WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 9, 910-919. Research Article ISSN 2277– 7105

# EVALUATION OF PHYTOCHEMICAL, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF LEAF EXTRACTS *VALLARIS SOLANACEA* (ROTH) KUNTZE

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Article Received on
26 June 2020,

Revised on 15 July 2020,
Accepted on 05 August 2020,

DOI: 10.20959/wjpr20209-18390

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## ABSTRACT

In this study phytochemical, antioxidant and cytotoxic properties of leaf of *Vallaris solanacea* was explored. Four solvent leaf extracts were prepared using soxhlet extraction method, these were namely hexane, chloroform, acetone and methanol. On phytochemical analysis sapponins, tannins, flavonoids and terpenoids were present in different extracts. Cytotoxic activity was found highest in chloroform extract(75.7%) at the dose 200µg/ml followed by hexane extract which showed 65% activity at the higher dose. Acetone extract showed highest antioxidant activity in FRAP assay while other extracts showed only mild activity.

**KEYWORDS:** Phytochemical, antioxidant, cytotoxic, DPPH, FRAP, *Vallaris solanacea.*

## INTRODUCTION

*Vallaris solanacea* belongs to family Apocynaceae, is commonly known as Bread flower in English and Ramsar in Hindi. Family Apocynaceae is endowed with a number of medicinal plants like *Alstonia scolaris, Catharanthus roseus, Nerium oleander and Plumeria rubra.*[1]

*Vallaris solanacea* founds mention in ayurvedic medicine, it is one of the ingredients of Visharbha taila.[2] Bark of *Vallaris Solanacea* is bitter and astringent and is chewed by the Kols for fixing loose teeth.[3] In the present study solvent leaf extracts of the plants were explored for their medicinal potential by studying the important class of compounds present

in them, followed by cytotoxic activity using brine shrimp assay and antioxidant potential using standard antioxidant methods like TLC and spectroscopic antioxidant assays.

## MATERIALS AND METHODS

### *Collection and processing of plant materials*

The leaves of *Vallaris solanacea* were collected from the medicinal germplasm garden of Regional Plant Resource Centre Bhubaneswar, Odisha, India. They were washed properly and dried in shade at room temperature. Dried leaves were made into fine powder using mechanical grinder.

### *Solvent extraction of plant material*

Extraction of plant material was done by Soxhlet method with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. During extraction the solvent diffuse into the plant material and solubilise compounds with similar polarity. The extracts were then concentrated under vacuum in Rota Vapour (Buchii) and semi solid extracts were stored in screw cap vials till further use.

### *Phytochemical tests*

Phytochemical tests were conducted as per the standard protocols.[4] For phytochemical tests stock solution of all the extracts (100mg/ml) was prepared.

### *Test for alkaloids*

***a) Mayer's test***: - 500µl of extract was taken in a test tube to which 2ml of dil. HCL was added and 1ml of Mayer's reagent was added in drop wise manner. Yellow buff or cream colour precipitate indicates the presence of alkaloid.

***b) Wagner's test:*** - 500µl of extract was taken to which 2ml of dil. HCL and 1ml of Wagner's reagent was added drop wise. The reddish brown precipitate indicates the presence of alkaloid.

***c) Dragondroff's test:*** - To 200 µl of extract, 2ml of dil. HCL and 1ml of reagent was added in a test tube; the orange brown precipitate exhibits the presence of alkaloid.

### *Test for saponins*

***Foam test***: - 1ml of extract was diluted with 20ml of water and solution was shaken in graduated cylinder for 15 minutes, stable foam exhibits the presence of saponins.

***Lead acetate test*:** - To 1ml of extract solution 1 % of aq. lead acetate solution was added. White precipitate indicates the presence of saponins.

***Test for flavonoids***

***Sodium hydroxide test*:** - 100 micro litre of extract was added to 1ml of water and filtered 10% NaOH was added to the filtrate, yellow colour indicates the presence of flavonoid.

***Test for Carbohydrates and Reducing sugars***

**Fehling's test:** - To 2ml of extract solution 5ml of Fehling's A and Fehling's B solution was added and boiled. The brick red precipitate depicts the presence of carbohydrates.

***Test for Terpenoids***

***Salkowski test*: -** 200mg of extract was dissolved in 2ml of chloroform and 2ml of sulphuric acid was added to it. Color change was observed.

***Test for Tannins***

To 1ml of extract solution 1ml of 5% ferric chloride solution was added. The greenish black precipitate shows the presence of tannins.

***Anthraquinone test*:** - To 1ml of extract 2ml of 5% KOH was added. Pink precipitate confirms the presence of anthraquinone.

***Glycoside test*:** - To 5ml of extract 25ml of dil. $H_2SO_4$ was added and boiled for 15 minutes. It was then neutralised with NaOH and 5ml of Fehling's solution A and B was added to it. Appearance of brick red colour indicates the presence of glycoside.

***Test for proteins/Ninhydrin test*:** - To the extract solution few drops of ninhydrin reagent was added. Yellow colour indicates the presence of protein.

***Test for Starch/Iodine test*:** - To the extract solution few drops of iodine solution was added. Appearance of blue black colour shows the presence of iodine.

**Determination of total phenolic content**

The total phenolic content (TPC) of the crude extracts of leaves was determined using the standard method.[5] To 0.5 ml of test sample (gallic acid or leaf extract), 1.5 ml (1:10 v/ v diluted with distilled water) Folin Ciocalteau reagent was added and allowed to stand for 5 min at 22ºC. After 5 min incubation, 2.0ml of 7.5% of sodium carbonate was added. These

mixtures were incubated for 90 min in the dark after slight shaking. Finally absorbance of different samples were measured at 725nm using spectrophotometer. The phenolic content was calculated as gallic acid equivalents GAE/ g on the basis of standard curve of gallic acid. The results were expressed as Gallic acid equivalents (GAE)/ g of the plant material. All the determinations were carried out three times.

**Determination of total flavonoid content**

The total flavonoid content (TFC) of Vallaris leaves was determined by using the aluminium chloride assay.[6] An aliquot (0.5 ml) of extracts was taken in different test tubes then 2ml of distilled water was added followed by the addition of 0.15 ml of sodium nitrite (5% $NaNO_3$, w/ v) and allowed to stand for 6 min. Later 0.15 ml of aluminium trichloride (10% $AlCl_3$) was added and incubated for 6 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4% w/ v) and volume was made upto the 5ml with distilled water. After 15 min of incubation the mixture turns to pink whose absorbance was measured at 510 nm using a spectrophotometer. Distilled water was used as blank. The TFC was expressed in mg of quercetine equivalents (QE) per gram of extract. All the experiments were conducted in triplicates.

***Cytotoxic activity of plant extracts***

The cytotoxic activity of leaf extracts of *Vallaris solaneacea* was done by brine shrimp motility assay.[7] A salt solution was prepared for hatching of brine shrimp larvae. 3.6 gm of potassium chloride (KCl) was dissolved in 200ml of distilled water and brine shrimp eggs were incubated for 24 hrs at 28 ± 2ºC. Different stock solutions of plant extracts were prepared. The cytotoxic activity assay was done in triplicates by taking control, positive control and experimental groups. Control test tubes contained only brine shrimps, positive control contained larvae plus ethanol (Vehicle) at different doses, while experimental test tubes contained extracts at different doses. The brine shrimp motility activity was viewed in each hour interval up to 4 hrs. After 24 hours all the samples were tested for live and dead parasites. Percentage inhibition was calculated by comparing experimental samples with the controlled samples.

***Antioxidant activity***

Antioxidant activity of all the extract was conducted using qualitative and quantitative antioxidant assays.

***Qualitative antioxidant assay*:** Thin layered chromatography based DPPH assay was performed for qualitative analysis.

A stock solution of 2 mg plant extracts in 500 µl of each solvent extract was prepared. The precoated TLC plates 60 $F_{254}$ (Merck Company) were activated at $100^0$C for 10 min. The samples were then spotted with the help of micro tips leaving 2 cm from the bottom of the sheet.

Three different types of solvents were prepared.

(I) Benzene: ethanol: ammonium hydroxide (BEA) (intermediate polarity/basic)in the ratio of 45:5:0.5,

(II) Chloroform: Ethyl acetate: Formic acid (CEF) (polar/acidic)(5:4:1) and

(III) Ethyl acetate: methanol: water (EMW) (polar neutral) (40:5.4:4).

0.2 % of DPPH solution was prepared in methanol. After drying of sheets DPPH solution was spread and the resulting bands were observed and Retardation factor ($R_f$) was calculated. Yellow bands in purple background represent the antioxidant bands of the extracts.

Retardation factor = Distance travelled by the compounds/ Total distance travelled by the solvents. Protocol of Mosoko & Eloff[8] was followed for TLC based DPPH assay.

***Quantitative antioxidant assays***

***DPPH radical scavenging assay***

The radical scavenging activity of different extracts against DPPH was determined by the method of Brand *et al*.[9] DPPH reacts with an antioxidant compound that can donate hydrogen and it gets reduced. The change in colour (from deep violet to light yellow) was measured. DPPH is a stable free radical and accepts an electron, or hydrogen radical to become a stable diamagnetic molecule. The intensity of the yellow colour depends on the amount and nature of radical scavenger present. A reaction mixture containing 500µl of 1 mM DPPH, various concentrations of plant extracts (7.81, 15.62, 31.25, 62.5, 125, 250 and 500 µg/ml) were prepared in methanol. A test tube containing only methanol and 500 µl of DPPH solution was taken as control. Then the tubes were incubated in dark for 30 min at room temperature. The yellow colour chromophore was measured at 517nm. Ascorbic acid was used as standard. The percentage scavenging of DPPH free radical was calculated by following formula.

% scavenged DPPH radical = A control – A sample/A control X 100; Where A control is absorbance of control and A sample is absorbance of sample.

**Ferric reducing anti-oxidant power assay (FRAP ASSAY)**

Total anti-oxidant activity was measured by ferric reducing anti-oxidant power (FRAP) assay of Benzie & Strain.[10]

## RESULTS AND DISCUSSIONS

Moisture content of leaves of Vallaris *solanacea* was found to be 76.10% and as per the yield of solvent extracts was concerned polar extract (methanol) had the highest yield of about 15% suggesting that polar component in the plants are more as compared to non polar molecules/compounds.

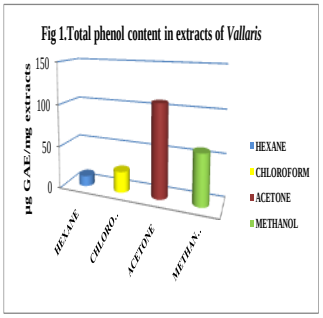
***Phytochemical analysis of Vallaris solanacea***

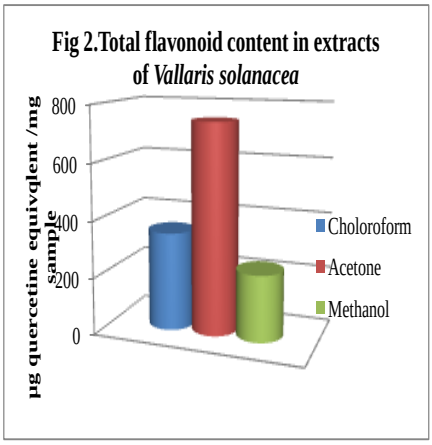
As can be seen from the Table 1, flavonoids and sapponins were present in chloroform, acetone and methanol but absent in hexane and fresh samples. Terpenoids was present in fresh and acetone extract. Tannin was present in fresh, acetone and methanol extracts. Cardiac glycosides were present only in hexane extract. Starch and anthraquinones were absent in all the samples. Phlobotanin was present in only fresh samples. Presence of tannins and sapponins is in confirmation with the earlier studies[11] and a number of flavonoids and cardiac glycosides have been isolated from the same as well as related species *Vallaris glabra.*[12]

Total phenolic content of four extracts namely hexane, chloroform, acetone and methanol was estimated as gallic acid equivalent/mg. As can be observed from Fig 1, Total phenolic content of acetone extract was maximum followed by methanol, chloroform and hexane in their respective order. Similarly total flavonoids content in acetone was highest whereas hexane extract lacked flavonoids(Fig 2) Phenolics and flavonoids are basically important medicinal molecules are often related to antioxidant and anti- inflammatory potential.[13] Amongst all the extracts acetone extract of the plant exhibited promising medicinal potential.

**Table 1: Phytochemical analysis of fresh and solvent leaf extracts of *Vallaris solanacea*.**

| Phytochemicals | Fresh sample | Hexane | chloroform | Acetone | Methanol |
|---|---|---|---|---|---|
| Alkaloid (Dragandroff test) | - | - | - | - | - |
| Alkaloid (Wagner's method) | - | - | - | - | - |
| Alkaloid (Meyer's test) | - | - | - | - | - |
| Flavonoid | - | - | + | + | + |
| Anthraquinone | - | - | - | - | - |
| Saponin | - | - | + | + | + |
| Terpenoids | + | - | - | + | - |
| Cardiac glycosides | - | + | - | - | - |
| Tannin | + | - | - | + | + |
| Starch | - | - | - | - | - |
| Phlobatannin | + | - | - | - | - |




**Bioevaluation of extracts of *Vallaris solanacea***

For biological evalution two parametres were selected these were cytotoxic activity using brine shrimp assay and antioxidant assays both qualitative (TLC based) and quantitative analysis (DPPH radical scavenging and FRAP assay).

**Cytotoxic activity using brine shrimp assay**

All the extracts were tested in three doses (50, 100, 200µg/ml). Cytotoxic activity was found highest in chloroform extract(75.7%) at the dose 200µg/ml followed by hexane extract which showed 65% activity at the higher dose. Remaining extracts showed only mild activities against brine shrimp mortality assay( Fig 3). Brine shrimp assay has a good correlation with anticancer activities. Glycosides isolated from *Vallaris solanacea* have shown promising activity against various cancer cell lines.[14]

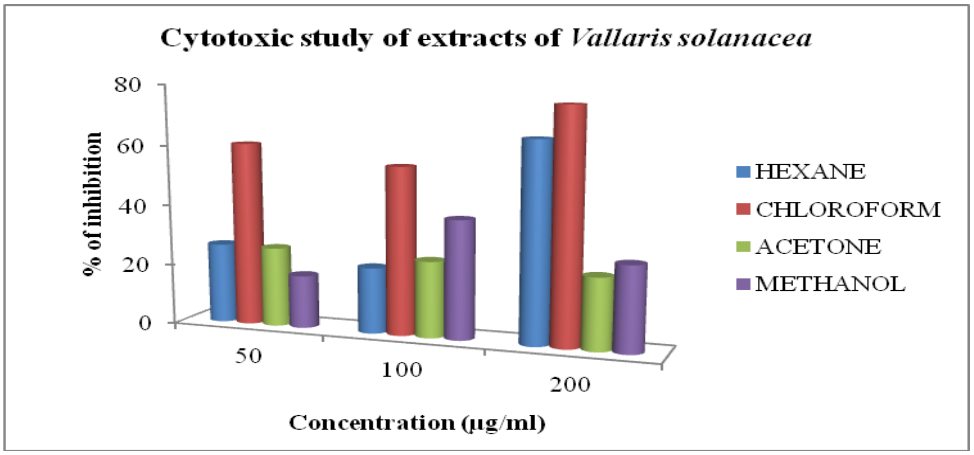
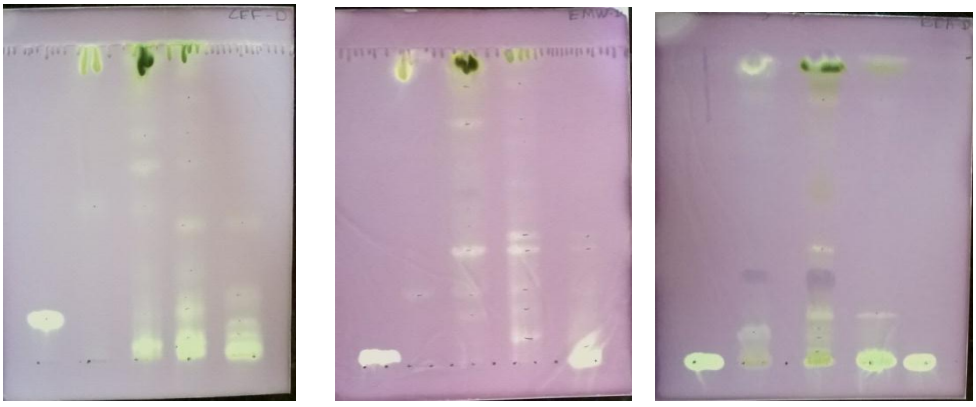



**Fig 3: Cytotoxic activity in different doses of different extract of *Vallaris solanacea.***

**Antioxidant activity of extracts of *Vallaris solanacea***

As can be seen from the chromatograms, a number of antioxidant bands were present in most of the samples. Chloroform extract showed highest number of antioxidant bands in TLC based antioxidant assay, but in quantitative assay acetone extract was most active in both the DPPH radical scavenging and FRAP assay. In DPPH radical scavenging assay acetone extract showed 58% activity, same extract showed highest FRAP value(1.838)(Table 2).

Overall chloroform extract of *Vallaris solanacea* showed cytotoxic as well as antioxidant activity in both the quantitative assays hence need further exploration.



**CEF** **EMW** **BEA**

**Fig 4: Chromatogram showing TLC fingerprinting of Four Extracts of *V. solanacea.***

**Table 2: FRAP values of four extracts of *Vallaris solanacea.***

| Solvent Extracts | Mm Ascorbic Acid Equivalent/Mg Extract |
|---|---|
| Hexane | 0.254 ± 0.075 |
| Chloroform | 0.364 ± 0.142 |
| Acetone | 1.838 ± 0.48 |
| Methanol | 0.582 ± 0.005 |

**ACKNOWLEDGEMENTS**

Acknowledgements are due to Regional Plant Resource Centre & Forest and Environment Department, Govt of Odisha for providing Laboratory facilities for the work.

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