Pharmacognostical, phytochemical and pharmacological studies in *Rauvolfia tetraphylla* L.

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SUMMARY

In the present study leaves of *Rauvolfia tetraphylla* L. were collected and analysed for the pharmacognostical, phytomicrographs, phytochemical and antimicrobial properties. Analytical values of leaves like total ash, water soluble ash, acid insoluble ash, sulphated ash and fluorescent analysis of the plant showed colour characteristics in both visible and ultra violet light. Cold extracts of the plants samples showed the presence of compounds for carbohydrates, alkaloids, tannins and phenols and flavonoids and absence of fixed oil and saponins. The extract exhibited positive antimicrobial activity against bacteria (*E. coli* and *Klebsiella pneumoniae*) and fungi (*Aspergillus flavus* and *Fusarium indicus*).

Key words : *Rauvolfia tetraphylla* L., TLC profile, Fluorescent behaviour, Biological compounds, Thytocomponents

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The main source of drugs for Indian system of medicine, majority of the Indian population depends on phytomedicine for their primary health care in this modern scientific world. In Indian flora, *Rauvolfia tetraphylla* L. (Family: Apocynaceae) is a small branched woody shrub cultivated in garden. Ethanobotanically, the extract of this herb mixed with castor oil is applied to skin diseases (Chaudhuri, 1965; Kannabiran and Krishnamoorthy, 1972; Ahmed, 1994). This plant is mainly used in major diseases, anti-hypertensive, sedative, antihelmentic (against worms), intestinal disorder, diarrhea and dysentery.

MATERIALS AND METHODS

The aerial parts of *Rauvolfia tetraphylla* L. were collected from Thiruvarur. Collected specimens were carefully examined and identified with the help of regional floras (Kirithkar and Basu, 1980). Specimens were further confirmed with reference to herbarium sheet available in the Botanical Survey of India, Suthern Circle, Coimbatore.

Pharmacognostical, phytomicrographs (Esau, 1964), powder preparation (Hardorne, 1973), total ash, water – soluble ash, acid – insoluble ash and sulphated ash (Anonymous, 1996; Kokate, 1994), powder analysis (Kokoshi *et al*., 1985; Chase and Pratt, 1949; Key, 1938; Johansen, 1940), Phytochemical – alkaloids, carbohydrates, tannins and phenols, flavonoides, gum and mucilage, fixed oils and fats, saponins and phytosterol (Kokate, 1994), total terpenoid (Ferguson, 1956), total alkaloids (Ferguson, 1956) were estimated.

Total alkaloids (TA), total terpenoides (TT), total glycoside (TG), alcohol, water and 50% alcohol extracts of the powdered drugs of all four samples were carried out. The chromatograms were observed under UV and visible light. The Rf value of the band can be obtained by using the following formula.

$$Rf = \frac{\text{Distance traveled by substance (cm)}}{\text{Distance traveled the mobile phase (cm)}}$$

The aqueous extract was examined in GC-MS. The antimicrobial activity was carried out by the method of Bauer *et al*. (1996).

RESULTS AND DISCUSSION

Quantitative microscopical analysis of *R. tetraphylla* was carried out as stomatal index, stomatal frequency, vein-islet number, vein termination and palisade radia measured (Table 1). Analytical values of *R. tetraphylla* like total ash, water soluble ash, acid-insoluble ash, sulphated ash solubility in alcohol, water and extractive values of water. Analytical values of leaves as water soluble extractive ash are higher (18.98 %) to total ash (16.45%) and sulphated ash was higher (16.99%) compared to acid insoluble ash (1.40%). Solubility percentage of leaves parts of *R. tetraphylla* in water is higher (16.81%). When compared,

Table 1 : Quantitative microscopic values of *R. tetraphylla* L.

Parameters	*R. tetraphylla* L.
Stomatal index upper surface	33.6-39.8-40
Lower surface	40-42-45
Stomatal frequency upper surface	34.8 – 39.7 – 41.6
Lower surface	42.3 – 45.8 – 48.8
Vein-islet number	20.7 - 23.8 – 25
Vein termination number	22.6 – 30.2 – 34.3
Palisade ratio	12.7 – 15.3 – 18.4

100% alcohol (2.90%) and 50% alcohol (9.08%) were present (Table 2).

Fluorescent analysis of *R. tetraphylla* leaves powders in different chemical reagents showed no major distinguishing feature. The powders showed colour characteristics in both visible light and UV light except in

Table 2 : Analytical values of leaves parts of *R. tetraphylla* L.

Sr. No.	Parameters	Value (%)
1.	Water soluble extractive ash	18.98%
2.	Total ash	16.45%
3.	Sulphate ash	16.99%
4.	Acid insoluble	1.40%
5.	Solubility in alcohol	
	100% alcohol	2.90%
	50% alcohol	9.08%
	water	16.81%

(HNO_3), nitric acid and ferric chloride (Table 3). The extractive value was obtained in water 44.90%, alcohol (25.67%) and other solvents extractive values ranging between 7.44 % to 11.60% (Table 4).

Cold extracts of the plant showed the presence of carbohydrate, alkaloids, tannins and phenols, saponins, flavonoids and absence of fixed oil and gum and mucilages phytosteral (Table 5) and successive extract of leaves

Table 3 : Fluorescent behaviour of dried leaves powder of *R. tetraphylla* L.

Sr. No.	Treatment with chemicals	UV light	Visible light
1.	P + H_2SO_4	Dark green	Dark green
2.	P + HNO_3	Yellowish green	Red
3.	P + HCl	Yellowish green	Yellowish green
4.	P + NH_4OH	Yellowish green	Yellowish green
5.	P + acetic acid	Yellowish green	Dark green
6.	P + Iodine	Yellowish green	Yellowish green
7.	P + $FeCl_3$	Yellowish green	Reddish green
8.	P + Picric acid	Yellowish green	Yellowish green
9.	P + NaOH	Yellowish green	Yellowish green

Table 4 : Successive extraction

Sr. No.	Solvents	Amount (%)
1.	Pet ether	4.74%
2.	Benzene	11.60%
3.	Chloroform	7.44%
4.	Alcohol	25.67%
5.	Water	44.90%

parts are given in Table 6. Quantitative estimation of the alcoholic (100%) extract of leaves parts of plant sample are shown in Table 7. The powder sample shared the total alkaloids content 0.06%, alkaloids fraction was yellowish in colour and had semisolid to oily in consistency. Total terpenoids content of the alcoholic extracts showed -392%, terpenoids was dark green and semisolid.

TLC was run for separation of various compounds in five different solvent extracts of *R. tetraphylla* using BAW, Ferosal, TBA, 60% alcohol and water. Mobile phases, RF values of alcohol, 50% alcohol and water extract of samples are presented in Table 8. The plant water extract was analysed in GC-MS for different components (Table 9 and Fig. 1).

Pharmacological results were obtained in the experiments were subjected to student't' test for their statistical significance (Table 10). The aqueous extract of *R. tetraphylla* leaf produced significant positive ionotropic effects, unaffected by propranalol, B- blocking drug, more affected by nifedipine, the Ca^{2+} channal

Table 5 : Qualitative phytochemical screening (cold extracts) of leaves of *R. tetraphylla* L.

Compound tested	Reagent used	50% alcohol	100% alcohol	H_2O extract
Carbohydrates	Fehling's	+	++	+++
	Molish's	+	++	+++
Alkaloids	Dragendraff's	+	+	++
	Wagner's	+	+	++
	Hager's	+	+	++
	Mayer's	+	+	++
Tannins and phenols	10% Lead acetate	-	-	+
Flavonoides	NaOH + HCl	+	+	+
Gum and mucilage	Alcoholic precipitation	+	-	+
Fixed oil and fats	Spot test	-	-	-
Saponins	Foam test	-	-	+
Phytosterol	LB test	-	-	-

(+++) = Rich amount, (++) = Moderate amount, (+) = Minimum, (-) = absent

Table 6 : Qualitative phytochemical succeeive extracts of leaves of *R. tetraphylla* L.

Compound tested	Reagent used	Pet ethe	Benz	Chloro	Alcoh	Water
Carbohydrates	Fehling's	-	-	-	+	++
	Molish's	-	-	-	+	++
	Dragendraff's	+	-	+	-	-
Alkaloids	Wagner's	-	+	+	-	+
	Hager's	-	-	+	-	-
	Mayer's	+	+	+	-	++
Tannins and phenols	10% Lead acetate	-	-	-	+	+
Flavonoides	NaOH + HCl	-	-	+	+	++
Gum and mucilage	Alcoholic precipitation	-	-	-	-	++
Fixed oil and fats	Spot test	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-
Phytosterol	LB test	+	+	+	-	-

(+++) = Rich amount, (++) = Moderate amount, (+) = Minimum, (-) = absent.

Table 7 : Quantitative estimation of biological compounds in alcoholic extract of *R. tetraphylla* L.

Sr. No.	Compound	Colour and physical nature	Quantity (%)
1.	Total alkaloids	Yellowish-semisolid to oily	0.06%
2.	Total terpenoids	Dark green colour semisolid	-392%

Table 8 : TLC profiles of extract of *R. tetraphylla* L.

Extract	Name of mobile phase (Rf value)				
	BAW	Ferosal	TBA	60% NaOH	H_2O
50% alcohol	0.72	0.92	0.84	0.82	0.74
100% alcohol	0.79	0.94	0.83	0.81	0.70
H_2O	0.40	1.08	0.74	0.96	0.83

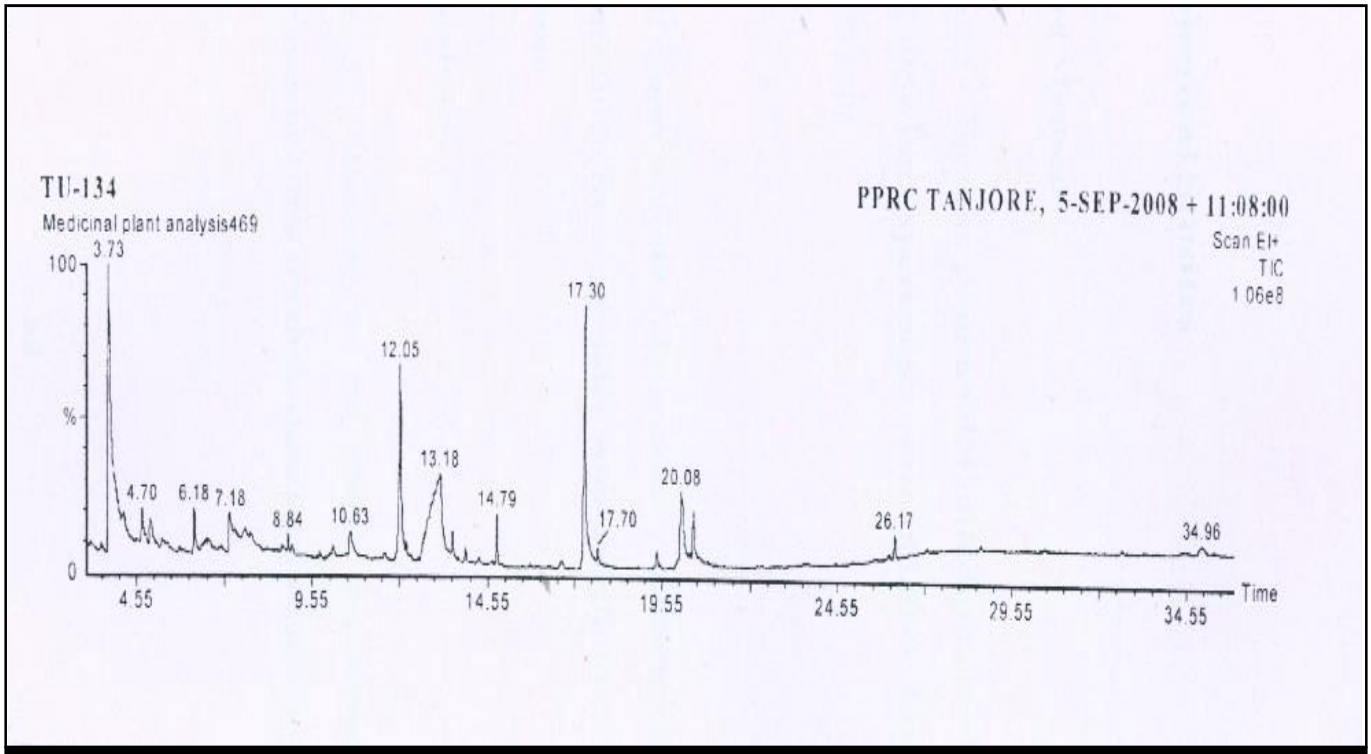

Fig. 1 : GC-MS identified phytocomponents in the extracts of *R. tetraphylla* L.

Table 9 : Phytocomponents identified in the extract of the plant samples

Sr. No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1.	3.73	2-Furancarboxaldehyde 5- methyl-	$C_6H_6O_2$	110	27.09
2.	4.70	3-Hydroxy-piperidine-1- Carboxylic acid, benzyl ester	$C_{13}H_{17}NO_3$	235	1.63
3.	4.93	2,5-Dimethyl-4-hydroxy-3(2H)- Furanone	$C_6H_8O_3$	128	1.81
4.	6.18	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	1.85
5.	7.18	Benzofuran, 2,3-dihydro-	$C_8H8_6O_4$	120	2.75
6.	7.63	1-Deoxy-d-mannitol	$C_6H_{14}O_5$	166	2.99
7.	8.84	Hexahydroindole	$C_8H_{13}N$	123	0.66
8.	9.75	D-Galactose, 6-deoxy-	$C_6H_{12}O_5$	164	0.24
9.	10.14	Carbonic acid, dithio-, O-ethyl S-(2-phenoxyethyl) ester	$C_{11}H_4O_2S_2$	242	0.68
10.	10.63	1,6-Anhydro-β-D-glucopyranose (levoglucosan)	$C_6H_{10}O_5$	162	2.47
11.	12.05	Spiro [1,3-dioxolane-2,2'-[6,7] diazabicyclo[3.2.2]non-6-ene]	$C_9H_{14}N_2O_2$	182	9.45
12.	13.18	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	20.71
13.	14.79	2,3-Bis (methylally) pyrrolidine	$C_{12}H_{21}N$	179	1.67
14.	16.68	Cyclopentaneundecanoic acid, methyl ester	$C1_7H_{32}O_2$	268	0.54
15.	17.30	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	15.13
16.	17.70	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200	1.40
17.	19.40	1,3-propanediol, 2-dodecyl	$C_{15}H_{32}O_2$	244	0.92
18.	20.08	Oleic Acid	$C_{18}H_{34}O_2$	282	5.24
19.	20.43	9,9-Dimethoxybicyclo [3..1]nona- 2,4-dione	$C_{11}H_{16}O_4$	212	1.99
20.	26.17	Phthalic acid, dodecyl 2- methoxyethyl ester	$C_{23}H_{36}O_5$	392	0.78

Table 10 : Effects RT leaf extract on frog heart *in situ* preparation (*=P < 0.05; **=P < 0.01; ***=P < 0.001)

Extract / Drug	Frog Ringer		Frog –Ringer ± Propranolol (10 µgm/ml)		Frog –Ringer ± Nifedipine (10 µgm/ml)		Frog –Ringer ± Atropine (10 µgm/ml)		Remarks
	HR%	FC%	HR%	FC%	HR%	FC%	HR%	FC%	
DX-Digoxin (10 µgm/ml)	80.16 ± 2.25***	303.9 ± 9.27***	-	-	62.63 ± 10.38***	229.96 ± 10.76***	-	-	Cardiotonic activity
ADR-Adrenaline (10 µgm/ml)	160.1 ± 5.915***	268.90 ± 5.26***	112.33 ± 3.18***	225.88 ± 4.63***	-	-	-	-	β-adrenergic activity
TQ-total aqueous extract (1mg/ml)	93.84 ± 1. 54***	164.0 ± 5.27***	94.38 ± 3.10 N S	174.39 ± 6.65 N S	48.78 ± 1. 49***	1484.48 ± 5.24***	95.44 ± 2.25NS	175.48 ± 7.68 N S	Cardiotonic like activity

N.S. = Not significant

blocker, while slightly less negative chronotropic effects unaffected by atropine, thus, suggesting the cardiotonic activity similar to cardiac glycosides. The extract has blood pressure lowering effect and acts as cardiotonic effect.

The antimicrobial activity of aqueous extract of leaf *R. tetraphylla* was tested against some human pathogenic bacteria (*E. coli, Serratia marcescens, Staphylococcus aureus, S. epidermitis* and *Klebsiella pneumoniae*) and fungi (*Aspergillus flavus, A. niger, A. fumigatus, Candida albicans* and *Fusarium indicus*). The extract exhibited positive antimicrobial activity against *E. coli* and *Klebsiella pneumoniae* in bacteria and *Aspergillus*

Table 11: Antibacterial activity of *R. tetraphylla* L.

Sr. No.	Bacterial spp.	Mean zone of inhibition (mm)		
		5 mg	10 mg	20 mg
1.	*Escherichia coli*	07	10	13
2.	*Serratia marcescens*	-	-	-
3.	*Staphylococcus aureus*	-	-	-
4.	*Staphylococcus epidermitis*	-	-	-
5.	*Klebsiella pneumoniae*	06	10	15

Table 12 : Antieungal activity of *R. tetraphylla* L.

Sr. No.	Fungal spp.	Mean zone of inhibition (mm)		
		5 mg	10 mg	20 mg
1.	*Aspergillus flaus*	11	14	17
2.	*Aspergillus niger*	-	-	-
3.	*Aspergillus fumigatus*	-	-	-
4.	*Candida albicans*	-	-	-
5.	*Fusarium indicus*	07	08	11

flavus and *Fusarium indicus* in fungi for 5-20 mg/ml and others had not inhibitory effect (Table 11 and 12).

Ash values and solubility values of *R. tetraphylla* in the present study are like those observed for the same species (Arokia doss, 2008). In *R. tetraphylla* TLC method has been described for the detection of various compounds. The method employed can be used for standardization of the drug (Thank and Radhika, 1997). similarly, GC-MS analysis of aqueous extract of *R. tetraphylla* was qualitatively and quantitatively analysed (Culea *et al*., 2003).

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