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QUANTITATIVE STUDY OF TOTAL PHENOLIC AND ALKALOID CONTENT IN HYDROALCOHOLIC CONTENT IN *RAUWOLFIA SERPENTINA*

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ABSTRACT

The objectives of this study are to screen the phytochemicals, estimate the content of flavonoid and alkaloids compounds and determine the antioxidant capacity of the *Rauwolfia serpentina* stem. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol and alkaloids were determined by the well-known test protocol available in the literature. The hydro alcoholic extract of stem of *Rauwolfia serpentina* was studied for antioxidant activity on different *in vitro* models namely 1,1-diphenyl, 2-picryl hydrazyl (DPPH) method. Phytochemical analysis revealed the presence of phenols and flavonoids. The total flavonoids and alkaloids content of *Rauwolfia serpentina* stem of hydroalcoholic extract was 1.086 and 2.364mg/100mg respectively. Ascorbic acid used as standards was also evaluated for comparison. The extract showed dose dependent free radical scavenging property in the tested models. *Rauwolfia serpentina* stem extract showed IC_{50} value 68.10µg/ml for DPPH method, which was comparable to that of ascorbic acid (IC_{50}=17.68µg/ml). The present study describes the phytochemical profile and antioxidant activity of *Rauwolfia serpentina* which will further used for medicinal applications.

Keywords: *Rauwolfia serpentina*, Qualitative, Quantitative phytochemical, Antioxidant activity

INTRODUCTION

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules viz nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc. (Halliwell and Gutteridge 1984; Maxwell, 1995). Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders (Rice *et al.,* 1996). Almost all organisms are protected up to some extent by free radical damage with the help of enzymes such as super-oxide dismutase, catalase and antioxidant compounds viz. ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids and glutathione. Prior and Cao (Prior and Cao 1999), reported that antioxidant supplements or dietary antioxidants protect against the damaging effects of free radicals. Presently, much attention has been focused on the use of natural antioxidants to protect the human body especially brain tissues from the oxidative damage caused by free radicals. In last two decades, several medicinal plants have shown such effectiveness through the traditional methods of psycho neuropharmacology (Dhawan, 1995). *Rauwolfia serpentina* is used as drug since ancient times. *Rauwolfia serpentina* belongs to family *Apocynaceae*. There were still about 121 chemical substances identified from different medicinal plant which are used throughout the world[6]. *Rauwolfia serpentina* is perceived as Rauwolfia in Hindi, Hindustani Snake Root in English, Amalpori in Malayalam, Chandra in Bengali, and so forth. It is a sage of restorative worth marked in ayurvedic, western arrangement of prescription. Its leaves, seeds, roots, and organic products are used in the cure of numerous disorders. *Rauwolfia serpentina* contains many phytochemical compounds including flavonoids, alkaloids, tannins, and phenols (Pandey *et al.,* 2010; Kumari *et al,*). Keeping this in view, the present study has been conducted to evaluate the comparative antioxidant activity of *Rauwolfia serpentina* which are traditionally well known for their various activities.

MATERIAL AND METHOD

Plant material

The stem of *Rauwolfia serpentina* was collected from rural area near Bhopal (M.P.). Plant material (stem) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their color, odor, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade.

Defatting of plant material

Stems of *Rauwolfia serpentina* were shade dried at room temperature. The shade dried plant material was coarsely

powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration method

70 gram of powdered stem of *Rauwolfia serpentina* was extracted with hydroalcoholic solvent (ethanol: water: 80:20) using maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extract (Khandelwal, 2005).

Determination of percentage yield

Calculation of percentage yield

The percentage yield of yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Phytochemical screening

Phytochemical examinations were carried out extracts as per the following standard methods (Kokate, 1994; Mukherjee, 2007; Roopashree *et al.,* 2008).

Detection of alkaloids

Extracts dissolved individually in dilute Hydrochloric acid and filtered.

a) Hager's Test

Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow colored precipitate.

Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test

Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

a) Legal's Test

Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Finding of pink to blood red color indicates the presence of cardiac glycosides.

Detection of saponins

a) Froth Test

Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.

Detection of phenols

a) Ferric Chloride Test

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Detection of flavonoids

a) Lead acetate Test

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the occurrence of flavonoids.

Detection of proteins

a) Xanthoproteic Test

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

Detection of diterpenes

a) Copper acetate Test

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formations of emerald green color indicate the presence of diterpenes.

Quantitative studies of phytoconstituents

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminum chloride method (Obasi *et al.,* 2010). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Total alkaloids content estimation

The plant extract (1 mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total

alkaloid content was expressed as mg of AE/100mg of extract (Audu *et al.,* 2007; Geeta and Deepak 2019; Olufunmiso and Afolayan 2011).

In-vitro antioxidant activity of extract of stems of *Rauwolfia serpentina* using DPPH method

DPPH scavenging activity was measured by the spectrophotometer[14]. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

RESULTS AND DISCUSSION

The percentage yields of Pet ether and hydroalcoholic extract obtained from *Rauwolfia serpentina* are depicted in the Table 1. Preliminary phytochemical studies of the extract were done according to the published standard methods. Phytochemical analysis revealed the presence of flavonoids and alkaloids Table 2. Total flavonoid content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y=0.032X + 0.018$, $R^2=0.998$, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table No. 1: % Yield of stems of *Rauwolfia serpentina*

S. No.	Extract	% Yield (W/W)
1.	**Hydro alcoholic**	3.75

Table No. 2: Result of Phytochemical screening of hydro alcoholic extract of *Rauwolfia serpentina*

S. No.	Constituents	Hydro alcoholic extract
1.	**Alkaloids** Hager's Test:	+ve
2.	**Glycosides** Legal's Test:	-ve
3.	**Flavonoids** Lead acetate Test:	+ve
4.	**Diterpenes** Copper acetate Test:	-ve
5.	**Phenol** Ferric Chloride Test:	-ve
6.	**Proteins** Xanthoproteic Test:	+ve
7.	**Carbohydrate** Fehling's Test:	-ve
8.	**Saponins** Froth Test:	+ve

Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: $Y=0.007X+ 0.024$, $R^2=0.995$, where X is the Atropine equivalent (AE) and Y is the absorbance. TFC of hydroalcoholic extract of *Rauwolfia serpentina* showed the content values of 1.086 and fallowed by TAC were 2.364 Table 3. DPPH radical scavenging assay measured hydrogen donating nature of extracts. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *Rauwolfia serpentina* hydroalcoholic stem extract was found to be 68.10µg/ml as compared to that of ascorbic acid (17.68µg/ml). A dose dependent activity with respect to concentration was observed Table 4.

Table No. 3: Estimation of total flavonoids and alkaloid content of *Rauwolfia serpentina*

S. No	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1	**Hydro alcoholic**	1.086	2.364

Table No. 4: % Inhibition of ascorbic acid and hydroalcoholic extract of *Rauwolfia serpentina*

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydro alcoholic extract
1	10	44.65	18.65
2	20	48.62	35.74
3	40	65.34	39.4
4	60	69.65	45.62
5	80	77.41	51.68
6	100	84.13	65.84
IC_{50}	**17.68**	**68.10**	

CONCLUSION

The results obtained in the present study clearly demonstrate that the extract, which can effectively scavenge various reactive oxygen species/free radicals under in vitro conditions. This may be due to the number of stable oxidized products that it can form after oxidation or radical scavenging. The broad range of activity of the extracts suggests that multiple mechanisms are responsible for the antioxidant activity. The above results showed that *L. parviflora* hydroalcoholic bark extract could exhibit antioxidant properties. Further studies, on the use of above plants for their antioxidant role in various systems may provide potential natural antioxidants.

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