have been identified on the basis of spectral data analysis and chemical reactions.

Keywords: *Artemissia annua* cultivar *jwarharti*, shoots, flavone, 3,4-dihydroxybenzy benzoate derviatives, structural elucidation.

1. Introduction
Artemisia annua L. (Asteraceae), known as sweet wormwood, sweet sagewort or annual wormwood (Chinese: qinghao), is an aromatic annual herb up to 2 m in height with yellow flowers. It is a native to temperate Asia, especially China but naturalized throughout the world including Argentina, Bulgaria, France, Hungary, India, Italy, Romania, Spain and USA. Currently, it is the source for the production of artemisinin and semi-synthetic artemisinin derivatives (including dihydroartemisinin, artesunate, artemether and arteether) which are cadinane-type sesquiterpenes lactones used for the production of combination therapies for treatment of malaria [1, 2]. The plant is highly pollinated and the seeds exhibited a great variation in maturity, biomass and the quantity of artemisinin. Artemisinin (Qinghaosu) presently is the most potent and efficacious compound against chloroquine and quinine-resistant *Plasmodium falciparum* and other malaria-causing parasites. Beside antimalarial effects, *A. annua* exhibited biological activities such as antibacterial, anti-inflammatory, angiotensin converting enzyme inhibitory, cytokininlike and antitumor effects [3]. In China, an aqueous preparation of the dried herb is prescribed to treat fever, malaria, skin diseases, jaundice, malignant ulcers and haemorrhoids. It is effective against pathogenic 'shu', a summer heat syndrome characterized by headache, fever, dry mouth, irritability, excessive sweating and full pulse. *A. annua* is one of the important ingredient in several Ayurvedic formulations. World Health Organization shows high interest with the active constituent artemisinin and its chemical derivatives. *A. annua* is included in the official Pharmacopoeia of China as Qinghao and in the drug directories of India, Japan and Vietnam.
The prominent coumarins identified from *A. annua* were aesculetin, iso-fraxidin, scopoletin, scopolin and tomentin. The main phenolic components of *A. annua* were quercetin glucoside, flaviolin, rhamnetin, chrysoplenol D, pillion and chlorogenic acid. In addition, other phenolic compounds such as 2, 4-dihydroxy-6-methoxy-acetophenone, 5-nonadecyl-3-O-methyletherresorcinol, 2, 2, 6-trihydroxychromene and 2,2-dihydroxy-6-methoxychromene have also been isolated from *A. annua* [4–11]. Apart from artemisinin, other terpenoidal lactones have been isolated from the aerial parts of the plant [12]. The plant also contained phytols, flavones, chrysoplenetin, chrysosplenol-D, friedelin, sterols and anthraquinones [12,13]. The root volatile oil was consisted mainly of cis-arteannuic alcohol (25.9%), β-farnesene (6.7%), β-maaliene, β-caryophyllene, its oxide and 2-phenylbenzaldehyde [14]. The stem volatile oil was rich in sesquiterpenes [15]. The paper describes isolation and characterization of three new

chemical constituents from the shoots of *A. annua* L. cultivar *jwarharti*.

2. Material and Methods

2.1 General procedures

Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India) and are uncurrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin-Elmer-Rotkreuz, Switzerland) in methanol. Infra-red spectra were recorded on Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Hong) spectrophotometer using KBr pellets; γ_{max} values are given in cm^{-1}. ^1H and ^{13}C NMR spectra were screened on advance DRX 400, Bruker spectrospin 400 and 100 MHz instrument in 5 mm spinning tubes at 27 ºC, respectively (Karlesruthe, Germany) using TMS as an internal standard. Mass spectra were scanned by effecting FAB ionization at 70 eV on a JEOL-JMS-DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G (Qualigen). Spots were visualised by exposing to iodine vapours, UV radiation, and spraying with ceric sulphate solution.

2.2 Collection of plant material

The shoots of *A. annua* cultivar *jwarharti* were collected from the experimental field of National Institute of Plant Genome Research (NIPGR), Aruna Asif Ali Road, New Delhi -110067 and identified by Dr. Sushil Kumar, Scientist Emeritus, NIPGR.

2.3 Extraction and isolation

The dried shoot powder (1.0 kg) was exhaustively extracted with methanol in a Soxhlet apparatus. The methanolic extract was evaporated under reduced pressure to get a brown viscous mass (50 g, 5.0% yield). The dried extract was dissolved in minimum quantity of methanol and added to silica gel (60-120 mesh) to prepare a slurry. It was air-dried, powdered and loaded on a silica gel column prepared in petroleum ether. The column was run with petroleum ether (b.p. 60- 80 °C), petroleum ether-chloroform (9:1, 3:1, 1:1, 1:3, v/v), chloroform, chloroform- methanol (99:1, 49:1, 19:5, 9:1, 17:3,4:1 7:3 and 1:1, v/v) and methanol. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having same R_f values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated:

2.4 Stigmasterol (1)

Elution of the column with chloroform furnished colourless crystals of **1**; recrystallized from methanol; 21 mg (0.025% yield); R_f**:** 0.24 (petroleum ether: chloroform, 1:9); comparable with the authentic sample of stigmasterol, m. p.: 168-169º C; +ve ion FAB MS *m/z* (rel. int.): 412 $[M]^+$ ($C_{29}H_{48}O$) (15.6), 397 (28.3), 394 (21.5).

2.5 Artimisiaflavone (2)

Elution of the column with chloroform–methanol (99:1) mixture afforded colourless amorphous powder of **2**; recrystallized from chloroform-methanol (1:1); 153 mg (0.188 % yield), R_f **:** 0.44 (chloroform-methanol, 9:1), m. p.: 163-165º C, UV λ_{max} (MeOH): 272, 313, 344 nm (log ε 4.8, 4.9. 6.9), UV λ_{max} (MeOH + NaOMe): 271, 313, 344 nm (log ε 6.7, 5.3), UV λ_{max} (MeOH + NaOAc): 273, 311 nm (log ε 2.9, 4.1), UV λ_{max} (MeOH + NaOAc + H_3BO_3): 273, 311, 346 nm (log ε 3.5, 4.8), UV λ_{max} (MeOH + $AlCl_3$): 270, 298, 310 nm (log ε 3.5, 3.6, 4.7), UV λ_{max} (MeOH + $AlCl_3$ + HCl): 273, 309, 345 nm (log ε 3.4, 3.5, 4.2), IR υ_{max} (KBr): 3335, 2990, 2945, 1702, 1612, 1564, 1506, 1423, 1288, 1190, 1134, 1014, 915, 863, 816, 740 cm^{-1}, ^1H NMR ($CDCl_3$): δ 7.68 (1H, dd, J = 2.8, 9.3 Hz, H-6′), 7.71 (1H, d, J = 2.8 Hz, H-2′), 6.93 (3H, brs, H-3), 6.84 (1H, brs, H-6), 6.16 (1H, J = 9.3 Hz, H-5′), 3,96, 3.90, 3.88 (3H each, brs, 3 x OMe); ^{13}C NMR ($CDCl_3$): δ 162.46 (C-2), 102.75 (C-3), 182.30 (C-4), 161.17 (C-5), 101.86 (C-6), 164,98 (C-7), 141,84 (C-8), 162.99 (C-9), 103.06 (C-10), 121.78 (C-1′), 110.91 (C-2′), 142.71 (C-3′), 150.61 (C-4′), 116.03 (C-5′), 119.11 (C-6′), 55.18, 55.31, 55.15 (3 x OMe) +ve ion FAB MS *m/z* (rel. int.): 344 $[M]^+$ ($C_{18}H_{16}O_7$) (2.2), 329 (5.2), 313 (3.6), 301 (4.2), 237 (5.2), 221 (5.3), 196 (10.3), 192 (100), 181 (11.3), 177 (14.5), 176 (10.6), 166 (18.3), 161 (18.5), 153 (52.8), 152 (13.7), 148 (39.1), 138 (33.5), 137 (41.2), 133 (21.5), 123 (45.3), 117 (21.8), 108 (37.6).

2.6 3, 4-Dihydroxybenzyl trihydroxybenzoate (3)

Elution of the column with chloroform–methanol (7:3) mixture furnished colourless precipitate of **3**; recrystallized from ethanol; 447 mg (0.562% yield), R_f : 0.17 (MeOH:$CHCl_3$; 3:7), m. p.: 209 - 210º C, UV λ_{max} (MeOH): 275 nm (log ε 3.1), IR υ_{max} (KBr): 3440, 3345, 3205, 3058, 2760, 1723, 1657, 1603, 1528, 1430, 1393, 1281, 1186, 1058, 1011, 818 cm^{-1}, ^1H NMR ($CDCl_3$): δ 8.08 (1H, d, J = 2.5 Hz, H-2), 6.93 (1H, d, J = 7.8 Hz, H-5′), 6,18 (1H, d. J = 8.5 Hz, H-5), 5.81 (1H, dd, J = 2.5, 8.5 Hz, H-6), 5.25 (1H, d, J = 7.8 Hz, H-6′), 3.39 (2H, brs, H_2-7); ^{13}C NMR ($CDCl_3$): δ 142.11 (C-1), 137.48 (C-2), 155.36 (C-3), 151,23 (C-4), 131,78 (C-5), 118.29 (C-6), 62.86 (C-7), 138.25 (C-1′), 156.81 (C-2′), 157.46 (C-3′), 156.13 (C-4′), 127.73 (C-5′), 116.18 (C-6′), 173.71 (C-7′), +ve ion FAB MS *m/z* (rel. int.): 292 $[M]^+$ ($C_{14}H_{12}O_7$) (2.5), 153 (18.6), 139 (10.1), 123 (11.3).

2.7 Hydrolysis of 3: Compound **3** (25 mg) was heated with alkaline alcoholic solution on a steam bath for 1 h. The reaction mixture was extracted with chloroform (2 x 5 ml) to isolate 3,4-dihydroxybenzyl alcohol, m. p. 116 – 117 °C; HPTL Rt 53.08 (C_{18} Beckman column, solvent : methanol-formic acid, 19:1). The reaction mixture was acidified with dilute hydrochloric acid, dried under reduced pressure and dissolved in ethanol to isolate colourless crystals of 2,3,4-dihyddroxybenzoic acid, m. p. 203 – 205 ºC, Rt 21.01 (C_{18} column, solvent: methanol and formic acid (0.1%).

2.8 3,4-Dihydroxybenzyl trihydroxybenzoate diglucoside (4)

Further elution of the column with chloroform–methanol (7:3) mixture afforded colourless crystals of **4**; recrystallized from methanol; 110 mg (0.138% yield), R_f : 0.82 (MeOH:$CHCl_3$; 3:7), m. p. : 169 - 170º C, IR υ_{max} (KBr): 3404, 3317, 3216, 2953, 2906, 1722, 1511, 1453, 1342, 1280, 1132, 1076, 1022, 959 cm^{-1}, ^1H NMR (DMSO- d_6): δ 8.02 (1H, d, J = 2.7 Hz, H-2), 6.91 (1H, d, J = 8.1 Hz, H-5′), 6.26 (1H, d, J = 8.5 Hz, H-5), 5.80 (1H, dd, J = 2.7, 8.5 Hz, H-6), 5.28 (1H, d, J = 8.1 Hz, H-6′), 4.73 (1H, d, J = 7.2 Hz, H-1″), 4.64 (1H, d, J = 7.3 Hz, H-1‴), 4.54 (1H, m, H-2″), 4.50 (1H, m, H-2‴), 4.37 (1H, m, H-3″), 4.35 (1H, m, H-3‴), 3.63 (1H, m, H-4″), 3,61 (1H, m, H-4‴), 3.29 (2H, d, J = 8.8 Hz, H_2-5″), 3.04 (2H, d, J = 9.3 Hz, H_2-5‴); ^{13}C NMR (DMSO- d_6): δ 140.39 (C-1), 138.15 (C-2), 155.61 (C-3), 150.84 (C-4), 131,75 (C-5), 117.52 (C-6), 63.16 (C-7). 140.23 (C-1′), 157.49 (C-2′),

156.14 (C-3′), 152.26 (C-4′), 127.68 (C-5′), 119.04 (C-6′), 173.68 (C-7′), 100.84 (C-1″), 82.86 (C-2″), 70.78 (C-3″), 72.67 (C-4″), 62.51 (C-5″), 100.26 (C-1‴), 81.63 (C-2‴), 70.15 (C-3‴), 72.04 (C-4‴), 60.13 (C-5‴); +ve ion FAB MS *m/z* (rel. int.): 556 $[M]^+$ ($C_{24}H_{28}O_{15}$) (1.1).

2.9 Hydrolysis of 4: Compound **4** (30 mg) was dissolved in ethanol (5 ml), 1N sodium hydroxide solution (2 ml) was added and the reaction mixture was refluxed for 1 h. It was dried under reduced pressure and the residue was dissolved in chloroform to isolate 3,4-dihydroxybenzyl alcohol, m. p. 116 – 117 °C; HPTL Rt 53.08 (C_{18} Beckman column, solvent : methanol-formic acid, 19:1). The reaction mixture was acidified with dilute hydrochloric acid to pH 3 and re-extracted with chloroform (3 x 5 ml) to separate 2,3,4-trihydroxybenzoic acid, m. p. 203-205 ºC, Rt 21.01 (C_{18} column, solvent: methanol and formic acid (0.1%). The mother liquor was chromatographed over SiO_2 TLC along with the authentic sample of D- xylose, R_f : 0.2 (*n*-BuOH: HOAc: H_2O; 4:1:5, top).

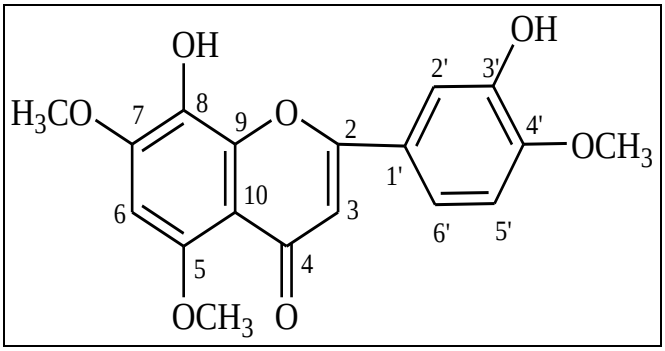
3. Results

Elution of the column with chloroform gave compound **1** as a colourless product. Compound 2 was obtained as a colourless amorphous powder on elution of the column with chloroform–methanol (99:1) mixture. Elution of the column with chloroform–methanol (7:3) mixture furnished compounds **3** and **4** as colourless products.

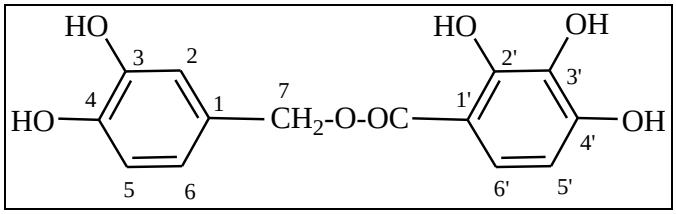
4. Discussion

Compound **1** was a known compound identified as stigmasterol [16].

Compound **2,** named artimisiaflavone, was obtained as colourless, amorphous powder from chloroform–methanol (99:1) eluants. It displayed UV absorption maxima at 272, 313 and 344 nm, a one-proton singlet at δ 6.93 and the corresponding upfield vinylic carbon signal at δ 102.75 characteristic of H-3 and C-3, respectively, in a flavone structure[17]. Shifting of band I to + 48 nm with no decrease of intensity with sodium methoxide indicated the presence of free hydroxy groups. The absence of any shift of bands with sodium acetate solution indicated bound nature of 7-hydroxyl group. There was no significant shift in band I with sodium acetate and boric acid ruling out the existence of B-ring dihydroxy groups. Absence of any shift of band I with aluminum chloride indicated the presence of bound 5-hydroxyl group. There was no shift of band I with aluminum chloride and hydrochloric acid ruling out the existence of B-ring *o*- dihydroxy groups [17-19]. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3335 cm^{-1}), carbonyl group (1702 cm^{-1}) and unsaturation (1612 cm^{-1}). On the basis of its FAB mass and ^{13}C NMR spectra the molecular ion peak of **2** was determined at *m/z* 344 consistent with the molecular formula of a flavonoid, $C_{18}H_{16}O_7$. The prominent peaks generated at *m/z* 196 and 148 due to retro-Diels-Alder fragmentation supported the presence of two and one hydroxyl groups in ring A and one-each methoxy and hydroxyl functions in ring B. The ^1H NMR spectrum of **2** showed two one-proton doublets at δ 7.71 (J = 2.8 Hz) and 6.16 (J = 9.3 Hz) assigned to meta-coupled H-2′ and ortho-coupled H-5′ protons, respectively, and a one-proton double doublet at δ 7.68 (J = 9.3, 2.8 Hz) attributed to ortho-, meta-coupled H-6′ suggesting ABX system of ting B. A one-proton broad singlet at δ 6.84 were ascribed to H-6. The broad singlets at δ 3.96, 3.90 and 3.88 integrating three protons each were accounted to three methoxy protons. The ^{13}C NMR spectrum of **2** showed a signal for carbonyl carbon at δ 182.30 (C-4) supporting the flavone type carbon framework of the molecule, methoxy carbons at δ 55.18, 55.31 and 55.15 and other flavone carbons between δ 162.46 - 101.86. The ^1H and ^{13}C NMR values were compared with the reported values of flavone molecules [17, 20]. On the basis of the spectral data analysis and chemical reactions, the structure of **2** has been elucidated as 5, 7, 4′- trimethoxy-8,3′-dihydroxyflavone. This has been found to be a new flavone.

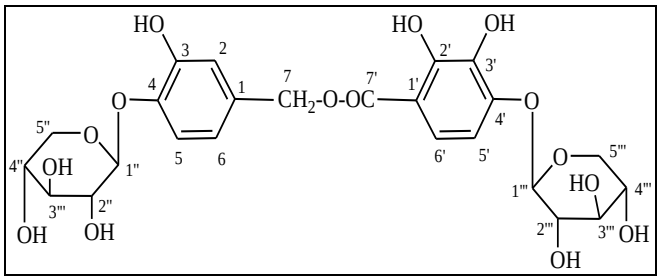


Compound **3,** designated as 3,4-dihydroxybenzyl trihydroxybenzoate**,** was obtained as a colourless mass from chloroform–methanol (7:3) eluants. It gave positive test for phenolic compounds. Its IR spectrum displayed absorption bands for hydroxyl groups (3440, 3345, 3205, cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra the molecular ion peak of **3** was determined at *m/z* 292 corresponding to a molecular formula of a dihydroxybenzyl trihydroxybenzoate, $C_{14}H_{12}O_7$. The prominent fragment ion peak generating at *m/z* 153 $[(HO)_3C_6H_2CO]^+$, 139 $[OCH_2C_6H_3(OH)_2]^+$ and 123 $[CH_2–C_6H_3(OH)_2]^+$ indicated esterification of trihydroxybenzoic acid with dihydroxybenzyl alcohol. The ^1H NMR spectrum of **3** exhibited four one-proton doublets δ 8.08 (J = 2.5 Hz), 6.93 (J = 7.8 Hz), 6.18 (J = 8.5 Hz) and 5.25 (J = 7.8 Hz) assigned correspondingly to meta-coupled H-2 and ortho-coupled H-5′, H-5 and H-6′ proton. A one-proton double doublet at δ 5.81 (J = 2.5, 8.5 Hz) and a two proton singlet at δ 3.39 were associated with aromatic H-6 and oxygenated methylene H_2-7 protons, respectively. The ^{13}C NMR spectrum of **3** showed important signals for ester carbon at δ 173.71 (C-7′), aromatic carbons between δ 157.46 – 118.29 and oxygenated methylene carbon δ 62.86 (C-7). Alkaline hydrolysis of **3** yielded 3,4-dihydroxybenzyl alcohol and 2,3,4-trihydroxbenzoic acid. On the basis of the above discussion, the structure of **3** has been established as 3,4-dihydroxybenzyl 2′,3′,4′-trihydroxybenzoate. This is a new dihydroxybenzyl trihydroxybenzoate.



Compound **4,** designated as 3,4-dihydroxybenzyl trihydroxybenzoate diglucoside, was obtained as a colourless crystalline powder from chloroform–methanol (7:3) eluants. It gave positive test for phenolic glycosides. Its IR spectrum displayed absorption bands for hydroxyl group (3404, 3317, 3216 cm^{-1}), ester group (1722 cm^{-1}) and aromatic ring (1511, 959 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra its molecular ion peak was established at *m/z* 556 corresponding

to a molecular formula of a dihydroxybenzyl trihydoxybenzoate diglycoside, $C_{24}H_{28}O_{15}$. The ^{1}H NMR spectrum of **4** exhibited four one- proton doublets at δ 8.02 (J = 2.7 Hz), 6.91 (J = 8.1 Hz), 6.26 (J = 8.5 Hz), and 5.28 (J = 8.1 Hz) and a one-proton double doublet at δ5.80 (J = 2.7, 8.5 Hz) assigned to meta-coupled H-2 and ortho-coupled aromatic H-5′, H-5 and H-6′ protons and meta-, ortho-coupled H-6 protons. Two one-proton doublets at δ 4.73 (J = 7.2 Hz) and 4.64 J = 7.3 Hz) were ascribed to anomeric H1″ and H-1‴ protons, respectively. The other sugar protons appeared from δ 4.54 to 3.04. The ^{13}C NMR of **4** displayed signals for ester carbon at *δ* 173.68 (C-7′), aromatic carbons between δ 157. 49 to 117.52, anomeric carbons δ 100.84 (C-1″) and 100.26 (C-1‴) and other sugar carbons between δ 82.86 – 60.13. Alkaline hydrolysis of **4** yielded 3,4-dihydroxybenzyl alcohol, 2,3,4-trihydroxybenzoic acid and D- xylose. On the basis of the spectral data analysis and chemical evidences, the structure of **4** has been established as 3,4-dihydroxybenzyl 2′,3′,4′-trihydroxybenzoate 4,4′-β-D-dixylopyranoside. This phytoconstituent is reported for the first time.



5. Conclusion

Phytochemical investigation of a methanolic extract of the shoots of *A. annua* cultivar *jwarharti* led to the isolation of stigmasterol and one each of flavone, dihydroxybenzyl ester and its dixyloside. This work has enhanced understanding about the phytoconstituents of the plant. These secondary metabolites can be used as an effective analytical marker, identity, purity and quality control of this plant.

6. Acknowledgements

The authors are thankful to the Head, Saphosticated Analytical Instrumentation Centre, Central Drug Research Institute (CSIR), Lucknow for recording spectral data.

7. References

1. Klayman DL, Lin AJ, Acton N. Isolation of artemisinin (qinghaosu) from *Artemisia annua* growing in the United States J Nat Prod. 1984; 47(4):715-717.
2. Mueller MS, Karhagomba IB, Hirt HM, Wemakor E. The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: agricultural, chemical and clinical aspects J Ethnopharmacol. 2000; 73(3):487-493.
3. Abdin MZ, Israr M, Rehmann RU, Jain SK. Artemisinin, a novel antimalarial drug. Biochemical and molecular approaches for enhanced production. Planta Med. 2003; 69:289-289.
4. Zheng GC. Cytotoxic terpenoids and flavonoids from *Artemisia annua.* Planta Med. 1994; 60(1):54-57.
5. Mohamed AEH, El-Sayed MA, Hegazy ME, Helaly SE, Esmail AM, Mohamed NS. Chemical constituents and biological activities of *Artemisia herba-alba*. Rec Nat. Prod. 2010; 4:1-25.
6. Ferreira JFS, Luthria DL, Sasaki T, Heyerick A. Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. Molecules. 2010; 15(5):3135-3170.
7. Lai JP, Lim YH, Su J, Shen HM, Ong CN. Identification and characterization of major flavonoids and caffeoylquinic acids in three Compositae plants by LC/DAD-APCI/MS. J Chrom B. 2007; 848:215-225.
8. Brown GD. Two new compounds from *Artemisia annua*. J Nat Prod. 1992; 55:1756-1760.
9. Bhakuni RS, Jain DC, Sharma RP, Kumar S. Secondary metabolites of *Artemisia annua* and their biological activity. Curr. Sci. 2001; 80:35-48.
10. Elford BC, Roberts MF, Phillipson JD, Wilson RJ. Potentiation of the antimalarial activity of qinghaosu by methoxylated flavones. Trans R Soc Trop Med. Hyg. 1987; 81:434-436.
11. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Rad Biol Med. 1996; 20:933-956.
12. Anonymous, The Wealth of India, a dictionary of Indian raw materials and industrial products, National Institute of Science Communication and Information Resources, CSIR, New Delhi, First supplement, 2006; 1:91-92.
13. Carbonara T, Pascale R, Argentieri MP, Papadia P, Fanizzi FP, Villanova L *et al*. Phytochemical analysis of a herbal tea from *Artemisia annua* L. J Pharma Biomed Analysis. 2012; 62:79-86.
14. Goel D, Goel R, Singh V, Ali M, Mallavarapu GR, Kumar S. Composition of the ssential oil from the roots of *Artemisia annua*. J Nat Med. 2007a; 61:458-461.
15. Goel D, Singh V, Ali M, Mallavarapu GR, Kumar S. Essential oils of petal, leaf and stem of the antimalarial plant *Artemisia annua*. J Nat Med. 2007b; 61:187-191.
16. Bhat ZA, Ali M, Ansari SH, Naquavi KJ. Phytochemical investigation of aerial parts of *Swertia tetragona* Edgew. Indian Drugs. 2012; 49(5):35-38.
17. Mabry TJ, Markham KR, Thomas MB. The systemic identification of flavonoids, New York, Springer- Verlag, 1970, 41-64.
18. Harborne JB, Williams CA. Flavone and flavonol glycosides, In: The Flavonoids, Harborne JB, Mabry TJ, Mabry H. Chapman and Hall, London, 1975.
19. Klyshev LK, Bandyukova VA, Alyukina LS. Plant Flavonoids, Nauka, Alma- Ata, 1978.
20. Agarwal PK. Carbon- 13 NMR of flavonoids. New Delhi, Elsevier, 1989.