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

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## Abstract

In the present work, flavonoid 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 flavonoid 4', 5, 7-trihydroxyflavone i.e., apigenin.

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

## 1. Introduction

The bioactive compounds are mostly plant secondary metabolites, which become medicine after processing to pure compounds; some are very useful dietary supplements, and many useful commercial products. Further modification of the active compounds lead to enhance the biological profiles and a large number of such compounds which are approved or undergoing clinical trials for clinical uses against different diseases like pulmonary diseases, cancer, HIV/AIDS, malaria, Alzheimer's and other diseases[1-2] .Crude herbs are used as drugs in different country of the world and therefore it take a basic part of many traditional medicines worldwide. In Asia, traditional Chinese medicine (TCM), Korean Chinese medicine, Japanese Chinese medicine (kampo), ayurvedic medicine (India) and jamu (Indonesia), phytotherapy and homeopathy in Europe, alternative medicines are typically named when herbal therapies use with various other traditional remedies in America. Integrative medicine came into being when the alternative medicine, mainly the aforementioned traditional and folk medicines used worldwide, with conventional medicine (Western medicine). In recent years, the popularity of complementary medicine has increased.

Flavonoids are secondary metabolites characterized by flavan nucleus[3] and a $C_6$-$C_3$-$C_6$ carbon skeleton. These are group of structurally related compounds with a chromane-type skeleton having phenyl substituent in $C_2$-$C_3$ position. The flavonoids belong to one of the most bioactive compounds which naturally exist in the plant kingdom. Till now,over 8000 varieties of flavonoids have been identified[4]. Different naturally occurring flavonoids have been described and sub-categorized into flavones, flavans, flavanones, isoflavonoids, chalcones, aurones and anthocyanidines. These flavonoids have remarkable biological activities, including inhibitory effects on enzymes, modulatory effect on some cell types, protection against allergies, antiviral, anti-malarial, anti-inflammatory and anti-carcinogenic properties. A number of flavones, flavonols, flavanones, and isoflavones, as well as some of their methoxy, isoprenyl, and acylated derivatives, show antibacterial activity[5]. Flavonoids are major components of medicinal plants and have been used in traditional medicine around the world. Flavonoids are phenolic compounds which are widely distributed in plants, and have been reported to exert multiple biological effects, including antioxidant, free radicals scavenging abilities anti-inflammatory and anticarcinogenic activity [6-8].

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*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[9]. *Abrus precatorius* possess various pharmacological activities such as, antidiabetic[10] antioxidative[11], antidepression[12], antiviral[13], memory enhancing[14], antimicrobial[15-20],antimalarial[21], anti-inflammatory [22-24], antiarthritic [25-26], anticancer[27], antifertility[28-32], antiallergic[33] ,antiasthamatic[34], anticataract[35], antiinsecticide[36], antitoxicity activity[37-38].

Leaves of *Abrus precatorius* resemble tamarind leaves having 20-40 leaflets. Its leaves are used in treating diseases like alopecia areata, dysmenorrhoea, urticaria, eczema, stomatitis, conjunctivitis, migraine, leukemia and urticaria. The main object of this study is to extract and characterize flavonoid in the leaves of *Abrus precatorius.*

## 2. 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 0.86. 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 falvonoids [39]:

**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. Aluminium Chloride Test:** To the small amount of extract, two drops of 1% aluminum chloride was added, yellow color was obtained.

**3. 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. Results

**Table 1 $^{1}$H NMR Spectral Data Compound**

| $^{1}$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 (6.59)s | 164.4(C-2) |
| 6 (6.82) d J=2.1Hz | 106.5(C-3) |
| 8 (6.71) d,J=2.1Hz | 180.4(C-4) |
| 3'(6.91) d J=8.8Hz | 164.9(C-5) |
| 5'(6.91) d J=8.8Hz | 104.8(C-6) |
| 2'(7.82)d J= 8.8 Hz | 160.2(C-7) |
| 6'(7.82)d J=8.8 Hz | 99.3(C-8) |
| | 160.7(C-9) |
| | 109.4(C-10) |
| | 123.1(C-1') |
| | 129.3(C-2') |
| | 117.1(C-3') |
| | 162.6(C-4') |
| | 117.1 (C-5') |
| | 129.3 (C-6') |

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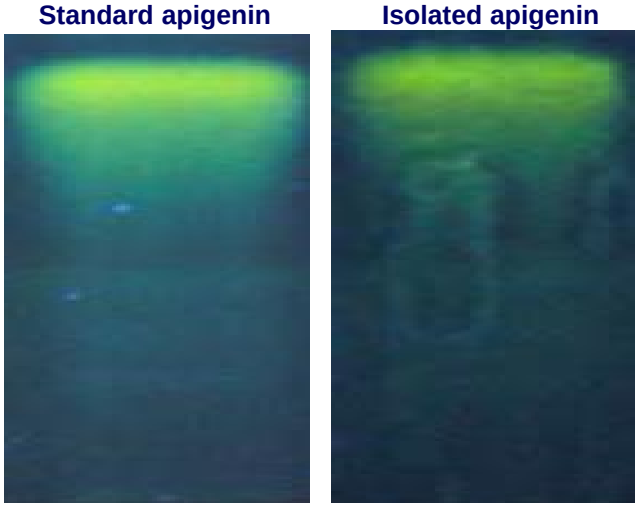


**Figure 1 Flavonoid finger printing of *Abrus Precatorius***

## 4. Discussion

The compound was isolated as yellow amorphous powder m.p203°C; $^{1}$HNMR (DMSO-$d_6$), $^{13}$CNMR (DMSO-$d_6$) Table1. The $^{1}$HNMR spectrum showed a doublet proton at δ7.82 corresponding to H-2' and H-6' proton. Another doublet proton occurs at δ 6.82 and δ 6.71 corresponding to H-6 and H-8 and doublet proton occurs at 6.91 corresponding to H-3' and H-5'proton. One singlet proton appeared at δ 6.59 corresponding to H-3 proton. The $^{13}$CNMR spectrum of the compound showed 15 signals for the apigenin. Carbon bonded to the carbonyl group C-4 appeared at δ 180.4. 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. Carbon bonded to the hydroxyl group C-5, C-7 and C-4' appeared at δ 164.9, δ 160.2, δ 162.6 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 268nm and 337nm which indicates the presence of flavonoid structure. Mass spectra of isolated compound show molecular ion m/z 270[M+] corresponding to the molecular formula $C_{15}$ $H_{10}$ $O_5$. On comparison with standard spectral data literature revealed that extracted compound was consistent to 4',5,7-trihydroxyflavone. The presence of apigenin in leaves of *Abrus precatorius* was not reported before the present study. The apigenin was reported earlier from *Parkinsonia aculeate, Ficus deltoidea, Alysicarpus onilifer* ' *Crataegus pinnatifida*, *Elsholtzia rugulosa* [40], *Matricaria chamomilla* [41], *Astragalus propinquus* [42] etc.

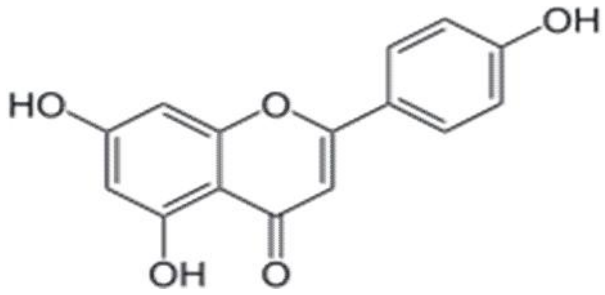


**Fig. 2 Structure of Apigenin**

## 5. Conclusion

Apigenin has been identified to induce significant neuroprotective effect against Parkinson disease[43], Alzheimer's disease [44-45] and ischemic stroke injury.The flavonoid apigenin is known to possess an antioxidant property and effectively militates against the prooxidative activity of cadmium [46-47]. Apigenin showed anticancer effect against lung cancer[48] as well as, growth inhibition of human colon carcinoma cell lines[49]. Literature revealed that apigenin is a promising flavone in inhibiting various kinds of cancer. Furthermore scientific evaluation are require to establish therapeutic efficacy. From the above studies it was concluded that 4', 5, 7,-trihydroxy flavone i.e., apigenin extracted from leaves of *Abrus precatorius.*

## Conflict of interest statement

We decline that we have no conflict of interest.

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