

# SCREENING OF PHYTO-CHEMICAL CONSTITUENTS, TRACE METAL CONCENTRATIONS AND ANTIMICROBIAL EFFICIENCY OF *RAUVOLFIA TETRAPHYLLA*

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## ABSTRACT

Phytochemical constituents and trace metals concentrations, and antimicrobial activity of ethanolic extract of *Rauvolfia tetraphylla* leaves were investigated. The phytochemical screening of the crude extract revealed the presence of steroids, reducing sugars, sugars, alkaloids, flavonoids, saponins, tannins and amino acids. The leave powder was subjected to analyze the trace metals using atomic absorption spectroscopy (AAS). The average concentrations of Cd, Cr, Cu, Fe, Ni, Pb and Zn were BDL, 0.03, 0.2, 0.5, BDL, 0.06 and 0.5 mg kg<sup>-1</sup>, respectively. And also the crude ethanol extract of *Rauvolfia tetraphylla* were analyzed for in-vitro antimicrobial activity against certain pathogens and the zone of inhibition were compared with control drug Methicillin and Itraconazole. The *Salmonella typhimurium* is the most sensitive strains (17mm) to test solution while smallest inhibition were recorded in *Micrococcus luteus* and *Cryptococcus* sp. (11 mm).

**Keywords:** *Rauvolfia tetraphylla*, Antimicrobial activity, Phytochemistry, Trace metals.

## INTRODUCTION

Indian medicinal plants are well-known universally for its main role in the primary health care systems. Most of the rural people still rely largely on traditional system to cure their ailments. Medicinal plant in their surrounding environments are collected and used largely. As they provide low cost or no cost for practicing knowledge on such medicinal systems have been in the process of transmittance from one generation to the other. It has increased the efficacy and reliability of the medicinal uses as it has been in continuous testing among the population over the years. In recent years, secondary plant metabolites or phytochemicals, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju et al. 2005). There were many reports on the antimicrobial activity of plant extracts against human pathogenic bacteria (White et al. 2002; Mishra et al. 2009; Haris Kumar et al. 2010).

*Rauvolfia* is a genus of evergreen trees and

shrubs in the *Apocynaceae* family. Approximately 85 species in the genus can mainly be found in tropical regions. *Rauvolfia tetraphylla* and *Rauvolfia serpentina* is the South African quinine plant species. *Rauvolfia tetraphylla* contains a number of bioactive phytochemicals and is mainly known for its phytochemical reserpine, which was widely used as an antihypertensive drug. But very least research has been published on the leaves of plant *Rauvolfia tetraphylla*.

Many plants throughout the world, including some with documented medicinal properties contain chemicals that are toxic to microorganisms (Hudson, 1987). The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants. The use of plant extracts and phytochemical, with established antimicrobial properties, could be of great significance in preventive and therapeutic approaches. The

most important antimicrobial compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. Although synthetic and semi-synthetic antimicrobial drugs abound in various markets today, there is a need for continuous search for new ones to cope with the increased evolution of multiple antimicrobial resistant strains of organisms (Hart et al. 1998). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of "untreatable" bacterial infections and adds urgency to the search for new infection-fighting strategies. In contrast to synthetic drugs, antimicrobial of plant origin usually are not associated with many side effects and have an enormous anti-infective potential in numerous infectious diseases. In present study aims to analyze the phytochemical constituents, trace metals and antimicrobial activity of the *Rauvolfia tetraphylla*.

## MATERIALS AND METHODS

### Plant Material

The plant materials were collected from Thanjavur District of Tamil Nadu in India during the period of January to February 2013. The shade dried plant powders (100 g) were successively extracted with ethanol by soxhlet apparatus and is used as test sample.

### Phytochemical screening

#### Qualitative analysis

The solvent extracts were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents present in sample (Koperuncholan et al. 2011).

**Steroids:** Three ml of test solution and minimum quantity of chloroform was added with 3-4 drops of acetic anhydride and one drop of concentrated  $H_2SO_4$ . Purple color thus formed changes into blue or green color indicating the presence of steroids.

**Triterpenoids:** A 3 ml of test solution was added with a piece of tin and 2 drops of thionyl chloride. Formation of violet or purple colour indicates the presence of triterpenoids.

**Reducing Sugars:** A 3 ml of test solution was added with a 2 ml of Fehling's reagent and 2 ml of water. Formation of reddish orange color indicates the presence of reducing sugar.

**Sugars:** A 3 ml of the test solution was added with very small quantity of anthrone reagent and a few drops of concentrated  $H_2SO_4$  and

heated. Formation of green or purple color indicates the presence of sugars.

**Alkaloids:** A 3 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity indicates the presence of alkaloids.

**Phenols:** A 3 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols.

**Flavonoids:** A 3 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange color indicates the presence of flavonoids.

**Saponins:** A 3 ml of test solution was added with water and shaken. Formation of foamy lather indicates the presence of Saponins. Tannins: A 3 ml of test solution was added with water and lead acetate. Formation of white precipitate indicates the presence of tannins.

**Anthroquinones:** A 3 ml of test solution was added with magnesium acetate. Formation of pink color indicates the presence of anthroquinones.

**Amino Acids:** A 3 ml of test solution was added with 1% ninhydrin in alcohol. Formation of blue or violet color indicates the presence of amino acids.

**Catechins:** A 3 ml of test solution in alcohol was added with Ehrlich reagent and a few drops of concentrated HCl. Formation of pink color indicate the presence of catechins.

#### Quantitative analysis of phytoconstituents

The chlorophyll pigments in the leaves were estimated following the method of Arnon (1949). After pre-cleaning, weighted fresh leaf material was homogenized and extracted thrice in chilled 80% acetone (v/v). The volume of the acetone extract was made up to a known one and the optical density was read at 645nm and 663nm wavelengths on a spectrophotometer. The concentration of the chlorophyll pigments was calculated and is expressed in mg/g fresh weight. Amino acids were estimated by Ninhydrin method which is calorimetrically measured at 570nm (Hwang, 1975). Proteins were estimated by Bradford method and the absorbance was measured at 595 nm against

blank/ sample (Bradford, 1976). Carbohydrates were estimated by anthrone method which can be measured by using colorimetrically at 620nm (or) by using a red filter (Morris 1948). All the trials were performed thrice and the mean values were presented.

#### Trace metal analysis

The *Rauvolfia tetraphylla* plant sample was collected from the Thanjavur district, Tamil Nadu. The leaves were carefully removed and washed with sterile distilled water. The cleaned leaves were dried in shadow area and were grinded with mortar and pestle. After drying, 1 g of plant samples was treated with aqua-regia mixture in Teflon bomb and was incubated at 140 °C for 2-3 days. After incubation, the reaction mixture was filtered with Whatman No.1 filter paper. Then the extraction was test for trace metals (Fe, Cu, Zn, Pd, Cd, Cr and Ni) analysis. The extraction of the studied metals in the solutions was determined by the 797 VA Computrace voltametry, Metrohm. To avoid the contamination, the devices were rinsed with acidified water (10% HNO<sub>3</sub>) and weighted to dissolve metals before analysis. And all the equipments and containers were soaked in 10% NHO<sub>3</sub> for 24 h then rinsed thoroughly in de-ionized water before use. Also find the below detectable limit of the instruments.

#### Determination of Antimicrobial Activity

##### Testing of antimicrobial activity

The test strains were: *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumonia* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholerae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1), *Cryptococcus* sp. MTCC 7076 (F2), *Microsporium canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4). The cultures were obtained from MTCC, Chandigarh and NCIM, Pune, India. Microbial strains were tested for antimicrobial sensitivity using the disc diffusion method (Koperuncholan et al, 2010). The antibacterial and antifungal activity of test samples was analyzed against certain microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively. A sterile cotton swab was used to inoculate the bacterial suspension on surface of agar plate. The 15 and 30 µL of sample coated disc were placed in agar plates, separately. For negative control study, the sterile triple distilled water was used. The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungus). After incubation, the zone of inhibition was measured with ruler. The assays

were performed in triplicate and the average values are presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control. All the media, standard discs and sterile disc were purchased from Hi-Media (Mumbai, India).

#### RESULTS AND DISCUSSION

##### Phytochemical constituents of secondary metabolites

The present study revealed that the medicinal plant such as *Rauvolfia tetraphylla* contains bioactive compounds. The phytochemical constituents were screened by qualitative and quantitatively methods and the results are presented in Table 1 and 2. In Tannin analysis, the brownish green colour formation indicates the presence of Tannin. Similarly, based on the presence or absence of colour change indicate positive and negative results are indicate. In these screening, the positive results were obtained from steroids, reducing sugars, sugars, alkaloids, flavonoids, saponins, tannins and amino acids while triterpenes, phenols, catechins, and anthroquinones gave negative results.

This shrub contains a number of bioactive phytochemicals and is mainly known for its phytochemical reserpine, which was widely used as an antihypertensive drug. It is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin et al. 1985). Tannins are dietary anti-nutrients that are responsible for the astringent taste of foods and drinks (Chikezie et al. 2008). Tannins bind to both proteins and carbohydrates which has several implications for commodities containing tannins. Their presence can cause browning or other pigmentation problems in both fresh foods and processed products. The presence of tannin in the plants implies they may have astringent properties and in addition, could quicken the healing of wounds and burns (Farquar, 1996). This justifies their usage in herbal medicine.

##### Trace metals analysis

Heavy metals such as Cu, Zn, Mn and Fe are essential for plant growth; many of them do not have any significant role in the plant physiology (Balandrin, et al 1985). Sometimes, it also affects the photosynthetic ability of leaves, closure of leaf stomata, plant growth, physiology and productivity of plants (Hart et al. 1998). Metal contents of plant samples, Cd, Cr, Cu, Fe, Ni, Pb and Zn concentrations are BDL, 0.03, 0.2, 0.5, BDL, 0.06 and 0.5 mg kg<sup>-1</sup>, respectively. Heavy metals such as Cu, Zn, Mn and Fe are essential for plant growth, many of them do not have any

significant role in the plant physiology (Shaw, 1990). Contaminated sites often support some plant species, which are able to accumulate or tolerate high concentrations of metals such as Pb and Zn (Kumar et al. 1995). A small number of species are capable of growing on soils containing high levels of metals, and also accumulate these pollutants in high concentrations in the parts above ground. These plants are known as hyper accumulators (Brooks et al. 1977). The uptake of these heavy metals by plants is an avenue of their entry into the human food chain with harmful effects on health (Ihekoronye and Ngoddy, 1985).

#### Antibacterial and Antifungal screening

The antimicrobial activity of *Rauwolfia tetraphylla* was examined with various microorganisms using the disc diffusion test. The results of the antimicrobial activities are summarized in Table 4. The two tested concentrations such as 15 and 30  $\mu\text{L}$  /disc produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively. In the present study, higher (30  $\mu\text{L}$ /disc) concentration of sample got greater sensitivity than (15  $\mu\text{L}$ /disc) lower concentration in most of the microorganisms. In bacteria, the test sample was most effective against *Salmonella typhimurium* NCIM 2501 (B5) while smaller effect was noticed from *Micrococcus luteus* NCIM 2871 (B4). In fungi, which was effective against *Trichophyton rubrum* MTCC 3272 (F4) whereas smaller effect was observed in *Cryptococcus* sp. MTCC 7076 (F2). All the microbial strains depict higher sensitivity to the higher concentration (30  $\mu\text{L}$ ) for the test sample when compared to the positive control except B3, B4 and B6. There is no antimicrobial activity in solution devoid of sample used as a vehicle control (sterile triple distilled water), reflecting that antimicrobial activity was directly related to the sample. Suresh et al. (2008) reported the best antimicrobial activity of *Rauwolfia tetraphylla*, which showed maximum activity against *E. coli* and *Enterobacter aerogenes*, and various tested fungi such as *A. niger* and *Penicillium sp.* were

found to be more sensitive to crude extract when compared to others. Several phytoconstituents such as terpenoids (Scortichini and Rossi, 1991), flavonoids (Tsuchiya et al. 1966) and tannins (Ya et al. 1988) are effective against certain microorganisms. The results of the present investigation clearly demonstrate the antibacterial and antifungal activities of the ethanol extracts of the leaves.

#### CONCLUSION

This study concluded that the presence of steroids, reducing sugars, sugars, alkaloids, flavonoids, saponins, tannins and amino acids are helps to the antimicrobial activity and is effective than positive control. The presence of heavy metals indicated that the plant was resistant to the trace metal and their secondary metabolites were not seriously affected by these metals and it may enter into the human food chain. In this endeavor, traditional herbal medicines must perform be granted the benefits of modern science and technology to serves further global needs. The *Rauwolfia tetraphylla* may act as an alternative antibiotic in near future.

**Table 1: Quantitative phytochemical constituent of *Rauwolfia tetraphylla***

Biochemical constituents	<i>Rauwolfia tetraphylla</i> (mg/g)
Chlorophyll A	0.117
Chlorophyll B	0.852
Total Chlorophyll	0.969
Amino acid	150
Protein	2.120
Carbohydrate	1.000
Phenol	0.025

**Table 2: Quantitative phytochemical constituent of *Rauwolfia tetraphylla***

Phytochemical Constituents	Result	Phytochemical Constituents	Result
Steroids	+	Catechins	-
Triterpenes	-	Flavonoids	+
Reducing sugars	+	Saponins	+
Sugars	+	Tannins	+
Alkaloids	+	Anthraquinones	-
Phenolics	-	Amino acids	+

**Table 3: Concentration of trace metals in *Rauwolfia tetraphylla***

Sampling Site Name	Sampling Site No.	Sample Name/ Family	Sample No.	S. Code	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Thanjavur, Tamil Nadu	S1	<i>Rauwolfia tetraphylla</i>	P1	TP1	BDL	0.03	0.2	0.5	BDL	0.06	0.5
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BDL – Below detectable limit

**Table 4: Antimicrobial activity of the ethanolic extracts of leaves in *Rauvolfia tetraphylla***

S.No	Test Microorganisms	Zone of inhibition (mm) Sample (15 & 30) $\mu$ L / disc				Diseases	Route of Transmission
		15 $\mu$ L	30 $\mu$ L	PC	Remarks		
1.	<i>Aeromonas liquefaciens</i> B1	13	16	14	> PC	Wound Infections / Gastroenteritis	Water / Food
2.	<i>Enterococcus faecalis</i> B2	12	14	8	> PC	Endocarditis / Bladder, Prostate, and Epididymal Infections / Nervous system Infections	Water / Food
3.	<i>Klebsiella pneumoniae</i> B3	13	15	28	< PC	Acute diarrhoea / Dysentery	Water / Food
4.	<i>Micrococcus luteus</i> B4	11	12	38	< PC	Skin & Pulmonary infections / Septic shock / Pneumonia endocarditis	Soil / Dust / Water / Airways / Food
5.	<i>Salmonella typhimurium</i> B5	14	17	0	> PC	Typhoid	Water / Food
6.	<i>Vibrio cholerae</i> B6	11	13	16	< PC	Cholera	Water / Food
<b>Fungi</b>							
7.	<i>Candida albicans</i> F1	12	15	10	> PC	Skin (Integument) Infections / Gastrointestinal tract Infection	Airways / Wound / Soil / Water
8.	<i>Cryptococcus</i> sp. F2	11	13	9	> PC	Cryptococcal disease / Bronchiectasis / Endophthalmitis.	Airways / Wound / Soil / Water
9.	<i>Microsporum canis</i> F3	12	14	9	> PC	Tinea capitis / Ringworm	Airways / Wound / Soil / Water
10.	<i>Trichophyton rubrum</i> F4	13	16	7	> PC	Tinea corporis / Tinea cruris / Tinea pedis / Onychomycosis	Airways / Wound / Soil / Water

PC -Positive Control (Using antibiotic disc: **Bacteria – Methicillin (10mcg/disc)** ; **Fungi – Itraconazole (10mcg/disc)** Samples - 15  $\mu$ L / disc & 30  $\mu$ L / disc; > PC – greater than positive control; < PC – less than positive control

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