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Research Article

Biological Evaluation of Leaf and Fruit Extracts of Wild Snake Root (*Rauvolfia tetraphylla* L.)

Dipti Ranjan Behera, Rashmi Ranjan Dash, Sunita Bhatnagar*

Regional Plant Resource Centre, Ekamrakanan, Nayapalli, Bhubaneswar-751015, India

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ABSTRACT

Leaf and fruit extracts of *Rauvolfia tetraphylla* were explored for their phytochemical, antioxidant and cytotoxic activity. Leaf and fruits were found to possess flavanoids and alkaloids in their extracts. Chloroform extract of leaf and acetone extract of fruits showed significant cytotoxic activity in brine shrimp assay. All the extracts showed good antioxidant activity in DPPH radical scavenging assay and nitric oxide antioxidant assays except hexane extract which showed only mild antioxidant potential. *Rauvolfia tetraphylla* could be a good substitute for endangered plant *Rauvolfia serpentina*.

Keywords: Antioxidant, Phytochemical, brine shrimp assay, Rauvolfia tetraphylla.

INTRODUCTION

Rauvolfia tetraphylla is a commonly available species of Odisha belonging to family Apocynaceae which is popularly used in Ayurvedic and unani system of medicines and are a part of folk remedies of most of the asian countries[1]. *Rauvolfia serpentina* is the most popular species of Rauvolfia genus commonly known as sarpgandha and since ancient times its roots are being used for their antihypertensive properties. A number of alkaloids have been isolated from this very plant and Serpentine is reported to inhibit histamine in in vitro models[2]. Other biologically important molecules from this plant are reserpine, ajmaline and deserpidiene etc. Besides this plant is also important ingredient in ayurvedic formulation sarpagandha vati. A significant medicinal property of sarpgandha plant has led to over exploitation of the plant and it is listed as endangered medicinal plant of Odisha as well as India[3]. Thus, there is a need for the search of its alternative. In this study *Rauvolfia tetraphylla* was explored as an alternative source for the popular medicinal plant. Biological evaluation of *Rauvolfia tetraphylla* was explored using phytochemical analysis, cytotoxic activity and antioxidant activities. These parameters basically provide important information regarding the medicinal potential of the plants.

MATERIALS AND METHODS

Collection and processing of planting material

Rauvolfia tetraphylla leaf and fruit samples were collected from Regional Plant Resource Centre's medicinal and germplasm garden. The collected plant material was dried in shade followed by grinding to a fine powder. Extraction of plant material (Leaf and fruit) was done using soxhlet extraction method. Successive extraction was carried out using solvents of increasing polarity namely Hexane, chloroform, Acetone and Methanol. About 20gm of powder plant sample was taken in a thimble and extracted using 250ml of solvents of increasing polarity. The extracted plant sample was then concentrated in a rotary evaporator. Semi solid extracts were kept in tight screw capped vials and same were used for all the tests.

Phytochemical Study

Phytochemical screening of all the extracts was done using standard protocols[4]. A brief account of the different tests conducted was as follows

Alkaloids

1 ml of methanolic extract *was* filtered. Then 2 ml of 1% aqueous HCl was added to it. Thereafter it was heated for few minutes. 2 drops of dragondroff reagent was added to the solution. Reddish brown precipitate with turbidity depicts alkaloid's presence.

Flavonoids

To 5 ml of methanolic extract, 1 ml of 10% NaOH solution was added. From the side of the beaker 2 drops of concentrated HCl was added. Yellow colour turning to colourless is an indication of presence of flavonoids.

Anthraquinone

To 1 ml of methanolic extract, 2 ml of 5% KOH was added. Then the solution was filtered. Change in colour was observed. Pink colour shows the presence of anthraquinones.

Saponins

About 2 ml of 1% sodium bicarbonate was added to 1 ml of methanolic bark extract and shacked. Lather like formation persistent for some time is indicative of presence of Saponins.

Tannin

1 gm of sample added with 100ml of distilled water, boiled and cooled, and then filtered. 1% ferric chloride was added drop wise to the filtrate. Green black

*Author for Correspondence: bhasunita@gmail.com

Table 1: Phytochemical analysis of Rauvolfia tetraphylla extracts

Phytoconstituent	Rauvolfia tetraphylla							
	Leaf extracts				Fruit extracts			
	Hex	Chl	Ace	Met	Hex	Chl	Ace	Met
Alkaloid	-	+	+	-	-	+	-	-
Flavonoid	+	-	+	+	-	-	+	+
Tannin	-	-	+	+	-	-	+	+
Saponnins	-	+	-	+	-	+	+	-

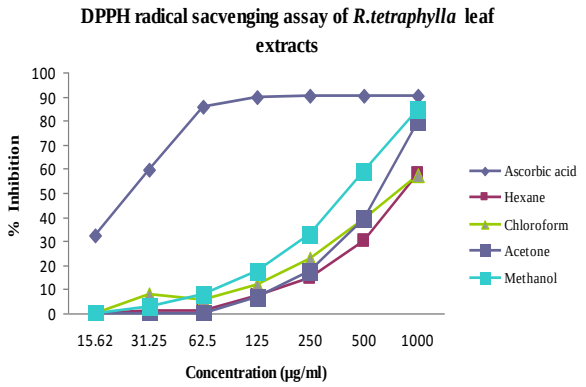


Figure 1: DPPH assay of leaf extracts

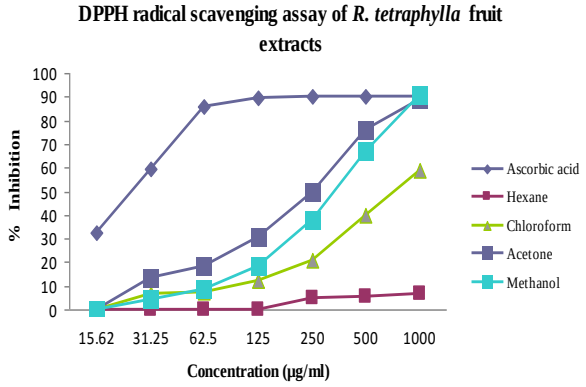
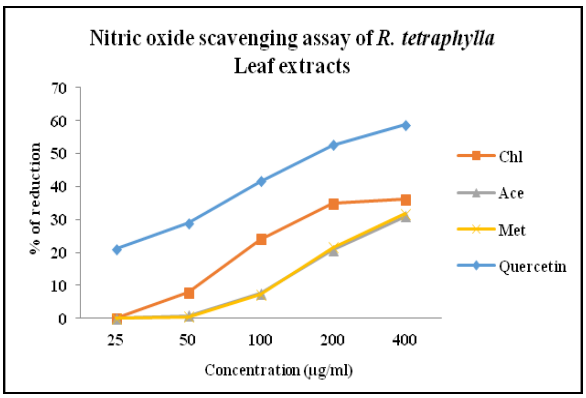


Figure 2: DPPH assay of fruit extract



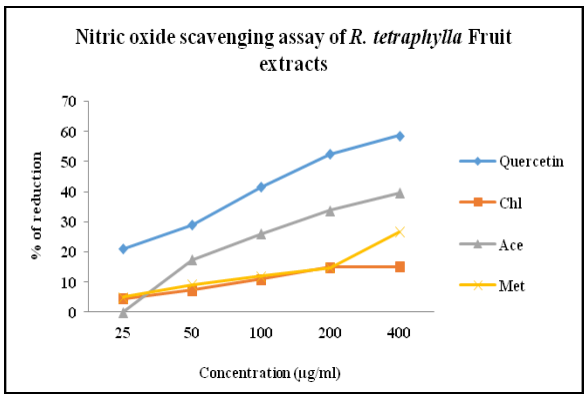


Figure 3 &4: Nitric oxide scavenging assay of leaf and fruit extracts.

Table 2: TLC based Qualitative antioxidant assay showing number of antioxidant bands in extracts.

Extracts	EMW		CEF		BEA	
	Leaf	Fruits	Leaf	Fruits	Leaf	Fruits
Hexane	2	4	3	3	Streak	4
Chloroform	2	7	Streak	7	6	4
Acetone	5	4	2	4	4	2
Methanol	5 Streak		4	1	No separation	4

precipitate shows the presence of tannin.

Terpenoid (Salkowski test)

About 2ml of extract was mixed with 1ml of chloroform and concentrated sulphuric acid was added drop wise to the test tubes. A reddish brown ring at the interface depicts the presence of terpenoids.

Antioxidant activity

TLC based antioxidant assay

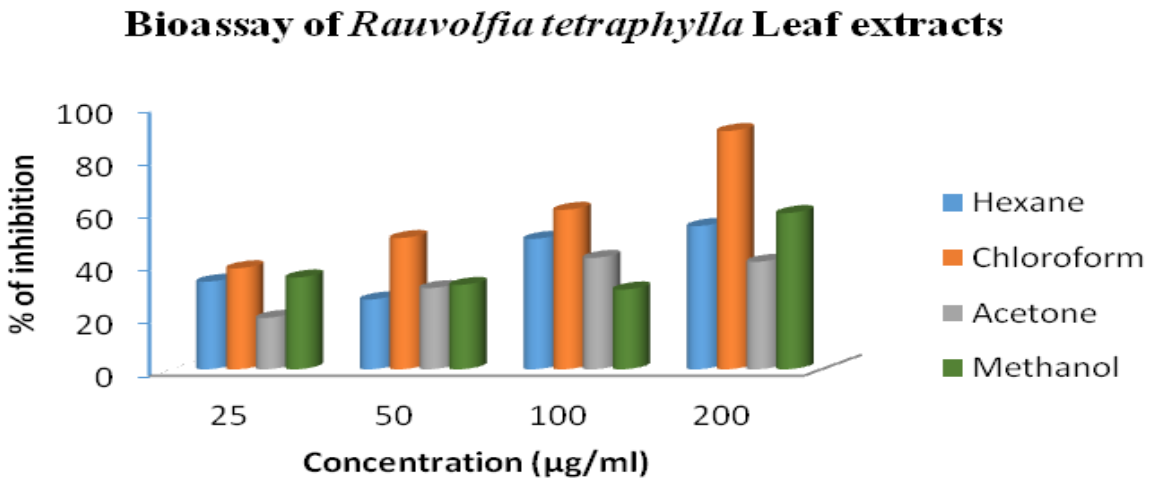
Thin layer chromatography based DPPH assay was performed for qualitative analysis of antioxidants. A stock solution of 2 mg plant extracts in 500µl of each solvent extract was prepared. TLC sheets 60 F_{254} (Merck Company) was used as stationary phase. Three different types of solvents were prepared as per the standard protocols[5].

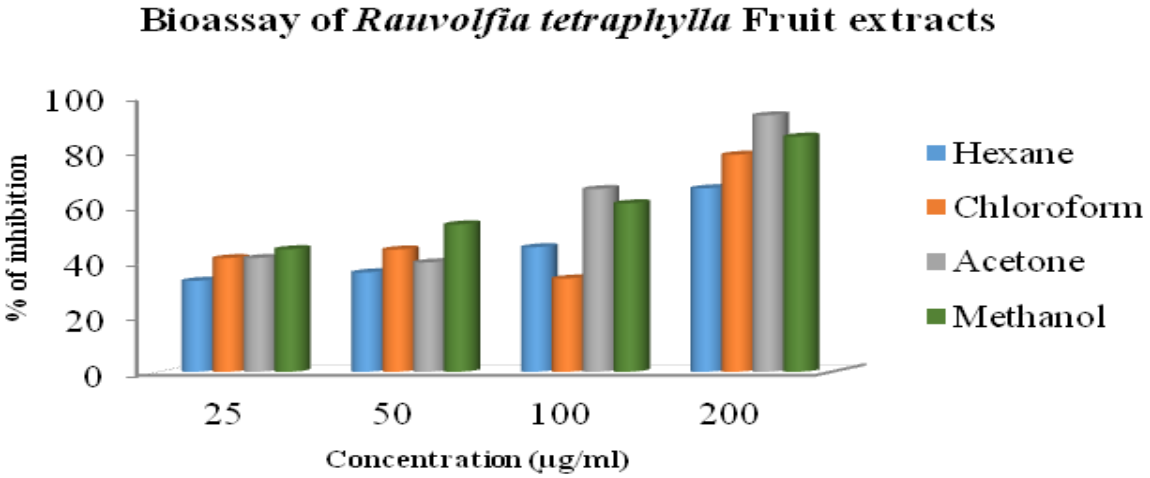
Ethyl acetate/Methanol/Water (40:5.4:4) [EMW] (polar neutral)

Chloroform/Ethyl acetate/Formic acid (5:4:1) [CEF] (intermediate polarity/acidic)

Benzene/Ethanol/Ammonium hydroxide (90:10:1) [BEA] (Non-polar/basic)

0.2 % DPPH solution was used as spraying reagent to develop chromatogram.





Retardation factors (R_f) values were calculated by the formula,

Retardation factor

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent}}$$

Numbers of yellow bands on purple background were recorded for each extract.

Quantitative antioxidant study

DPPH radical scavenging assay

A reaction mixture containing 50µl of 1mM DPPH solution and 100 µl of various concentrations of plant extracts (31.25, 62.5, 125, 250, 500 and 1000µg/ml) were prepared in methanol. Control consisted of only 100µl of methanol and 50 µl of DPPH. Samples were incubated in dark for 30 min at room temperature. The yellow colour chromophore formed was measured at 517nm using Multiplate Reader. Ascorbic acid was used as standard. The percentage scavenging of DPPH free radical was calculated by following formula.

$$\text{\% of inhibition } (\%I) = \frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \text{ X } 100$$

Where $A_{control}$ is absorbance of control and A_{sample} is absorbance of sample.

Nitric oxide radical scavenging Assay

Sodium nitroprusside 5 mM was prepared in phosphate buffer (pH 7.4). To 1 ml of various concentrations of test compound, sodium nitroprusside 0.3 ml was added. The test tubes were incubated at 25 ºC for 5 hr after which, 0.5 ml of Griess reagent was added. The absorbance of the chromophore was read at 546 nm. The experiment was performed in triplicates.

Cytotoxic activity

Cytotoxic activity was conducted using brine shrimp assay[6]. Brine shrimp (*Artemia salina*) eggs were incubated for 48hrs (1.8gm of black salt in 100ml of distilled water) to get the desired growth of the larvae for biological evaluation. Stock solution of different extracts was prepared at a concentration of 10µg/ml. Extract was evaluated at the doses 25, 50 & 100µg/ml. For each dose level three replicates were used. Motility, readings were taken every hour up to 4hours. After 24hrs, number of live parasites were counted in all the samples, percentage inhibition was calculated by comparing the treated samples with the controls. Standard deviation was also calculated.

$$\%\ of\ inhibition = \frac{\text{Control sample} - \text{Treated sample}}{\text{Control sample}} \text{X } 100$$

RESULTS AND DISCUSSIONS

Phytochemical analysis

As can be seen from the table 1, Alkaloids were present in leaf as well as fruit samples, this was expected as a number of alkaloids have been reported from the related species of this family which are namely serpentine,

reserpine, ajmalacine and many of these have proven medicinal records[7]. Beside this acetone extracts of both leaf and fruits showed the presence of flavanoids, tannin and saponin as well. This suggests the medicinal potential of aerial parts of the medicinal plants.

Presence (+), Absence (-)

Qualitative antioxidant assay

Stable free radical 1, 1-diphenyl-2-picrylhydrazil (DPPH) is reduced to corresponding hydrazine in the presence of antioxidant molecules, which gives a yellow colour[7].

$DPPH + AH \rightarrow DPPH - H + A^{-}$

(Purple color) (Yellow color)

As can be seen in Table 2, All the extracts showed good number of antioxidant bands. In some of the extracts antioxidant bands were so very close that a streak of yellow bands was obtained which depicts large number of antioxidant molecule in the extract.

Quantitative antioxidant activity

DPPH radical scavenging assay

As can be observed from Figure 1 and 2, none of the extract had similar activity as compared to the standard antioxidant ascorbic acid but at higher doses activity of methanol and acetone extracts of both leaf and fruit were very close by. This could be due to the fact that extracts are mixture of a number of molecules present in them whereas standard anti-oxidant is a pure molecule. Thus, it can be derived that there is definitely some molecule which is responsible for antioxidant potential of the crude acetone and methanol extracts of fruit and leaf. As flavonoids were present in both the extracts, they might be contributing towards the antioxidant property of extracts as a number of flavonoids have been reported to show antioxidant potential[8].

Nitric oxide radical scavenging assay

Results of nitric oxide scavenging assay were not as promising as other assay, here extracts showed mild antioxidant activity.

Cytotoxic activity (Brine shrimp assay)

Amongst leaf extracts, chloroform extract showed the highest cytotoxic potential whereas in fruit extracts acetone extracts showed significant cytotoxic activity. Majority of the extracts showed dose dependent activity. Bioassay guided isolation using brine shrimp assay has often resulted into important active principles like ethyl p cinnamoic acid and ethyl cinnamate[9]. Overall *Rauvolfia tetraphylla* leaf and fruit extracts indicated significant medicinal potetnial and warrants for detailed analysis for the isolation of active principles.

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