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Estimation of toxic heavy metals and antioxidant potential of Robinia pseudoacaia

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Abstract

The present contribution was aimed to evaluate antioxidant potential of *Robinia pseudoacaia*, Furthermore, this study was also aimed to estimate quantitatively toxic heavy metals. *Robinia pseudoacaia* (Fabaceae) is one of the medicinal plant , Native to north America , commonly distributed in various region of Kashmir. The plant is used as an antispasmodic, febrifuge, antioxidant, diuretic, emollient etc. Antioxidant activity was assessed as free radical scavenging capacity (RSC) towards 2, 2- diphenyl-1-picrylhydrazil (DPPH) radicals. Estimation of toxic heavy metals was performed by using atomic absorption spectrometery. The physicochemical parameters were done by standard procedures. The percentage scavenging effects of ethyl acetate on DPPH radical were 12.89 %, 15.74 %, 19.36 % at 50 µg/ml, 100 µg/ml, 200 µg/ml respectively. Whereas methanolic extract showed IC_{50} value 210.82 µg/ml. Total ash was found to be 8.4gm%. Water soluble ash 6.0gm%, alcohol soluble matter 16.6 gm%, acid insoluble ash 7.4% and the moisture content i.e loss of drying at 105 ^{o}c was found 6.7 gm%. The quantity of lead, cadmium, cobalt was found 0.042 ppm, 0.002 ppm, 0.1ppm respectively. *Robinia pseudoacaia* possessed significant antioxidant activity and contained safe levels of toxic heavy metals. It should be noted such study is first report from the *Robinia pseudoacaia*,

Key words: AAS, DPPH, *Robinia pseudoacaia*

Contents

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1. Introduction

A natural product is a chemical compound or substance produced by a living organism, found in nature that usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design (DJ Newman et al 2007). Many crude drugs were observed by the local healers to have some medicinal value. Many of these aqeous, ethanolic, distilled, dried and condensed extracts did exhibit a real and beneficial effects (Danbensky et al 2004). Natural antioxidants occur in all parts of plants. Plants may contain many different antioxidant components

such as phenolic compounds, nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity (G Cao, 1996: RA Larson, 1988: YS Velioglu, 1998: W Zheng, 2001: YZ Cai 2003: AL Branen, 1975). There is currently immense interest in natural antioxidants and their role in human health and nutrition (OI Aruoma, 1994). The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs

Robinia pseudoacaia (Fabaceae) is one of the medicinal plant , Native to north America , commonly distributed in various region of Kashmir like Kupwara, Banks of Manasbal Lake, Ganderbal, Pulwama(Puchal) (NR Wani, 2012) etc. It is a medium sized, melliferous tree which grows upto 6 meters. The plant is used as an antispasmodic, febrifuge, antioxidant, diuretic, emollient, laxative, antitumor and antimicrobial [AF Rosu, 2012]. Many potent flavonoids has been reported from various parts of this plant such as acacetin, secundiflorol, mucronulato, isomucronulato, isovesitol. In ad-dition to this four oligomeric flavonoids like robinetinidol leucorobin-etinidins, robinetinidol dihydrorobinetins robinetinidolrobinetin and robinetinidol flavone has also been reported (T Feifei 2001). Keeping in view, various reported flavonoids of *Robinia pseudoacaia*, this study was conducted to evaluate antioxidant potential of the plant. Further it has been documented that plants may contaminate with toxic heavy metals. Thus the aim of this study was also to estimate the toxic heavy metals from *Robinia pseudoacaia.*

2. Materials and methods

The plant material was collected from Pulwama (Puchal). After shade dried total ash content was determined and Sample for heavy metal analysis were prepared as per method given in Unani formulary. Then 300gms of material was extracted with ethyl acetate and methanol by using soxhlet and obtainted extract was reduced through rotary evaporator and finally dried in decicator.

Determination of Physicochemical properties.

Physicochemical properties of the drug and its ingredients were determined following standard methods as prescribed in the format issued by CCRUM, New Delhi ,India.

Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical scavenging activity: The free radical scavenging activity of the *plant* ethanolic extract was measured in terms of hydrogen donating or radical- scavenging ability using the stable radical DPPH (Blois, 1958). 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (50-250 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following

Formula:

$$\% \text{ inhibition} = ((A_0 - A_t) / A_0 \times 100).$$

Where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Estimation of Heavy metals

Heavy metal analysis of drug was performed on atomic absorption spectrometer (Thermofisher M Series, 650902 VI.27 model) as per procedures recommended by WHO, 1998 and AOAC, 2005.. Standard operating parameters for working elements were set, The operating parameters for Lead and Cadmium are: instrument technique—flame technique, wave length (Lead)—219nm, wave length (Cadmium)—228.8 and wave length(Co)-240.2nm, slit width—0.5mm, lamp current (Pb)—4.0mA, lamp current (Cd)—3.0mA, carrier gas and flow rate—Air and acetylene, 1.1L/min, flow rate—2ml/min. The concentrations of analytes were directly obtained from calibration graphs and all measurements were run in triplicate for the samples and standard solutions.

Table ; 1 Antioxidant potential of Ethyl acetate and methanolic extract of *Robinia pseudoacaia*

Samples	Concentration (µg/ml)	% protection
Ethyl acetate extract	50	12.89
	100	15.74
	200 IC50 = 0.86mg/ml	19.36
	250	22.43
Methanolic extract	50	15.80
	100	29.92
	200 IC50 = 210.82µg/ml	44.71
	250	59.62
Ascorbic Acid	50	74.47
	100	78.77
	200	86.58
	250	89.57

3. Results and discussion

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Positive DPPH test suggests that the samples were free radical scavengers. The scavenging effect of ethyl acetate (EA), methanolic (M) extract and ascorbic acid on DPPH radical was compared. On the DPPH radical, Extract (EA) had significant scavenging effects with increasing concentration in the range of 50-250 µg/ml when compared with that of ascorbic acid, the scavenging effect of both the extract was lower. Further, the DPPH activity of the M extract (50, 100 , 200 , 250 µg/ml) was increased in a dose dependent manner, which was found in the range of 15.80 to 59.62% as compared to ascorbic acid (74.47 to 89.57%). (Table 1; Fig 1). The physicochemical parameters data shown in (Table 2) below. Total ash was found to be 8.4 gm%. Water soluble ash 6.0gm%, alcohol soluble matter 16.6 gm%, acid insoluble ash 7.4% and the moisture content i.e loss of drying at 105 ^{o}c was found 6.7 gm%. Results of toxic heavy metals are tabulated (Table 3). The contents of toxic metals like lead (Pb), cadmium (Cd), Cobalt (Co) have been analyzed by using Atomic Absorption Spectroscopy. From quantitative estimation by atomic absorption spectroscopy, the concentrations of analysed toxic heavy metal such as Lead (0.042), cadmium (0.002ppm), cobalt (below the 0.1ppm) were found. All the monitored toxic metals in this drug were within the safe limit approved by WHO limit for drugs. On conclusion our findings suggest that consumers of this drug would not be exposed to any health risk generally associated with contamination of analysed toxic metals. It should be noted that evaluated antioxidant potential and estimated toxic heavy metals are the first report from the *Robinia pseudoacaia*.

Table.2 Physicochemical parameters from the leaves of *Robinia pseudoacaia*

Parameters	Obtained
Total ash	8.4%
Water soluble	6.0%
Alcohol soluble	16.6%
Acid insoluble	7.4%
Moisture content	6.7%

Table.3 Analysis of Heavy metals by Atomic absorption spectroscopy

S.No.	Name of the Toxic Element	Results	Permissible Limit
1	Lead	0.042 ppm	10 ppm (WHO)
2	Cadmium	0.002 ppm	0.3 ppm (WHO)
3	Cobalt	0.100 ppb	1.0 ppm (API,2008)

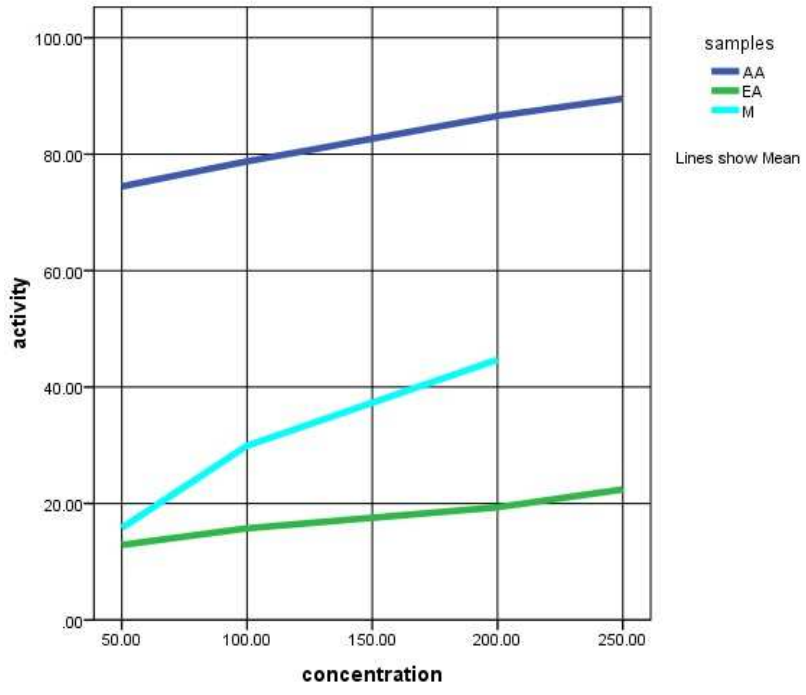

Fig 1. Antioxidant potential of EA, M, AA.

AA; Ascorbic Acid, EA; ethyl acetate, M; methanolic extract; Concentration (µg/ml)

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