International Journal of Chemical Studies

P-ISSN2349–8528
E-ISSN 2321–4902
IJCS 2016; 4(1): 14-17

Received: 03-11-2015
Accepted: 04-12-2015

**Jaya Gupta**
Department of Chemistry,
Agra College, Agra, India.

**Amit Gupta**
Department of Chemistry,
Agra College, Agra, India.

**Correspondence:**
**Jaya Gupta**
Department of Chemistry,
Agra College, Agra, India.

# Isolation and characterization of flavonoid glycoside from leaves of *Abrus precatorius*

**Jaya Gupta, Amit Gupta**

**Abstract**
In the present work, flavonoid glycoside compound was isolated from the leaves of *Abrus precatorius* and characterized by using thin layer chromatography. By using spectroscopic technique their structure and chemical bonds were analyzed. Phytochemical studies reveal the presence of apigenin flavonoid glucoside i.e., Vitexin.

**Keywords:** *Abrus precatorius*, flavonoids, TLC, NMR etc.

## Introduction
The popularity of complementary medicine has increased in recent years. Dietary measures and traditional plant therapies as described by ayurvedic and other indigenous systems of medicines are used commonly in India. The natural compounds are not considered as chemicals and they are readily accepted by the consumers. Also, they have a GRASS (Generally Recognized as Safe) status and do not require toxicological testing. Flavonoids are major components of medicinal plants and have been used in traditional medicine around the world. *Abrus precatorius* Linn. Belongs to family fabaceae is an indigenous plant found all throughout India from Himalayan region to down the southern India. It is known as Gunja in Sanskrit, Gunchi in Hindi, Jequirity and Crab's eye in English. Commonly it is known as 'Ratti' or Rosary Pea'. Its seeds have remarkably uniform weight of 1/10$^{th}$ of a gram. So its seeds are used by goldsmiths; in old time to weight gold and silver. The plant has been used in Hindu medicines from very early times, as well as in China and other ancient cultures [1]. *Abrus precatorius* possess various pharmacological activities such as, antioxidative [2], antidiabetic [3] antiviral [4], antidepression [5], memory enhancing [6], antimicrobial [7-12], antimalarial [13], anti-inflammatory [14-16], antiarthritic [17-18], anticancer [19], antifertility [20-26], antiallergic [27], antiasthmatic [28], anticataract [29], antiinsecticide [30], antitoxicity activity [31-32]. Leaves of *Abrus precatorius* resemble tamarind leaves having 20-40 leaflets. Its leaves are used in treating several diseases. The main object of this study is to extract and characterize flavonoid in the leaves of *Abrus precatorius.*

## Experimental
$^{1}$H NMR and $^{13}$C NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer. The EI-mass was recorded on Shimadju QP 2000 mass spectrometer. UV-spectra were recorded on Shimadju UV-160 spectrophotometer. The leaves of *Abrus precatorius* was washed thoroughly with tap water followed by rinsing with double distilled water and shade drying for fifteen days. The fine powder was obtained from dried leaves by using kitchen mixer grinder (Philips electronics). The leaves powder was sterilized at 120$^{o}$C for 15 minutes. The leaves powder was stored under dessicator for further studies. Solvent extraction of dried powder (25gm) of *Abrus precatorius* was done using 250ml. of 80% methanol in a soxlet extractor for 36 hours. The extract was concentrated by evaporation (40$^{o}$-50$^{o}$) in vaccum rotatory. The concentrated methanolic extract (10ml.) was suspended in 50ml. of distilled water and was further extracted twice with hexane and then with ethyl acetate. The ethyl acetated fractions were washed two times with distilled water. The ethyl acetate fraction was analyzed for flavonid using chromatographic separation. The glass plates (20x20cm) coated with silica gel (0.2-0.3mm) were dried naturally (atmosphere). Subsequently they were activated at 100$^{o}$C for 30 minutes and were cooled at temperature 25$^{o}$C. Diluted samples of

leaves of *Abrus precatorius* were qualitatively studied by TLC, butanol: acetic acid: water (4:1:5) upper layer was used as mobile phase. TLC plates coated with silica gel were used as stationary phase. The plates were sprayed with a solution of 1% ethanolic 2- amino ethyl diphenyl borinate followed by 5% ethanolic solution of polyethylene glycol-400.Flavonoid appears in color zone under UV-365nm. Standard flavonoids were used for identification. Retention time is 0.65.With both reagent A and B light green color is obtained. The remaining extract was evaporated and residue was obtained, it was subjected to various physical and spectral analyses.

**Chemical Identification of flavonoids [33]**

1. **Shinoda Test:** To the small amount of extract in alcohol, magnesium ribbon was added followed by addition of drops of concentrated hydrochloric acid, formation of pink color confirms the presence of flavonoids.
2. **Zn- Hydrochloride Reduction Test:** To the extract add a mixture of zinc dust and concentrated hydrochloric acid. Heat the solution, after few minutes, color of the solution changes to red.
3. **Aluminium Chloride Test:** To the small amount of extract, two drops of 1% aluminum chloride was added, yellow color was obtained.

**Results and Discussion**

The compound was isolated as yellow amorphous powder m.p 203°C; $^{1}$HNMR (DMSO-$d_6$), $^{13}$CNMR(DMSO-$d_6$) Table1. The $^{1}$HNMR spectrum showed a doublet proton at δ 8.02 corresponding to H-2' and H-6' proton. Another doublet proton occurs at δ 6.88 corresponding to H-3' and H-5'. Two protons appeared at δ 6.60 and δ 6.26 as singlets corresponding to H-3 and H-6 protons respectively. The $^{13}$CNMR spectrum of the compound showed 21 signals for the vitexin. Carbon bonded to the carbonyl group C-4 appeared at δ 182.0. The carbonyl carbon, C-4 resonates around δ 175-178, when the carbonyl is not hydrogen bonded. But in the presence of H-bonding to 5-hydroxy group, it moves downfield to about δ 182. When 3-hydroxy group is alone it resonates at δ 171-173. When both 3- hydroxyl and 5-hydroxyl groups are present, it resonates at δ176.Carbon bonded to the hydroxyl group C-5, C-7 and C-4' appeared at δ 61.1, δ 162.4, δ 160.2 respectively. Signals of C-6 from C-8 and signals of C-5 from C-9 are distinguished with the help of $^{13}$C-$^{1}$H coupling data. The degree of coupling identifies each carbon and demonstrates that C-9 resonates at higher field from C-6 while C-8 resonates at higher field from C-6.The degree of coupling identifies each carbon and demonstrates that C-9 resonates upfield from C-5 while C-8 resonates up field in comparison to C-6.

The UV spectrum of this compound exhibited two major peaks in the region 335nm and 271nm which indicates the presence of flavonoid structure. Mass spectra of isolated compound show molecular ion m/z 433 [M+] corresponding to the molecular formula $C_{21}$ $H_{20}$ $O_{10}$.The presence of vitexin in *Abrus precatorius* was not reported before the present study. The vitexin was reported earlier from leaves of *Parkinsonia aculeate* [34], *Ficus deltoidea* [35], *Alysicarpus monilifer* [36], *Crataegus pinnatifida* [37], etc. From the above studies it was concluded that this compound is 5,7,4'-trihydroxy flavones -8-glucoside i.e., apigenin 8-C glucoside or vitexin.

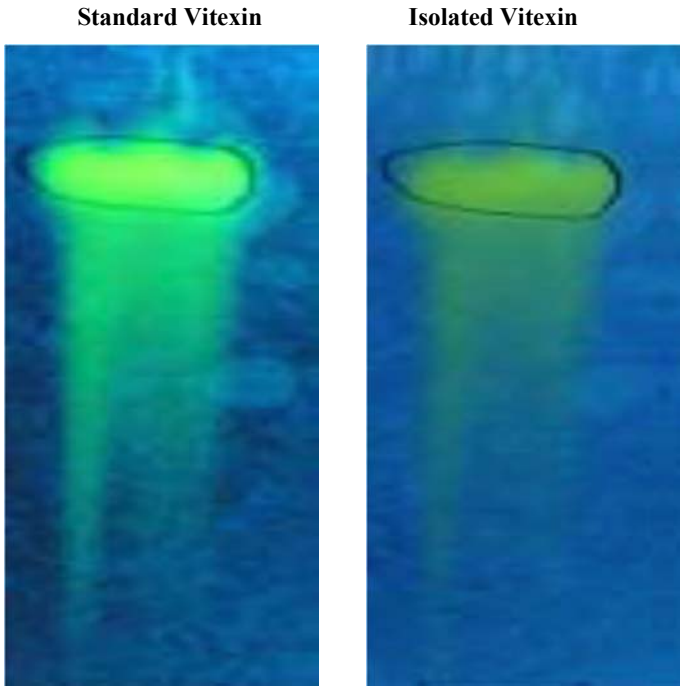


**Fig 1:** Flavonoid finger printing of *Abrus precatorius*

Vitexin possess hepatoprotective, antidiabetic [36], analgesic [40] activity. Vitexin is able to protect nerve cells against oxidative injury [38]. Vitexin is antineoplastic agent, platelet aggregation inhibitor possess neuroprotective properties [39]. It has a long term effect and improved the neurobehavioral outcomes. Vitexin is HIF-1α inhibitor. It is natural flavonoid antioxidant and may be useful in treatment of various diseases. The suspicious carcinogenic effect of synthetic antioxidants such as BHA and BHT has raised significant concerns about the use of chemical additives in food. The possible mechanism of action of bioactive flavonoid glycosides vitexin could be to block the active sites of reactive oxygen species to which they have greater affinity and cause cellular damage, there by offering protection to the targeted tissue acting as antioxidant.

Flavonoids have been identified as a new type of neuromimetic ligand with *in vivo* possess anxiolytic properties [41]. Flavonoid glycosides, showed to exert central nervous system mediated activities, particularly as sedative-hypnotics, analgesics and anxiolytic [42-43].

Thus, recently the demand of natural antioxidants has increased enormously and production of vitexin from leaves of *Abrus precatorius* may be useful if it is used in place of synthetic antioxidants. This study confirms flavonoid vitexin in the leaves of *Abrus precatorius.*

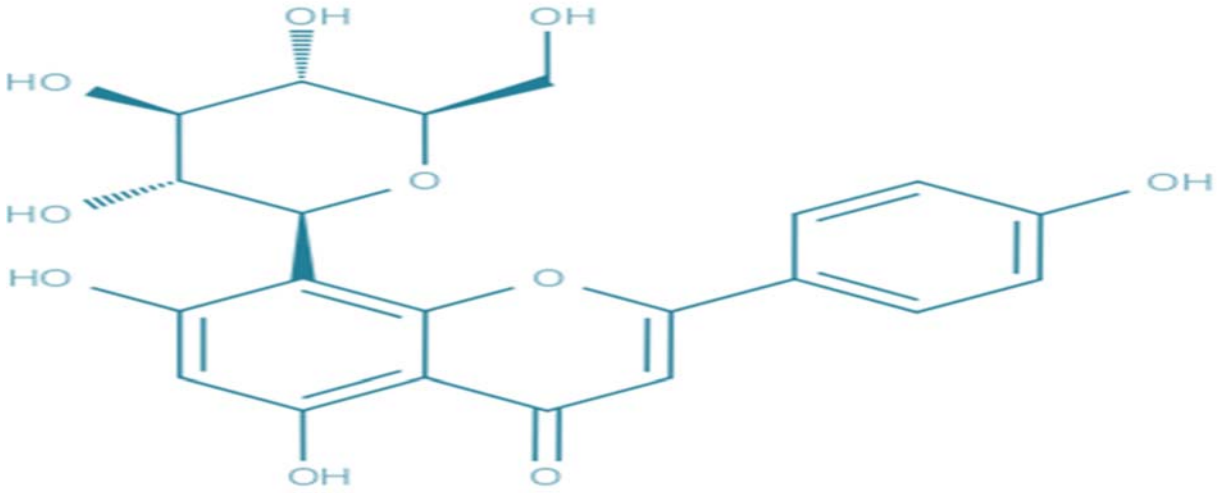


**Fig 2:** Vitexin

**Table 1**

| $^{1}$H NMR Spectral Data for Compound (400MHz, DMSO, δ(ppm)) | $^{13}$C NMR Spectral Data for Compound (100 MHz, DMSO, δ (ppm)) |
|---|---|
| **H- Position** | **C-position** |
| 3 (6.60)s | 163.8(C-2) |
| 6 (6.26)s, | 102.3(C-3) |
| 2’ (8.02 ) d J=8.4 | 182.0(C-4) |
| 3’(6.88)d J= 8.4 | 161.1(C-5) |
| 5’(6.88)d J= 8.4 | 98.2 (C-6) |
| 6’ (8.02)d J=8.4 | 162.4(C-7) |
| 1” (4.68)d | 104.5(C-8) |
| | 155.8(C-9) |
| | 104.0 (C-10) |
| | 121.4(C-1’) |
| | 128.7(C-2’) |
| | 115.6(C-3’) |
| | 160.2(C-4’) |
| | 115.6 (C-5’) |
| | 128.7 (C-6’) |
| | 73.2(C-1”) |
| | 7.0.8 (C-2”) |
| | 78.6(C-3”) |
| | 70.4(C-4”) |
| | 81.5(C-5”) |
| | 61.8(C-6”) |

**Acknowledgements**

I am very grateful to University Grants Commission, New Delhi, India for their financial assistance (Grant No.F.15-39/12 (SA-II). I am also very thankful to Dr. A.K. Gupta Head of the Chemistry Deptt. and Dr. M.K. Rawat, Principal Agra College, Agra, for their full support.

**References**

1. Craig WJ. Health promoting properties of common herbs. Am J Clin Nutr. 1999; 70(3):491S-499S.
2. Arora R. Phytopharmacological evaluation of ethanolic extract of the seeds of *Abrus precatorius* L. J Pharmacol Toxicol. 2011; 6(6):580-588.
3. Dhawan BN, Patnaik GK, Rastogi RP, Singh KK, Tandon JS. Screening of Indian plants for biological activity. Indian J Exp Biol. 1977; 15:208-219.
4. Premanand R, Ganesh T. Neuroprotective effects of Abrus precatorius Linn. Aerial extract on hypoxic neurotoxicity induced rats. Int. J Chem Pharmac Sci. 2010; 1(1):9-15.
5. Attal AR, Otari KV, Shete RV, Upasani CD, Nandgude TD. Abrus precatoriusLinnaeus: a phytopharmacological review. J Pharm Res. 2010; 3(11):2585-2587.
6. Frohne D, Pfander HJ. A colour atlas of poisonous plants. London: Wolfe Publishing Ltd. 1983, 291.
7. Adelowotan O, Aibinu I, Aednipekun E, Odugbemi T. The *in vitro* antimicrobial activity of *Abrus precatorius* (L.) fabaceae extract on some clinical pathogens. Niger Postgrad. Med J. 2008; 15(1):32-37.
8. Bobbarala V, Vadlapudi V. *Abrus precatorius* L. seed extracts antimicrobial properties against clinically important bacteria. Int. J Pharm Tech Res. 2009; 1(4):1115-1118.
9. Parekh J, Jadeja D, Chanda S. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turk. J Biol. 2005; 29:203-210.
10. De Britto AJ, Jeya PB, Kumar R, Gracelin S, Herin D. Abrus precatorius L. A medicinal plant with potential as antibacterial agent. J Pharmacy Res. 2012; 5(2):1207-1209.
11. Prashith Kekuda TR, Vinayaka KS, Soumya KV, Ashwini SK, Kiran R. Antibacterial and antifungal activity of methanolic extract of *Abrus pulchellus* Wall and *Abrus precatorius* Linn – a comparative study. Int. J Toxicol Pharmacol Res. 2010; 2(1):26-29.
12. Saxena VK, Sharma DN. A new isoflavone from the roots of *Abrus precatorius.* Fitoterapia 1999; 70:328-329.
13. Saganuwan SA, Onyeyili PA, Ameh EG, Etuk EU. *In vivo* antiplasmodial activity by aqueous extract of *Abrus precatorius* in mice, Rev. Latinoamer Quim. 2011; 39(1-2):32-44.

14. Gogte VM. Ayurvedic pharmacology and therapeutic uses of medicinal plants (Dravyagunavignyam), India. Bharatiya Vidya Bhavan 2000, 600-601.
15. Georgewill OA, Georgewill UO. Evaluation of the anti-inflammatory activity of extract of *Abrus precatorius.* Eastern J Med. 2009; 14:23-25.
16. Kuo SC, Chen SC, Chen LH, Wu JB, Wang JP, Teng CM. Potent antiplatelet, anti-inflammatory and allergic isoflavoquinones from the roots of Abrus precatorius. Planta Med. 2009; 61:307-312.
17. Sudaroli M, Chatterjee TK. Evaluation of red and white seed extracts of *Abrus precatorius* Linn. Against freund's complete adjuvant induced arthritis in rats. J Med Plants Res. 2007; 1(4):86-94.
18. Nagaveni P, Saravana Kumar K, Ramesh Y, Ramesh CN. Pharmacognostic properties and analgesic activity studies of *Abrus precatorius* leaves. JITPS. 2012; 3(1):18-23.
19. Anbu J, Ravichandiran V, Sumithra M, Chowdary BS, Kumar SKSLVVSN, Kannadhasan R*et al*. Anticancer activity of petroleum ether extract of *Abrus precatorius* on Ehrlich Ascites Carcinoma in mice. International Journal of Pharma and Bio Sciences. 2011; 2(3):24-31.
20. Zia-Ul-Haque A, Qazi MH, Hamdard ME. Studies on the antifertility properties of active components isolated from the seeds of *Abrus precatorius* Linn., Pakistan J Zool.1983; 15(2):129-139.
21. Jahan S, Rasool S, Khan MA, Ahemad M, Zafar M, Arshad M. Antifertility effects of ethanolic seed extract of *Abrus precatorius* L. on sperm production and DNA integrity in adult male mice. J Med Plant Res. 2009; 3:809-814.
22. Kusumot IT, Shimada I, Kakiuchi N, Hattori M, Namba T, Supriyatna S. Inhibitory effect of Indonesian plant extracts on reverse transcriptase of an RNA tumour virus. Phytother Res.1992; 6(5):241-244.
23. Rao MV. Antifertility effects of alcoholic seed extracts of *Abrus precatorius* Linn. In male albino rats. Acta Eur. Fertil 1987; 18(3):217-220.
24. Sarwat J, Rasool S, Khan MA, Ahmad M, Zafar M, Arsahd M. Antifertility effects of ethanolic seed extract of *Abrus precatorius* L. on sperm production and DNA integrity in adult mice, J Med Plant Res. 2009; 3:809-814.
25. Sinha R. Post-testicular antifertility effects of *Abrus precatorius* seed extract in albino rats. J Ethnopharmacol 1990; 28(2):173-181.
26. Talukder S, Hossain MA, Sarker S, Khan MAH. Investigation into effect of crude mixture of *Abrus precatorius* seed on hypothalamopituitary gonadal axis and development of antifertility in male rats. Bangladesh J Agric Res. 2011; 36(1):103-109.
27. Chinnappan A, Rathinam S. Studies on wound healing activity of red and block coloured seed, white coloured seed extracts of *Abrus precatorius* L. Int. J Pharm Bio Sci. 2011; 2:302-312.
28. Taur DJ, Patil RY. Mast cell stabilizing and antiallergic activity of *Abrus precatorius* in the management of asthma. Asian Pac. J Trop Med. 2011; 4(1):46-49.
29. Umamaheswari M, Dhinesh S, Asokkumar K, Sivashanmugam T, Subhadradevi V, Puliyath J. Anticataractic and antioxidant activities of *Abrus precatorius* Linn. against calcium-induced cataractogenesis using goat lenses. Eur. J Exp Biol. 2012; 2(2):378-384.
30. Khanna P, Kaushik P, Bansal V, Sharma A. New sources of insecticides: rotenoids. Proc. Natl. Acad. Sci. India1989; 59(1):83-86.
31. Sivakumar R, Alagesaboopathi C. Studies on cytotoxicity and antitumor screening of red and white forms of *Abrus precatorius* L. Afri. J Biotech. 2008; 7(22):3984-3988.
32. Subbaiah MV, Yuvaraja G, Vijaya Y, Krishnaiah A. Equilibrium, kinetic and thermodynamic studies on biosorption of Cu(II), Cd(II), Pb(II) and Ni(II) from aqueous solution by chitosan *Abrus precatorius* blended beads. J Chem Pharm Res. 2011; 3(2):365-378.
33. Harborne JB. Phytochemical Methods. A guide to modern techniques of plant analysis. London, Chapmann and Hall 1984; IIIrded.
34. Sharma KK, Sharma AK, Sharma MC, Tanwar K. Isolation of Orientin and Vitexin from Stem Bark of *Parkinsonia aculeata* (*Caesalpiniaceae*) and their Successive Blending on Sheep Wool Fiber, International Journal of Pharmacognosy and Phytochemical Research. 2014; 6(3):557-561.
35. Choo CY, Sulong NY, Man F, Wong TW. Vitexin and isovitexin from the Leaves of Ficus deltoidea with in-vivo α-glucosidase inhibition. Journal of Ethnopharmacology. 2012; 142(3):776-781.
36. Manikya KK, Ganga RB, Padmaja V. Role of vitexin and isovitexin in hepatoproctective effect of *Alysicarpus* monilifer Linn. against $CCl_4$ induced hepatotoxicity. Phytopharmacology 2012; 3(2):273-285.
37. Shan Cheng, Feng Qiu, Jia Huang, Junqi He. Simultaneous determination of vitexin-299-Oglucoside, vitexin-299-O-rhamnoside, rutin, and hyperoside in the extract of hawthorn (Crataegus pinnatifida Bge.) leaves by RP-HPLC with ultraviolet photodiode array detection. J Sci. 2007; 30:717-721.
38. Yang ZB, Tan B, Li Ting-Bo, Lou Zheng, Jiang Jun-Lin, Zhou Ying-Jun *et al.* Protective effect of vitexin compound B-1 against hypoxia/reoxygenation-induced injury in differentiated PC12 cells via NADPH oxidase inhibition. Naunyn- Schmiedeberg's Archives of Pharmacology 2014; 387(9):861-871.
39. Min JW, Hu Jiang-Jian, HeMiao, Sanchez Russell M, Huang Wen-Xian, Liu Yu-Qiang*et al.* Vitexin reduces hypoxia- ischemia neonatal brain injury by the inhibition of HIF-1 α in a rat pup model., Neuropharmacology 2015; 99:38-50.
40. Borghi SM, Carvhlho TT, Staurengo- Ferrari L, Hohmann MS, Pinge-Filho P, Casagrande R *et al.* Vitexin inhibits inflammatory pain in mice by targeting TRPV1, oxidative stress, and cytokines. J Nat Prod. 2013; 76(6):1141-49.
41. De Castro PCF, Hoshino A, Da Silva JC, Mendes FR. Possible anxiolytic effect of two extracts of Passiflora quadrangularis L. in experimental models. Phytother Res 2007; 21:481-484.
42. Elsas SM, Rossi DJ, Raber J, White G, Seeley CA, Gregory WL *et al. Passiflora incarnata* L. (Passionflower) extracts elicit GABA currents in hippocampal neurons *in vitro*, and show anxiogenic and anticonvulsant effects *in vivo*, varying with extraction method. Phytomedicine 2010; 17:940-949.
43. Fernandez SP, Nguyen M, Yow TT, Chu C, Johnston GAR, Hanrahan JR *et al.* The flavonoid glycosides, myricitrin, gossypin and naringin exert anxiolytic action in mice. Neurochem Res 2009; 34:1867-1875.