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Isolation and Extraction of Flavonoid from the Leaves of *Rauwolfia serpentina* and Evaluation of DPPH-scavenging Antioxidant Potential

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ABSTRACT

In the present work, methanolic extract from the leaves of *Rauwolfia serpentina* were analyzed phytochemically for the presence of flavonoids. Phytochemical studies revealed the presence of flavonoidal structure, by using chromatographic and spectroscopic techniques, 3,5,7,4'- tetrahydroxy flavone i.e., Kaempherol is identified. Antioxidant potential was determined by DPPH method.

Key words: *Rauwolfia serpentina,* flavonoids in leaves, isolation, Identification, NMR, antioxidant potential, etc.

INTRODUCTION

Since time immemorial humans have been dependent on plants for nutritional and medicinal requirements. Without plant kingdom human cannot survive on the earth for long time. Plants have been used in traditional medicines for several years. According to WHO, as many as 80% of the world's population depends on traditional medicine for their primary health care needs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases. *Rauwolfia serpentina* is an herb of medicinal value described in ayurvedic, western system of medicine. It is evergreen, woody glabrous shrub belongs to family apocyanaceae. It is known as sarpagandha in Hindi,Indian snake root in English, amalpori in Malayalam,chandra in Bengali etc[1] In India, it is found in Northern Himalayas especially in gharwal region, gangetic plains etc. Its leaves, seeds, roots, fruits are used in treatment of various ailments[2]. It is used in the treatment of arrhythimia,[3] hypertension[4] , high blood pressure[5], human promyelocytric[6], fever[7], malaria[8], eye diseases, pneumonia[9], AIDS, anticancer[10] ,spleen disorder,

skin diseases, asthma[11] etc. Dietary flavonoids are natural antioxidants[12].Flavonoids have existed for over one billion years and possess anti-ischemic[13],anti-inflammatory[14], antiapoptotic [15], anti-hypertensive[16] , anti-thrombic [17]activity. Flavonoid serves as antioxidant by scavenging singlet oxygen [18], superoxide anion[19] and lipid peroxy radicals[20].

Antioxidants are the molecules which safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Antioxidant donates their electrons to free radicals, so they are those substances that protect the cells against the effects of free radicals. When from an antioxidant, a free radical gains an electron it gets stabilized and thereby never damage cells further. The antioxidants of food are thought to prevent diseases caused by oxidative stress [21-22]. Free radicals are believed to be one of the causes of over sixty health problems. These problems include cancer, aging, and atherosclerosis. By increasing antioxidant intake and reducing exposure to free radicals can help lower health risks and problems.

The main object of this study is to extract and characterize flavonoid antioxidant in the leaves of *Rauwolfia serpentina*.

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. Leaves of *Rauwolfia serpentina* was collected from Chalesar, Agra. The leaves were air dried under shade for fifteen days. Then the leaves were finely powdered with the help of kitchen mixer grinder. The powdered leaves (100gm) of *Rauwolfia serpentina* were subjected to hot extraction in a Soxhlet apparatus with methyl alcohol containing 1% concentrated HCl, until complete discoloration. Now at 30^{0}C this extract was evaporated in vaccum rotatory, crude extract (5gm) was obtained. This crude extract was hydrolyzed with HCl. The obtained solution was extracted with amyl alcohol. Until the pH of the aqueous layer remained constant, the organic layer was washed with water. The extract was evaporated in vaccum rotatory to leave a residue. The dried residue dissolve in methanol and using the silica gel as stationary phase and mobile phase contain formic acid-water –methyl alcohol(1:9:20),performed thin layer chromatography.

Yellow bands of kaempherol on the chromatograms were located with Rf value 0.20.These bands were carefully eluted and extracted .The solvent was evaporated from the resulting extracts and residue was obtained. It was subjected to various physical and spectral analysis.

Chemical identification of flavonoids:-Following chemical tests was performed for flavonoid in isolated compound[23]

Shinoda Test

To a small amount of test solution in alcohol, magnesium ribbon was added followed by addition of drops of concentrated hydrochloric acid, formation of pink color confirms the presence of flavonoids.

Alkaline Reagent Test

To the extracts add a few drops of sodium hydroxide solution, yellow color were obtained which turns to colorless on addition of few drops of dilute HCl.

Zn-HCl reduction Test

To the small amount of extract add a mixture of Zn- dust and concentrated hydrochloric acid. Heat the solution after few minutes, color of the solution changes to red.

Kaempherol

Slightly yellow powder; m.p 276°C; ^{1}H NMR (400 MHz, DMSO): δ (ppm) = 6.18 (1H, *d, J* = 2.0 Hz, H-6), 6.42 (1H, *d, J* = 2.0 Hz, H-8), 6.92 (2H,*d, J* = 8.0 Hz,H-3', H-5'), 8.04 (2H, *d*, *J* = 8Hz,H-2', H-6'), ^{13}C NMR (DMSO,100MHz,): δ (ppm) = 146.4 (C-2), 135.2 (C-3), 175.4 (C-4),160.7(C-5), 97.4 (C-6), 163.1 (C-7), 93.2 (C-8), 155.6 (C-9), 102.7 (C-10), 121.2 (C-1'), 129.2 (C-2', C-6'), 115.2 (C-3',C-5'),160.1 (C-4').

Antioxidant potential

To determine antioxidant activitiy 3.96mg of 1,1-diphenyl-2-picrylhydrazyl was dissolved in 20ml of methanol to make a stock solution. 5gm

leaves of *Rauwolfia serpentina* was extracted with 50ml methanol for 5 days with shaking at regular interval. The extract was filtered and concentrated by vaccum rotatory evaporator. 5mg of this extract was dissolved in 20ml methanol to make a stock solution. 0.5ml of sample solution was added to 1ml of DPPH solution separately. These solutions were incubated for 30 minutes at room temperature in dark. The absorbance was measured at 517 nm. Lower absorbance of the solution indicated higher free radical scavenging activity by equation :

Percentage of Scavenging DPPH free radical = 100 X (1- AE/AD)

Where AE is absorbance of the sample solution and AD is the absorbance of the DPPH solution with nothing added.

RESULT AND DISCUSSION

The UV spectrum of this compound exhibited two major absorption peaks in the region 240-400nm. Two peaks are at 366.1nm and 266nm. Peak of flavones occurs in the range of 304-350nm. Peak of 3-hydroxy flavones occur at 352-385nm[24].

The ^{1}HNMR spectrum showed a two doublet proton at the region δ 6.18 and δ 6.42 corresponding to H-6 and H-8 protons respectively. These protons at C-6 and C-8 of flavones contain the common 5, 7 dihydroxy substitution pattern, give rise to two doublets in the range 6.0-6.5. The H-6 doublet occurs at higher field in comparison to signal for H-8[25].

Another ,two proton signals were observed in the region of spectrum at δ6.92 which are bonded to C-3' and C-5'atoms.These two doublet proton corresponds to H-3'and H-5'.Two doublet proton at δ8.04 corresponds to H-2'and H-6'.The position of doublet for the C-3' and C-5'protons appears upfield (6.65-7.1)in comparison to C-2'and C-6'.The position of the C-2' and C-6'doublet appears at lower field (7.1 - 8.1).^{13}C NMR of the compound showed 15 signals for flavonoid skeleton. The carbon bonded to –OH group appears at δ135.2, δ160.7, δ163.1, δ160.1 corresponds to C-3, C-5, C-7 and C-4' respectively. The carbonyl carbon C-4 appears at δ 175.4. The carbonyl carbon, C-4 resonates around 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. 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. 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.

Mass spectra of isolated constituent show molecular ion m/z 286 coresponding to the molecular formula $C_{15}H_{10}O_6$. From the above, studies it was concluded that this compound is 3, 5, 7, 4'-tetrahydroxy flavones i.e., Kaempherol.

In this study antioxidant potential of the leaves of the leaves of *Rauwolfia serpentina* was determined using DPPH method. At room temperature DPPH is a stable free radical and accepts electron or hydrogen radical to become a stable diamagnetic molecule. The decrease in DPPH absorbance at 517nm induced by antioxidants shows its reduction capability. The antioxidants causes decrease in absorbance of DPPH radical because when reaction between antioxidant molecules and radicals occurs then it causes scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to red .The free radical scavenging activity of leaves of *Rauwolfia serpentina* is 62.5%.

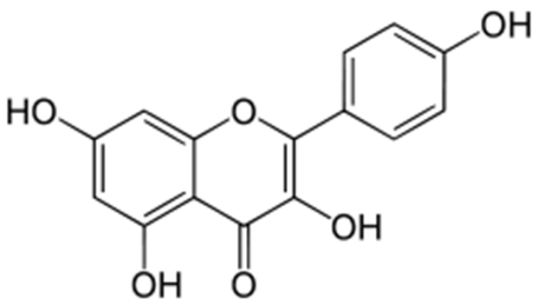

Fig.1: Kaempherol

CONCLUSION

Kaempherol posses antioxidant, anti-inflammatory, anticancer, antimicrobial, antidiabetic, analgesic, antiallergic, anti-osteoporotic, cardioprotective, neuroprotective, properties[26]. It inhibits the enzyme fatty acid amide hydrolase (FAAH) [27]. Kaempherol is natural antioxidant and may be useful if used in place of artificial ones, so production of kaempherol from *Rauwolfia serpentina* leaves may be of economic benefit. The antioxidant supplements reduce level of oxidative stress and slow down or prevent the development of complications associated with diseases. Antioxidants help to reduce the number of free radicals that form in the body, lower the energy levels of existing free radicals, and stop oxidation chain reactions to lower the amount of damage caused by free radicals. Flavonoids are a part of human diet. So commercial interest in these compounds as well as in flavonoid - rich plant sources is considerable. That's why these aspects justify the intense interest in flavonoids which has been manifested over several decades. This study confirms flavonoid Kaempherol in the leaves of *Rauwolfia serpentina* which have free radical scavenging activity due to which leaves of this plant possess antioxidant properties.

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