Isolation of flavonoid from Abies webbiana leaves and its activity

Dinesh Kumar Yadav1*, Mohammed Ali2, Ashoke Kumar Ghosh1, Babita Kumar1

1College of Pharmacy, Shree Ganpati Institute of Technology, Ghaziabad (U.P.), INDIA.
2Pharmacognosy & Phytochemistry, Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, INDIA.
3School of Pharmaceutical Sciences, IFTM University, Moradabad (U.P.), INDIA.

ABSTRACT
Background: Abies webbiana commonly known as Talispatra in Bengali and Hindi, Talispatram in Sanskrit and Indian Silver Fir in English. This is a large, tall, evergreen tree occurring in the Himalayan region from Kashmir to Assam in India. It comes under the Family: Pinaceae. The present study was designed for isolation of flavonoid from ethyl acetate extract of A. webbiana leaves and assessed their toxic effect on liver and kidney. Materials and Methods: The isolation of flavonoid using different chromatographic methods (thin layer and column chromatography). The isolated flavonoid was identified; Structures and chemical bonds were analyzed by using MP, FTIR, 1-H NMR and MS spectral analysis. Effect of flavonoid on liver and kidney was assessed by inducing (0.1 ml/kg) CCl₄ (i.p.) and (6 mg/kg) Cisplatin (i.p.) respectively measured by biochemical marker of liver and kidney. Results and Discussion: It was identified that isolated compound was as 4’-hydroxy quercetin on the basis of FTIR, 1-H NMR and MS spectral analysis. Isolated flavonoid reduced the increased biochemical marker (BM) of liver and kidney. The BM was increased by inducing CCl₄ and Cisplatin respectively. Conclusion: Isolated compound was 4’-methoxy quercetin and significantly protect the liver and kidney.

Keywords: Abies webbiana, Quercetin, 1-H NMR, Cisplatin, CCl₄.

Corresponding author: Mr. Dinesh Kumar Yadav, College of Pharmacy, Shree Ganpati Institute of Technology, Opp-Jindal Pipes Ltd., NH-24 Ghaziabad, Uttar Pradesh, INDIA.
Phone no: 8765918721
Email: dineshnbr108@gmail.com
DOI: 10.5530/pj.2016.4.6

INTRODUCTION
Abies webbiana commonly known as Talispatra in Bengali and Hindi, Talispatram in Sanskrit and Indian Silver Fir in English, is a large, tall, evergreen tree occurring in the Himalayan region from Kashmir to Assam in India. It comes under the Family: Pinaceae. The leaves of this plant have different uses in Ayurveda, the traditional system of Indian medicine and have been described for using against swasa, kasa, amadosha, hikka, chhardi and mukharoga. A. webbiana leaves have been reported as antibacterial and antifungal, cell stabilizing, anti-tumor, anti-inflammatory, anti-tussive, female antifertility, febrifuge, anti-spasmodic properties, Central nervous system (CNS) depressant actions and have different uses in Ayurveda, in Sanskrit and Indian Silver Fir in English. This is a large, tall, evergreen tree occurring in the Himalayan region from Kashmir to Assam in India.

MATERIALS AND METHODS
Collection of plant material
The leaves of A. webbiana were collected from the forest of Tungnath (Garhwal, Utarakhand). Plant material was authenticated by Head, Department of Pharmacognosy and Ethno-Pharmacology, NBRI, Lucknow. The voucher specimen was preserved for the future reference. The leaves were separated from the branches and dried at the temp of 40°C for one hour before pulverization by mechanically grinder. The powder was passed through 40 mesh sieve and preserved for future purpose in tightly sealed container.

Extraction
800 gm of dried, coarsely powdered of leaves of A. webbiana was extracted with 99% ethanol using soxhlet apparatus. The extract was filtered and the solvent recovered by distillation. The filtered extract was evaporated under vacuum to give semisolid mass (20% w/w) which further dried. Alcoholic extract was suspended in small portion of water, extracted with ethyl acetate and then resulting solution were concentrated to provide ethyl acetate soluble parts. TLC finger prints and phytochemical tests were performed.

Isolation of flavonoid
Ethyl acetate extract was loaded on silica gel (60-120 mesh) column chromatography for the isolation of phytoconstituent gave the various fractions, out of these five fraction no (16-20) from column chromatography were collected. These fractions have the same R₁ value when TLC was performed. Fractions were combined based on TLC analysis (developed in Toluene Ethyl acetate Formic acid (6:2:0.8). detected by ferric chloride solution and performed for flavonoid test (Alkaline test, Shinoda test and ZN-HCl). Concentrated fraction kept in refrigerator overnight for crystallization and melting point was measured.

Experimental
UV spectrum was measured with UV/Visible spectrophotometer (UV-1800 Shimadzu, Japan) in CH₃OH at room temperature. TLC was performed on a 0.25 mm thick Silica gel G (CDH, New Delhi). The TLC was detected by their UV fluorescence and by spraying with 0.5% FeCl₃. Column chromatography was performed with Silica gel 60-120 mesh (CDH, New Delhi). Melting points were determined on open capillaries using a Cintex melting point apparatus. IR Spectra were recorded on Perkin-Elmer spectrometer BX series FTIR spectrometer. 1H-NMR spectrum was recorded on Bruker 500 MHz spectrometers using TMS as internal standard. The chemical shifts are reported in ppm (δ) and coupling constants (J) are in Hz. Mass spectra were recorded on Bruker 75 MHz spectrometer.
**Identification of flavonoid: 4'-methoxy quercetin**

Yellow coloured solid compound was obtained and having m.p. 304°C, Rf value (Toluene Ethyl acetate Formic acid, 6:2:0.8) at 0.38, UV–Vis λmax in Ethanol: (nm) 359, IR (KBr), m=862 (C–H, Ar), 1091 (C=O), 1129(C–C–…), 1165, 1259(C-O-stretching) 1356, 1454 (C=O), 1515 (C=C, Ar), 1678, 1645 (C=C stretching) and 3416, 3360, 3251 (Ar-OH). 1HNMR(DMSO-d6), d=δ 7.67(1 H, d, J=1.8 Hz H-2'), 7.54 (1 H, d, J=8.4 Hz H-5'), 7.54 (1 H, d, J=6.3 Hz H-5), 6.18(1 H, d, J=1.6 Hz H-6),3.33(3 H, brs, Ome), 4'-methoxy quercetin.

**Activity of flavonoid on Liver and Kidney**

**Animals**

Albino rats (Wistar) weighing 150-200 g of either sex were used in the present study. The animals were acclimatized for one week under laboratory conditions in SGIT, Ghaziabad (U.P.). They were housed in polypropylene cages and maintained at 22°C ± 2°C under 12 hrs dark/ light cycle. They were fed with standard rat feed and water ad libitum. The experimental protocol was approved by the Institutional animals ethical committee (IAEC, Registration No SGIT/2014/04) prior to the beginning of the work.

**Acute toxicity study**

Flavonoid doses (0.5, 5, 300, 500 mg/kg, p.o.) were used for acute toxicity in accordance to Organization for Economic Cooperation Development (OECD, 2002) guideline 423. Three rats, each sequentially dosed at intervals of 48 hrs, were used for the test. Once daily cage side observations included changes in skin, fur, eyes, mucous membrane (nasal), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsions) changes. Mortality, if any, was determined over a period of 2 weeks and dose was taken for isolated flavonoid (50 mg/kg, p.o.).

**Method for Evaluation of Hepatotoxicity activity**

In the dose response experiment, Wistar albino rats were taken randomly assigned into three groups of 6 individuals each. Group-I (-ve control) were administered 1 ml distilled water p.o., Group-II (+ve control) were administered (0.1 ml/kg) CCl4 (i.p.) and Group-III were administered (CCl4, 50 mg/kg) isolated flavonoid p.o., for 5 days. Animals were sacrificed on the 6th day under mild ether anesthesia. Blood samples were collected for evaluating the serum biochemical parameters such as Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), Serum alkaline phosphates (SALP), Serum total bilirubin and Serum direct bilirubin were estimated by commercially available kits.

**Method for Evaluation of Nephrotoxicity activity**

In the dose response experiment, albino rats were taken randomly assigned into three groups of 6 individuals each. Group-I (-ve control) were administered 1 ml distilled water p.o., Group-II (+ve control) were administered (6 mg/kg, i.p.) Cisplatin and Group-III Animals were administered (Cisplatin+50 mg/kg) isolated flavonoid p.o., for 7 days. The body weight of all the animals was taken on every day. The animals were sacrificed on day 7 under mild ether anesthesia. Kidney blood samples were collected and kidneys were weighed. The blood samples were used to measure serum creatinine and Blood urea nitrogen (BUN).

**Statistical Analysis**

The values were expressed as Mean ± SE. Statistical analysis was performed by tukey multiple comparison test One way analysis of variance (ANOVA) by Tukey multiple comparison test, was carried out and p<0.05 was considered as significant P<0.01 represent more significant and ***P<0.001 represent highly significant. Groups were compared with positive control and negative control group.

**RESULTS AND DISCUSSION**

Yellow coloured solid compound which was having sharp melting point m.p. 304°C that revealed its purity and in TLC single spot with value at Rf=0.38 was obtained. UV λmax in Ethanol: (nm) was obtained 359 nm, IR (KBr), m=862 (C–H, Ar) showed aromatic carbon stretching, 1091 (C=O) showed presence of carbon oxygen, 1129(C–C–…) carbon stretching, 1165, 1259(C-O-stretching) 1356, 1454 (C=O), 1515 (C=C, Ar), 1678, 1645 (C=C stretching) and 3416, 3360, 3251 and was showing presence of hydroxyl aromatic (Ar-OH) (Figure 1). 1-HNMR (DMSO-d6) (Figure 2) spectrum the presence of the H-2’ was provided by the presence of one signal doublet at d 6.77 ppm J=1.8 Hz representing, signal as doublet at d 7.54 ppm J=1.8 Hz representing H-6’, presence of H-5’ was provided by one signal doublet at d 6.88 ppm J=8.4, presence of H-8 was provided by the presence of one signal doublet at d 6.44 ppm J=1.6 Hz representing, presence of H-6 was provided by one signal doublet at d 6.18 ppm J=1.6 Hz representing and H-3 and methoxy was provided by the presence d 3.33 ppm. Molecular formula was found by MS C31H33O9 [M+], and isolated falvonoid was found as 4'-methoxy quercetin and their structure (Figure 3). No toxicity effects were found by acute toxicity studies but the higher dose of flavonoid has increased respiration of rats and lower dose was safe. Assessment for activity, one dose level were chosen in such a way that, high dose was approximately one-tenth of the maximum dose during acute toxicity studies, which was (50 mg/kg, p.o.). Preliminary phytochemical investigation of extract led to the presence of alkaloid, flavonoid, terpenoids, tannins, phenolic compound and glycosides. Hepatotoxicity study was performed and level of SGOT, SGPT, SALP and total bilurubin (Total and Direct) was (Showed in Table 1). It was found that the biochemical measurement were significant increased as compared to control (Group I) after administration of CCl4 (Group II). Isolated flavonoid (Group III) significant decreased the increased level of biochemical parameter as compare to negative control (Group II). Nephrotoxicity study was assessed and level of serum BUN, serum creatinine and serum protein and % change of body weight shown in (Table 2). The levels of serum BUN, serum creatinine and serum protein and % change of body weights were increased significantly in cisplatin treated animals (Group II) when compared to normal control animals (Group I). The extent of elevation was reduced significantly in animals which received isolated flavonoid (Group III). Exposure of CCl4 has been reported that free radical generated in tissue such as liver heart, brain, blood and testis. Free radical of CCl4 is believed to process leading the oxidative stress which is indirect cause the many pathological condition such as diabetes, cancer; liver damage and kidney damage. Most protein found in the plasma are synthesized by the hepatocytes and secreted in circulation. Reduction in total protein level at administration of CC4. Cisplatin significantly elevated the levels of serum BUN, serum creatinine and serum protein; and body weight. Cisplatin induces oxidative stress causing damage to intracellular organelles and alters this functions which lead to inhibition of protein synthesis glutathione depletion lipids peroxidation and mitochondrial damage. Flavonoids are a group of polyphenolic compounds which are present widely in plant kingdom both in the free state as glycosides and poses wide biological activities. The capability to interact with protein phosphorylation and the antioxidant iron chelating and free radicals scavenging activity. A number of flavonoids are known to possess good anti-inflammatory antibacterial, antiheptotoxic activity. Flavonoids have reported that protective against environmental toxic agents and phytochemical analysis revealed that various chemical constituents like; monoterprenes (from essential oil), flavonoids, biflavonoid glycosides, phytosterols, amino acids, saponins, tannins, alkaloids, lipids, triterpenoids, steroids, diterpene glycosides and alkaloids are present in the leaf of A. webbiana. In the present investigation it was observed that pretreatment.
Table 1: Effect of isolated flavonoid from *A. webbiana* on liver biomarkers

| Group                        | SGOT (units) | SGPT (units) | SALP (units) | Serum Bilirubin (units) |
|------------------------------|--------------|--------------|--------------|-------------------------|
| Control                      | 36.4 ± 1.92  | 43.66 ± 1.83 | 111.66 ± 4.32| 0.35 ± .023             |
| CCl₄                         | 84 ± 2.60*** | 86.33 ± 1.21*** | 212.5 ± 2.88*** | 0.86 ± 0.33***          |
| Isolated Flavonoid (50 mg/kg) + CCl₄ | 39.5 ± 2.88*** | 54.66 ± 3.14*** | 100.66 ± 3.07*** | 0.498 ± 0.01**          |

Values are mean ± SEM (N=6) one way ANOVA followed by Tukey’s multiple comparison column test. Where* represent P<0.05 represent significant, **P<0.01 represent more significant and ***P<0.001 represent highly significant compare with CCl₄ group and * represent significant compare with Control group.

Table 2: Effect of isolated flavonoid from *A. webbiana* on Nephrotoxicity

| Group                        | % change in body weight | Serum creatinine (mg/dl) | Serum BUN (mg/dl) | Serum protein (g/dl) |
|------------------------------|-------------------------|--------------------------|-------------------|----------------------|
| Control                      | 1.275 ± 0.06            | 0.781 ± 0.01             | 16.92 ± 0.04      | 3.887 ± 0.09         |
| Cisplatin                    | 13.55 ± 0.95***         | 2.079 ± 0.01***          | 26.87 ± 0.06***   | 9.491 ± 0.25***      |
| Isolated Flavonoid (50 mg/kg) + Cisplatin | 1.78 ± 0.075***        | 1.010 ± 0.08***          | 22.01 ± 0.06***   | 5.745 ± 0.11***      |

Values are mean ± SEM (N=6) one way ANOVA followed by Tukey’s multiple comparison column test. Where* represent significant at P<0.05, ** represent more significant at P<0.01 and *** represent highly significant compare with Cisplatin group and * represent significant compare with Control group.

**Figure 1:** FTIR spectra of flavonoids.
flavonoid (50 mg/kg p.o.) for 5 days has significantly reduced the elevated biochemical markers of liver and kidney.

**CONCLUSION**

It may be concluded that flavonoid has isolated successfully from the *A. webbiana* leaves and on the basis of spectral data; the compounds were identified as 4’-methoxy quercetin. It was found that no toxic effect of flavonoid on liver and kidney, instead of whereas it shows protective effect on liver and kidney strongly.
2. Sarkar SK, Poddar G, Mahato SB. Glucoside from Abies webbiana. Planta Medica. 1986;42(2):219.
3. Rawat AKS, Mehratra S, Shome U. Comparative pharmacognostical studies on the leaves of Abies spectabilis and Taxus wallichiana. International Journal of Pharmacognosy. 1996;34(5):378.
4. Nayak SS, Ghosh AK, Debnath B, Jha T. Anti tussive activity of A. webbiana Lindl. Leaf extract against sulphur dioxide-induced cough reflex in mice. Phytotherapy Research. 2003;17(8):930.
5. Nayak SS, Ghosh AK, Debnath B, Vishnoi SP, Zaman TJ. Synergistic effect of methanol extract of Abies webbiana leaves on sleeping time induced by standard sedatives in mice and Anti-inflammationary activity of extracts in rats. J Ethnopharmacol. 2004;93(2):397.
6. Kumar VPN, Chauhan S, Padh H, Rajani M. Search for antibacterial and anti-fungal agents from selected Indian medicinal plants. J Ethnopharmacol. 2006;107(2):182.
7. Visnoi SP, Basu A, Alam SKM, Samanta S, Jha T. Evaluation of the antipyretic potential of methanol extract of the leaves of Abies spectabilis(D. Don) Spach. Natural product of Radiance. 2007;6(5):369.
8. Visnoi SP, Ghosh AK, Debnath B, Samanta S, Gayen S, Tha T. Antibacterial activity of Abies webbiana. Fitoterapia. 2007;78(2):153.
9. Ghosh AK, Bhattacharya S. Pharmacognostic Studies on Leaves of Abies webbiana Grown in Sikkim Himalayan Region of India. Pharmacognosy Journal. 2009;12(2):171.
10. Ghosh AK, Bhattacharya S. Planar chromatographic studies on Abies webbiana leaves. International Journal of Chem Tech Research. 2009;11(4):807.
11. Ghosh AK, Sen D, Bhattacharya S. A nitrogenous compound isolated from Abies webbiana leaf. Der Pharma Chemical. 2010;23(3):205.
12. Ghosh AK, Sen D, Bhattacharya S. A new alkaloid isolated from Abies webbiana Leaf. Pharmacognosy Research. 2010;2(3):186.
13. Nadkarni AK. Indian material medica, Bombay Popular Prakashan, 1976:1st ed.3.
14. Selvaraj K, Chowdhury C, Bhattacharjee C. Isolation and structural elucidation of flavonoids from aquatic fern azolla microphylla and evaluation of free radical scavenging activity. Int J Pharm Pharm Sci. 2013;5(3):943-9.
15. Harborne JB. Phytochemical methods. A Guide to modern techniques of plant analysis, Chapman and Hall, 1973:1st ed. 29.
16. Kakate CK, Purohit AP, Gokhale SB. Pharmacognosy, Nirali Prakashan, 2004:18.
17. Adil A, Mujwah A, Mohammed A, Mohammed B, Mohammed H, Ahmed C. First isolation of a flavonoid from Juniperus procera using ethyl acetate extract. Arabian Journal of Chemistry. 2010;3(2):85-8.
18. OECD, Guideline for testing of chemicals, Acute oral toxicity, Environmental Health and Safety monograph series on testing and adjustment No 423, 2002:1.
19. Zeeuwaqin K, Setty RCS, Majid MSA, Ibrahim M. Evaluation of hepatoprotective activity of Boswellia serrata leaves extracts in albino rats. Indian Drugs. 2010;47(2):14.
20. Kumar SVS, Mishra SH. Hepatoprotective activity of rhizomes of Cyperus rotundus Linn against carbon tetrachloride-induced hepatotoxicity. Indian J of Pharm Sci. 2005;67(1):84.
21. Girish C, Koner BCS, Jayanthi KR, Rao B, Pradhan RSC. Hepatoprotective activity of six polyherbal formulations in paracetamol induced liver injury in mice. Indian J Med Res. 2009;129(5):569.
22. Shashtra NY, Biradar SM, Mahadevan KM, Habbu PV. Isolation and characterization of secondary metabolite from Amorphophallus paeonifolius for Hepatoprotective activity. Research J Pharm Bio and Chem Sci. 2010;14(4):429.
23. Suja SR, Latha PG, Pushpangadan P, Rajasekharan S. Evaluation of hepatoprotective effects of Helminthostachys zeylanica (L.) hook against carbon tetrachloride induced liver damage in Wister rats. J Ethnopharmacol. 2004;92(1):61.
24. Sreedevi KB, Prasad KVSRG. Protective effect of rutin against cisplatin-induced nephrotoxicity in rats. Journal of Natural Remedies. 2010;10(2):144.
25. Corcostegui R, Labeaga L, Arteche JK, Ojales A. Protective effect of hidrosmin against cisplatin induced acute nephrotoxicity in rats. J Pharm Pharmacol. 1998;49(7):465.
26. Devi PS, Shaymal DCS. Protective effect of quercetin in cisplatin-induced cell injury in the rat kidney. Indian J Pharmacol. 1999;31(6):422.
27. Chioma AA, Uchenna BU, Ogechi N. Effect of ethanol extract of Pyrenacantha staudtii leaves on carbon tetrachloride induced hepatotoxicity in rats. Biochemistry. 2008;20(1):172.
28. Kostova I. Platinum complex as anticancer agents, recent patents anticancer drug Discover. 2006;1(1):1.
29. Saia A, Scales M, Lanza M, Marullo D, Bonina F. Flavonoids as antioxidant agents: Importance of their interaction with biomembranes. Free Rad Biol Med. 1996;19(4):491.
30. Saia A, Tomario A, Trombetta D, Giacchi M, Pasquale AD, Bonina F. Influence of different penetration enhancers on in vitro skin permeation and in vivo photo-protective effect of flavonoids. Int J Pharm. 1998;175(1):85.
31. Trease, EVC. Pharmacognosy, Saunders company London, (W.B.),1996:249.

PICTORIAL ABSTRACT

SUMMARY

- It may be concluded that flavonoid has isolated successfully from the A. webbiana leaves and on the basis of spectral data of Mass, IR and NMR; the compounds were identified as 4’-methoxy quercetin.
- Flavonoid is highly responsible for protection of liver and kidney.
- It was found that no toxic effect of flavonoid on liver and kidney, instead of whereas it shows protective effect on liver and kidney strongly.

ABOUT AUTHOR

Dinesh Kumar Yadav: Is an associate professor, college of pharmacy, SGIT, Ghaziabad, U.P, India. His research field focuses on Phytochemistry and biological activity testing of natural products.