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Phytochemical screening, total antioxidant activity and quantitative determination of reserpine in *Rauwolfia* spp.

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Abstract : *Position of Rauwolfia in pharmaceutical industry is emerging. Reserpine is the first herbal constituent included in modern medicine system. Due to its high demand over the world market the genuine plant is on the track of extinction. Therefore, the present study was attempted to search reserpine from parts of Rauwolfia serpentina and Rauwolfia tetraphylla other than only roots of the plant. So that different parts of both the species can be explored for the bioactive reserpine and the root of commercial plant Rauwolfia serpentina can be minimized from extraexploitation and thus the plant from extinction. The quantitative determination of Reserpine was done by UV-spectrophotometer method.The result showed the presence of reserpine in root alongwith leaf and stem in both the species. In addition to this, antioxidant activity was measured spectrophotometrically using phosphomolybdenum method. Antioxidant activity was found to be 49.01% for Rauwolfia serpentina and 48.1% for Rauwolfia tetraphylla. This may be mainly due to the presence of phytochemical flavonoid in the leaf , stem and root.*

Key Words : *UV Spectroscopy, Reserpine, Rauwolfia serpentina, Rauwolfia tetraphylla.*

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Introduction :

Tropical plant *Rauwolfia serpentina* is a small,woody, perennial medicinal shrub. It is a medicinally famous herb in Ayurveda, Siddha, Unani, and Western system of medicines. The International Union for the Conservation of Nature and Natural Resources (IUCN) has assigned an endangered status to *Rauwolfia serpentina.* It has been reported to contain 50 indole alkaloids that are mainly localised in the root bark. Among these alkaloids, reserpine, yohimbine, ajmalicine, ajmaline etc. are the rich source found in the root of *Rau*

wolfia serpentina (Kumar *et al.,* 2008). The *Rauwolfia* species is mainly known for its phytochemical reserpine, which is widely used as an antihypertensive drug. Its alkaloid called sreserpine is a powerful sedative and hence it has important medicinal values. The present study deals with phytochemical analysis, antioxidant activity and quantitative determination of reserpine in different parts of the plant *Rauwolfia serpentina* and *Rauwolfia tetraphylla*.

Materials and Methods :

Collection of Plants Materials : The fresh plant material of *Rauwolfia serpentina and Rauwolfia tetraphylla* was collected from Patna Women's College and Patna Science College campus respectively. The leaves, stem and roots were collected in bulk and washed under running tap water to remove the adhering dirt.

Sample Preparation and Extraction: Plant materials were air dried under shade for two weeks. The dried plant materials were ground with a grinder. The coarse powder was stored in air dried container. From each plant material 5 gm of powder was extracted with 100 ml of methanol. The extracts were filtered. The filtrates were centrifuged at 2500 ppm for 10 minutes to remove any plant debris. Supernatant was used for assays.

Phytochemical Screening of Plants: The methanolic extract of root, stem and leaves of *Rauwolfia serpentina* and *Rauwolfia tetraphylla*, were subjected to preliminary phytochemical testing for the detection of major chemical groups. The details of the tests are as follows:

1. **Test for Tannin:** About 2-3ml of methanolic extracts of plants were taken in test tubes separately. Then, 1 ml of alcoholic ferric chloride ($FeCl_3$) was added in each test tube. Dark blue coloration of the solution indicated the presence of tannin in the sample.
2. **Test for Flavonoid:** About 2-3ml of methanolic extracts of plants were taken separately in test tubes and few pieces of magnesium turnings and 1ml of conc.HCL were added to the test tubes. Pink red coloration of the solution indicated the presence of flavonoids in the sample.
3. **Test for Alkaloid:** About 3ml of methanolic extracts of plants was taken separately in test tube with the Wanger's reagent which included 1.27gm of Iodine (I), 2gm potassium iodide (KI) and 100ml of distilled water. Brown precipitate indicated the presence of alkaloid in the sample.
4. **Test for Steroid and Terpenoid:** To 1ml of methanolic extract of plant, 1ml of chloroform, 2-3ml of acetic anhydride and 1 to 2 drops of concentrated sulphuric acid were added. Dark green coloration of the solution indicated the presence of steroid while no red coloration of the solution indicated the absence of terpenoid.
5. **Test for Saponin:** About 2ml of extract was vigorously shaken with 5ml of distilled water in a test tube, then allowed to stand for a while at room temperature. No persistent frothing indicated the absence of saponin.

Hydrogen peroxide scavenging activity: The ability of the extracts to scavenge hydrogen peroxide was determined as described by Kumar *et al*. (2008). One ml of the extract was rapidly mixed with 2 ml of 10mM phosphate buffered (0.1M, pH 7.4) hydrogen peroxide solution. The absorbance was measured at 230 nm with the UVvis-spectrophotometer after 10 minutes of incubation at 37°C against a blank (without hydrogen peroxide). The percentage of inhibition of hydrogen peroxide was calculated using the following formula.

% inhibition H_2O_2= ([A0]-[A1])/ [A0] X 100

Where,

(A0 – Absorbance of control; A1 – Absorbance of sample)

Total antioxidant capacity: The total antioxidant capacity of the crude extracts of plant materials was evaluated by the method of Goa *et al*. (2000).

The antioxidant capacity of the extract was measured spectrophotometrically using phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate / Mo (V) compounds. A 0.3 ml aliquot of sample solution was combined with 2.7 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). All the samples were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm. For the blank, 0.3 ml methanol was mixed with 2.7 ml of the reagent. A typical blank solution contained 2.7 ml of reagent solution and the appropriate volume of methanol used for the dissolution of the samples and it was incubated under the same conditions as the rest of the samples.

Reducing power assay: The reducing power of the extracts was determined according to the method of Harisaranraj *et al*. (2009).

All the plant extracts and standard antioxidants in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml; 10 g/l). The mixtures were incubated at 50°C for 20 min. Then, a portion of tri carboxylic acid (10%; 2.5 ml) was added to each mixture and centrifuged at 3000 rpm for 20 min. Finally, the supernatants (2.5 ml) were mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml; 0.1%). The absorbance of the solution was measured at 700 nm. A higher absorbance of the reaction mixture with an average value of 3.4206 for all the parts of two species indicated that the reducing power had increased.

Quantitative Determination of Reserpine in different parts of *Rauwolfia serpentina and Rauwolfia tetraphylla* by UV-spectroscopy.

The quantitative determination of reserpine was done according to the method of Bacher and Lewis (1984).

100 mg of reserpine was taken and dissolved in 100 ml of methanol, and various dilutions were made from it having concentration ranging from 100 µg/ml-1000 µg/ml, all the dilutions of reserpine were observed under UV vis-spectrophotometer at 268nm. 5gm of plant material was extracted with 25 ml methanol and the absorbance was taken. Absorbance of all the samples and standard was calculated by extraploting from the absorbance data the unknown concentration was determined.

Results and Discussion:

Phytochemical screening: Chemical test were carried out on methanolic extracts for determination of phytochemical constituent according to the method of Harisaranraj *et al*., (2009).

Table 1. Phytochemical Screening of Plant

NAME OF PLANT	TANNIN NOID	FLAVO-LOID	ALKA-OID	STER-NOID	TERPE-	SAPONIN
Rauwolfia serpentine						
Leaf	+	+	+	+	–	–
Stem	+	+	+	+	–	–
Root	+	+	+	+	–	–
Rauwolfia tetraphylla						
Leaf	+	+	+	+	–	–
Stem	+	+	+	+	–	–
Root	+	+	+	+	–	–

Where,

'+'-present ; '_'-absent

In case of *Rauwolfia serpentina* all the phytochemicals were present except saponin and

terpenoid. The same was seen in the case of *Rauwolfia tetraphylla* (Table 1).

Hydrogen peroxide scavenging activity of plants: Tests for H_2O_2 scavenging activity, total antioxidant capacity and reducing power assay were carried out for determination of potential antioxidant activity according to the method of Arouma (1998).

Table 2. H_2O_2 Scavenging activity of plant

PLANT	% INHIBITION
Rauwolfia serpentina	
a) Leaf	25.67
b) Stem	28.79
c) Root	26.84
Rauwolfia tetraphylla	
a) Leaf	24.58
b) Stem	27.32
c) Root	26.45
Standard ascorbic acid	**33**

Of all the parts of *Rauwolfia serpentina and Rauwolfia tetraphylla,* stem of *Rowolfia serpentina* showed maximum % of inhibition (Table 2).

The inhibitive effect of plant extract were subjected to H_2O_2 scavenging assay and found to be moderate when compared to ascorbic acid taken as standard. Of all the parts of *Rauwolfia serpentina and Rauwolfia tetraphylla,* stem showed maximum of inhibition (Table 3).

Table 3. Total Antioxidant capacity

PLANT	% INHIBITION
Rauwolfia serpentina	
a) Leaf	47.42
b) Stem	50.48
c) Root	49.15
Rauwolfia tetraphylla	
a) Leaf	46.40
b) Stem	49.97
c) Root	47.93
Standard ascorbic acid	**55.40**

The results presented above indicated that there is a potential antioxidant activity in all the parts of both the species. Flavonoids have been shown to have potent antioxidant activity. Stem has showed highest % of inhibition.

Table 4. Reducing power assay

Plant	Absorbance at 700 nm
Rauwolfia serpentina	
a) Leaf	2.978
b) Stem	3.981
c) Root	3.303
Rauwolfia tetraphylla	
a) Leaf	2.871
b) Stem	3.886
c) Root	3.637
Standard ascorbic acid	**4.538**

Plants exhibited reducing power ranging from 2.871 to 3.886. All of the parts of both species showed more or less significant reducing power at the same level as standard antioxidant, because of some degree of electron donation capacity in a concentration dependent manner (Table 4). Extracts containing the highest amounts of total phenolics had weaker reducing power than compounds although results were closed.

All the parts of the two species showed more or less significant reducing power at the same level as standard antioxidant.

Quantitative Determination of Reserpine

Analysis of different samples : With respect to absorbance of different sample the unknown concentration were determined according to the method of Bacher and Lewis (1984).

Table 5. Absorbance and content of Reserpine in *R.serpentina* an *R.tetraphylla*

		R.serpentina		R.tetraphylla	
Methanolic Extract	Concen-tration	Absorb-ance	Concen-tration of reserpine (mg/50 ml)	Absorb-ance	Concen-tration of reserpine (mg/50 ml)
Root	500mg/50ml	4.495	450	4.444	425
Stem	500mg/50ml	3.959	150	4.004	180
Leaf	500mg/50ml	3.086	125	3.769	160

Values showed that concentration of reserpine in root of *R.serpentina* was highest i.e 450 mg/ml.

The content of Reserpine was seen to be highest in root of *R.serpentina*. But its presence in other parts of *R.serpentina* and parts of *R.tetraphylla* in considerable amount were also seen (Table 5).

The phytochemical screening of different parts of *Rauwolfia serpentina* and *Rauwolfia tetraphylla* showed the presence of alkaloid, tannin and steroid which is similar to the results of previous works of Koche *et.al*. (2010). Flavanoid was found to be present in all parts of both the species and terpenoid and saponin were absent. The result differed from the previous works of Koche *et. al*. (2010).

The results for antioxidant capacity was found to be 49.01% for *Rauwolfia serpentina* and 48.1% for *Rauwolfia tetraphylla* which was different from the earlier works of Gordon (1990).

The quantitative determination of reserpine revealed that the concentration of reserpine was maximum in root rather than stems and leaves of both the species. The results were similar to the previous works of Bacher and Lewis (1984).

Conclusion :

On the basis of the results of this study, it was concluded that the methanolic extracts of plant *Rauwolfia* have significant antioxidant activity compared to other well characterized, standard antioxidant ascorbic acid in vitro. In addition, the antioxidant activity may be due to the presence of phytochemicals such as steroids, flavonoids, alkaloids, terpenoids, tannins and phenols in plant extracts.

Due to its high demand over the world market the genuine plant is almost on the track of extinction. The analytical data revealed that Reserpine is present in leaves, stem and roots of both the species. So, other parts of both the species can be explored for the isolation of bioactive compound reserpine.

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