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Full Length Article

Phytochemical Screening and Antimicrobial Activity of Leaf, Stem, Root and their Callus Extracts in *Rauwolfia tetraphylla*

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Abstract

Phytochemical screening and Antimicrobial studies were carried out in a medicinally important plant species, *Rauwolfia tetraphylla.* The aim of the present study was to screen leaf, stem and root extracts and their callus (NaCl stressed & unstressed) extracts of *R. tetraphylla* (24 months old) for the qualitative analysis of phytochemicals like alkaloids, flavonoids, saponins, tannins and gums and mucilages and to study antibacterial and antifungal activities in these extracts. Phytochemical screening revealed that Alkaloids are major phytochemicals in leaf, stem and root extracts of *R. tetraphylla* (24 months old). In antimicrobial activity studies, maximum bacterial growth inhibition zone (25.0 ± 2.4 mm) was observed in methanol based leaf extracts against *Staphylococcus aureus* and maximum fungal growth inhibition zone (22.0 ± 2.1 mm) was observed in *Fusarium oxysporum* in methanol based root extracts. In NaCl stressed callus extracts both antibacterial and anti-fungal activities were observed but the results were not comparable to extracts of *in vivo* plant based leaf, stem and roots. © 2016 Friends Science Publishers

Keywords: Phytochemical Screening; Antimicrobial Activity; Callus Extracts; *Rauwolfia tetraphylla*

Introduction

Since the existence of human life on the planet earth, plants have been the source of food, clothing, shelter, fiber, fuel and medicine. Since time immemorial plants are the principle raw materials of traditional medicinal system that has been practiced and continue to provide mankind with novel therapies (Cragg and Newmann, 2005). In Plants secondary metabolites are stored in different organs like root, leaf, bark, flower, seed etc. Since roots and other underground plant parts like rhizome, tubers etc; are the principle sites for secondary metabolite storage, indiscriminate collection of these plant parts for usage as raw material in preparation of pharmaceutical products has resulted in endangering of plants.

The family Apocyanaceae consists of about 250 genera and 2000 species of tropical trees, shrubs and vines (Ng, 2006). Important feature of this family is that most of the species produce milky sap. In traditional medicine, this family species are used to treat gastrointestinal ailments (Wiart, 2006). The roots, leaves and latex of this family species are used to treat skin prolems and liver disorders, worms, ulcer problems, tumors and ear aches (Rajakaruna *et al.,* 2002). Important plant species in this family are *Thevetia peruviana*, *Allamanda cathartica*, *Nerium oleander*, *Catharanthus roseus*, *Rauwolfia serpentina* etc. *Rauwolfia* species are very much important due to their traditional medicinal use such as insanity, scorpion bite, purgative, edema, snake sedative, antihelmintic, rheumatic pain, epilepsy, relief cough antidiarrheal and intestinal disease (Panda *et al.,* 2012).

Rauwolfia is an important genus of the family Apocynaceae, has a unique position as it is a source of therapeutically significant alkaloids, this fact was first pointed by Greshoff in 1890. The discovery of the genus *Rauwolfia* dates back to the 16th century and up to now approximately 130 species of *Rauwolfia* have been described which are found in most tropical and semi-tropical regions of both northern and southern hemispheres. In 1703 Charles Plumier named the genus *Rauwolfia* in the honour of Rauwolf Leonhard (1535–1596), a German botanist for exploring the potential of the plant as drug.

Among the species of *Rauwolfia*, (i) *R. serpentina* Benth, (ii) *R. tetraphylla* Linn and (iii) *R. vomitoria* Afzuelia have the commercial importance, and presently they are as used as the best source of therapeutically active antihypertensive alkaloids. Dried roots of *Rauwolfia* genus of Apocynaceae family is used for centuries for the treatment of insomnia and anxiety (Vakil *et al.,* 1949), psychotic, insanity, epilepsy, sleeplessness and several other ailments (Ojha *et al.,* 1985). The root extract of this plant is also used to hasten expulsion of the fetus, diarrhea etc.

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(Tona *et al.,* 1999), dysentery, cholera and colic (Ghani, 1998).

Among the related species, the most important in terms of pharmaceutical application is wild snake root plant, known as *Rauwolfia tetraphylla.* This species is been known by different synonyms (Jyothi *et al.,* 2012): *R. canescens, R. heterohylla, R. hirsuta, R. latifoloia, R. odontophora, R. subpubescens* and *R. tomentosa*. In India it is called by several local names (Table 1).

Stoll *et al.* (1955) named wild snake root plant as *R. tetraphylla*, because of the presence of four leaves at each node, which is a characteristic feature of this species. *R. tetraphylla* is regarded as a rich source of different types of important alkaloid constituents such as reserpine, deserpidine, reserpiline, raujemidine, isoreserpiline, ajmaline, ajmalicine, yohimbines, serpentine, tetraphylline, sarpagine and vellosimine (Haack *et al.,* 1954; Mukherjee, 1961;). Roots of *R. tetraphylla* are used as sedative, used for treating the blood pressure; used for the treatment of mentalness, hypochondria and disorders of the central nervous system (Pullaiah, 2002). Root extracts are also used in treatment of diarrhea, colic, dysentery, cholera, and fever (Gupta, 1989) and valuable for intestinal troubles (Tona *et al.,* 1999).

The roots also used for anti-inflammatory, syphilis, tranquilizing actions diuretic, stomatitis, expectorant, swelling, used in toothache, fever, malaria, ulcer, gingivitis, sore throat, and nervousness (Panda *et al.,* 2012). *R. tetraphyll*a alkaloids which are in use of cardiovascular and psychiatric treatments (Anitha and Kumari, 2006), alkaloids has anti-hypertensive activity (Faisal *et al.,* 2005; Rohela *et al.,* 2013). The root is also used to promote uterine contraction and it is recommended for use during child birth in difficult ones (Villar *et al.,* 1998; Tona *et al.,* 1999).

Besides the above uses, the plant parts of *R. tetraphylla* in combination with other natural products can also be employed in medicinal uses. Fruits yield black dye is applied to skin ailments (The useful plants of India, Ramchandran and Kashyapa, 1986). Root paste along with orange peel is used against fever; leaf juice is used against piles and against sterility in women (Yusuf *et al.,* 2009).

In view of all the medicinal applications of this important plant species (*R. tetraphylla*), we have screened most of the phytochemical compounds present in leaf, stem, root and their callus extracts in different solvent systems and tested antimicrobial activity of all these extracts against different bacterial and fungal species.

Materials and Methods

Rauwolfia tetraphylla plants were procured from Forest Department, Warangal, Andhra Pradesh, India in January, 2009. They were maintained in the medicinal arboretum of the Department of Biotechnology, Kakatiya University, Warangal, Andhra Pradesh, and were used in the present investigation.

Bacterial cultures: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia* were obtained from the Department of Microbiology, Kakatiya University, Warangal, Telangana, India and were used in the present study.

Fungal cultures: *Aspergillus niger*, *Pencillium* sp, *Candida albicans* and *Fusarium oxysporum* were obtained from the Department of Microbiology, Kakatiya University, Warangal, Telangana, India and was used in the present study.

Extract Preparation for Phytochemical and Antimicrobial Analysis

The plant material (leaf, stem and roots) of *R. tetraphylla* were allowed to dry and grinded by using mortar and pestle. Five to ten (5−10) grams of grinded material was dissolved in 50−100 mL of solvent (Petroleum ether, Benzene, Chloroform and Methanol) and kept overnight in an orbital shaker at 100 rpm and 28ºC. The extracts were then filtered with Whattman No. 42 filter paper (125 mm) and the resulting extract filtrate was used for further investigation.

Methods for Phytochemical Analysis

Test for alkaloids: Measured with the method of Salehi *et al.* (1992). Three mL of each extract was allowed to dry and it was heated on a boiling water bath with 5 mL of 2N HCl. The mixture was cooled and filtered and the resultant filtrate was subdivided into two parts. To one of them with, few drops of Mayer's reagent (Potassiomercuric iodide solution) was added and to the other same amount of Wagner's reagent (Rizk, 1982) was added. The Samples were then observed for the formation of turbidity. Positive score was recorded for presence of compound; negative score was recorded for the absence of compound.

Test for flavonoids: Measured with the method of Somolenskin *et al.* (1972). Five mL of each extract was treated with few mL of concentrated hydrochloric acid and turnings of magnesium (0.5 g). Flavonoids presence is confirmed by the appearance of pink color.

Test for phytosterols: One g of the extract was treated with few drops of glacial acetic acid; followed by 3 mL of acetic anhydride, and at last few mL of concentrated sulfuric acid was added. Green color formation is the positive test (Ling and Jones, 1955).

Test for saponins: Determined following the method of Segleman and Farnsworth (1969), 2–3 g of the dry sample was heated with boiling water for 10 min. After incubation time it was cooled and shaken continuously to form the froth and then left it to stand for 10–15 min. Presence of froth in less amounts is a negative result, and more amount of froth formation is a positive result.

Test for tannins and phenolic compounds: Following the method of Kapoor *et al.* (1969), 10 mL of extract was allowed to dry and later treated with10 mL of heated0.7% NaCl solution. It was subdivided into three portions. To

one of the portion of the test extract 1% NaCl solution was added, gelatin salt was added to another portion; precipitation in the second one indicates the presence of tannins. Addition of $FeCl_3$ solution to the extract and gives green or blue green colored precipitate, which is a positive result.

Test for gums and mucilage: About 5–8 mL of different extracts was taken and added separately in 15–20 mL of 100% alcohol with continuous mixing and then later on filtered. The resultant precipitate was allowed to dry and examined it for swelling properties which indicates presence of gums and mucilages (Amelia *et al.,* 2011).

Antibacterial Assay

Antibacterial activity was determined by using agar diffusion method (Bauer *et al.,* 1966; Wilkins *et al.,* 1972). Nutrient Agar Medium (NAM) (Green and Larks, 1955) (Table 2) was spread with bacterial cells on solid media containing petriplate by a spreader. Then wells were formed by using sterile borer followed by addition of 100 µL of test samples (Petroleum ether, Chloroform, Methanol, Benzene, and Aqueous leaf, stem, root and their callus extracts) including controls (pure solvents) in each well (6mm diameter). The petriplates were incubated for 24 h at 37˚C. Sterile water was used as a control. At the end of incubation period, inhibition zone was measured surrounding each of the well loaded with specific solvent extract. Three replicates were maintained to calculate average of zone of inhibition.

Antifungal Assay

For the detection of antifungal activity, Potato Dextrose Agar medium (Table 3) (Japanese Pharmacopoeia, 2007) containing plates were inoculated with fungal organisms. Then wells were formed by using sterile borer followed by addition of 100 µL of test samples (petroleum ether, chloroform, benzene, methanol and aqueous leaf, stem, root and their callus extracts) including controls (pure solvents) in each well (6 mm diameter). Incubation of plates for 5 days at 25ºC. Sterile water was used as a control. At the end of incubation period, inhibition zone was measured surrounding each of the well loaded with specific solvent extract. Three replicates were maintained to calculate average of zone of inhibition.

Results

Phytochemical Screening

Phytochemical screening of leaf, stem and root extracts (Fig. 1) and their callus (NaCl stressed) extracts of *R. tetraphylla* (24 months old) is given in Table 4. Alkaloids, tannins and phenolic compounds are observed in leaf, stem and root extracts, while alkaloids are observed only in root callus extracts. Flavonoids are present in both stem and leaf extracts, while they are observed only in leaf callus extracts. Phytosterols are observed in both leaf and root extracts, while none of the callus extracts possess phytosterols. Saponins were not observed neither in root, stem and leaf extracts nor in their callus extracts. Gums and mucilages were observed only in root, stem and leaf based callus extracts.

Tannins, phenolic compounds and gums and mucilages are extracted in all the solvent systems, while alkaloids, flavonoids and phytosterols were extracted in all the solvent extracts, except aqueous extracts.

Table 1: List of vernacular names of *R. tetraphylla* (www.flowersofindia.net)

Vernacular Names	
English	Wild snake root, Devil pepper, be still tree, American serpent wood, Milk bush.
Sanskrit	Vanasarpagandha; Sarpanasini
Hindi	Barachandrika; Chandrabhaga
Telugu	Papataku
Tamil	Pampukaalachchedi
Malayalam	Pampumkolli; Kattamalpori
Kannada	Dodda Chandrike
Oriya	Patalagarudi
Bengali	Barchandrika, Gandhanakuli

Table 2: Composition of Nutrient Agar Medium (NAM)

Ingredients	Composition (g/L)
Peptone	5
beef extract/yeast extract	3
NaCl	5
Agar	15
pH	7

Table 3: Composition of Potato Dextrose Agar (PDA) Media

Ingredients	Composition (g/L)
Potato	4.0
Dextrose	20.0
Agar	15.0
pH	5.6

Antimicrobial Activity

Antibacterial: Antibacterial activity of leaf, stem and root extracts is given in (Fig. 2, 3 and 4) and their callus (NaCl stressed) extracts in (Fig. 5, 6 and 7). Compared to leaf, stem and root extracts their callus (NaCl stressed) extracts has shown less antibacterial activity. Among all the solvent extracts tested, methanol based leaf stem and root extracts showed maximum antibacterial activity against *S. aureus.*

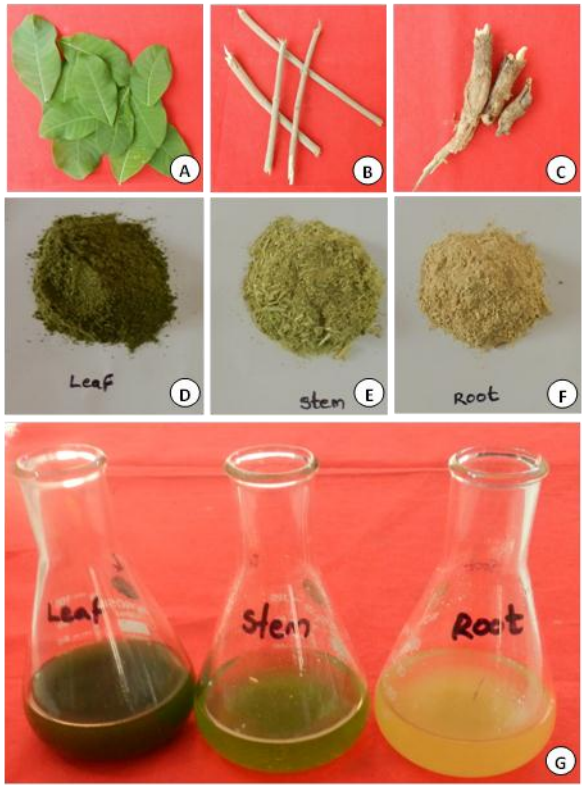

Fig. 1: Phytochemical screening of leaf, stem and root parts of *R. tetraphylla* (24 months old). (A), (B) & (C) Leaf, stem and root parts of *R. tetraphylla* (D), (E) & (F) Powdered leaf, stem and root of *R. tetraphylla* (G) Methanolic extracts of leaf, stem and root of *R. tetraphylla*

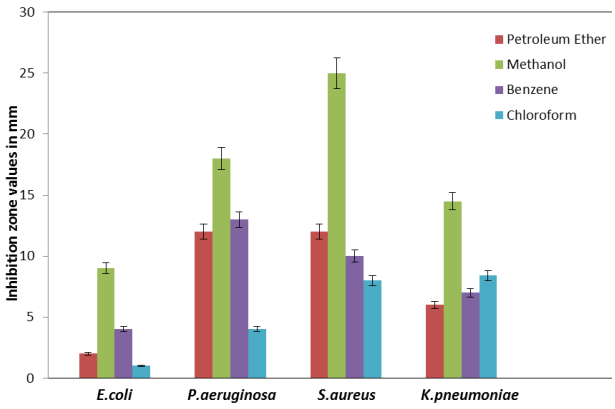

Fig. 2: Antibacterial activity of leaf extracts of *R. tetraphylla* in different Solvent systems, measured by average inhibition zone values in mm

Maximum bacterial growth inhibition zone (25.0 ± 2.4 mm) was observed in methanol based leaf extracts against *S. aureus* (Fig. 8) and least inhibition zone (0.1±0.02 mm) is observed in all the solvent extracts against *E. coli*. In unstressed callus extracts, bacterial growth inhibition was not observed.

Antifungal: Antifungal activity of leaf, stem and root extracts of *R. tetraphylla* (24 months old) is given in (Fig. 9, 10 and 11). Antifungal activity of leaf, stem & root callus (NaCl stressed) extracts is given in (Fig. 12, 13 and 14). Maximum inhibition zone (22.0 ± 2.1 mm) was observed in *F. oxysporum* in methanol based root extracts. Similarly, maximum inhibition zone (8.4± 2.1 mm) was observed in *F. oxysporum* in methanol based root callus (NaCl stressed) extracts.

Discussion

In this study, for phytochemical screening of leaf, stem and root extracts and their callus (NaCl stressed) extracts of *R. tetraphylla* (24 months old), alkaloids are the major phytochemicals. Maximum inhibition zone against *S. aureus* and *F. oxysporum* was observed in leaf and root extracts of *R. tetraphylla*, respectively.

Phytochemical screening has been reported in *R. serpentina* (Harisharanraj *et al.*, 2009; Panda *et al.*, 2012) and in *R. tetraphylla* (Kumar *et al.*, 2010; De Britto *et al.*, 2011). Plants are the natural sources of antimicrobial compounds like phenols, polyphenols, terpenoids, sesquiterpenes, flavonoids etc (Bagde *et al.*, 2013). Screening of plant extracts with antimicrobial activity represents a continuous effort to find the novel compounds with potential to act against antibiotic resistant bacteria and fungi (Doss *et al.*, 2011), because medicinal plants secrete secondary metabolites of varied structural configuration (Mazid *et al.*, 2011).

Tannins have been used in medicinal formulations for treatment of sprains, bruises and external injuries. It is well known that *R. tetraphylla* root extract possess several alkaloids like reserpine, recinnamine, ajmalicine, serpentine, yohimbine deserpidine, raujemidine, reserpinine, Isoreserpinine, tetraphylline, tetraphyllicine etc (Iqbal *et al.*, 2013). Therefore traditionally they are used for the treatment of various diseases (Hasan *et al.*, 2004; Usman and Osuji, 2007).

Methanolic root extracts of *R. tetraphylla* showed maximum activity against *S. aureus*. Similar results were reported in *R. tetraphylla* (Suresh *et al.*, 2008), (Kumar *et al.*, 2010) and (Rao *et al.*, 2012), has revealed *in vitro* antibacterial activities of root bark extract in *R. tetraphylla*.

Methanolic root callus extracts (NaCl unstressed) callus didn't show any antimicrobial activity. However, methanolic root based callus extracts (NaCl stressed) exhibited antimicrobial activity. Shariff *et al.* (2006), reported antimicrobial activity of leaf and callus extracts of *R. tetraphylla* and *Physalis minima.*

Varuna *et al.* (2010), reported that *Acyranthus aspera* ethanolic extracts of root, leaf and stem showed antibacterial and antifungal activity. Clinical microbiologists are involved in screening the leaf, stem and root extracts for phytochemicals with antibacterial and antifungal activities, with the purpose of identifying new phytochemical drugs

Table 4: Phytochemical screening in roots, stem and leaf extracts and their NaCl stressed callus extracts of *R. tetraphylla*in different solvent systems

Phytochemical Constituents	Petroleum Ether Extract						Benzene Extract						Chloroform Extract						Methanol Extract						Aqueous Extract					
	Tissue			Callus			Tissue			Callus			Tissue			Callus			Tissue			Callus			Tissue			Callus		
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L
Alkaloids	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Flavonoids	-	+	+	-	-	+	-	+	+	-	-	+	-	+	+	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-
Phytosterols	+	-	+	-	-	-	+	-	+	-	-	-	+	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tannins and Phenolic Compounds	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-
Gum and Mucilage	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+

R=Root; S=Stem; L=Leaf; + indicates presence and – indicates absence

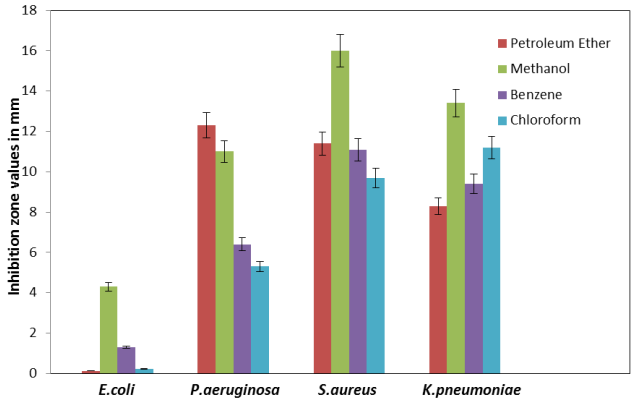

Fig. 3: Antibacterial activity of stem extracts of *R. tetraphylla* in different solvent systems, measured by average inhibition zone values in mm

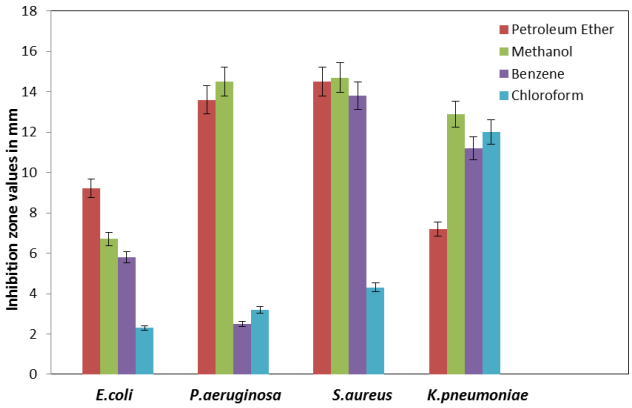

Fig. 4: Antibacterial activity of root extracts of *R. tetraphylla* in different solvent systems, measured by average inhibition zone values in mm

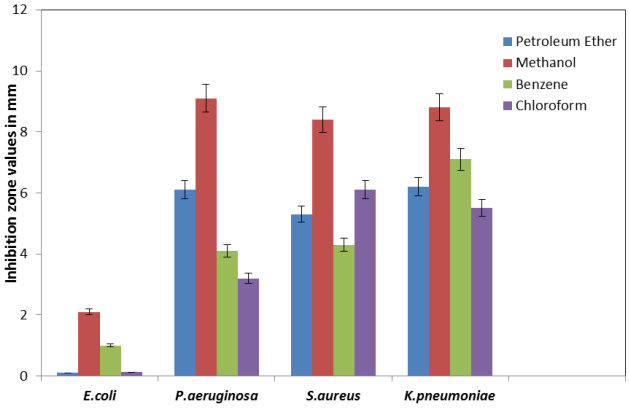

Fig. 5: Antibacterial activity of NaCl stressed leaf callus extracts of *R. tetraphylla* in different solvent systems, measured by average inhibition zone values in mm

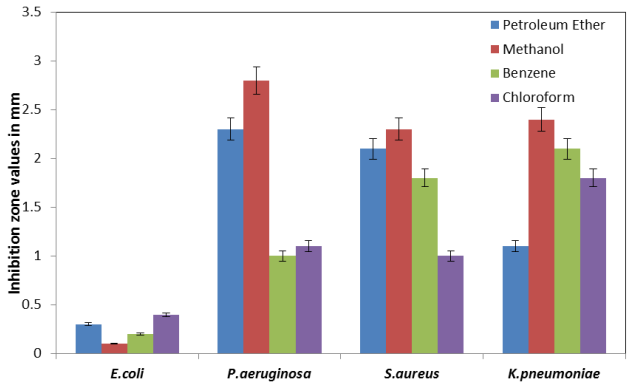

Fig. 6: Antibacterial activity of NaCl stressed stem callus extracts of *R. tetraphylla* in different solvent systems, measured by average inhibition zone values in mm

with potential to act against antibiotic resistant bacteria and fungi (Doss *et al.*, 2011).

Rauwolfia species are used by folk healers for treating chronic as well as infectious diseases (Deshmukh *et al.*, 2012). Leaf, stem, root etc collected from *Rauwolfia* species growing in the wild are used as raw material in many herbal formulations (Panda *et al.*, 2012). In India the roots of *R. serpentina* were used by ayurvedic and other practioners for treating mental illness and snakebite, hence it was called as snake root or insanity herb (Srivastava *et al.*, 2006).

R. tetraphylla leaf extract has been used in the treatment of fever, cholera and eye diseases. It is used as an

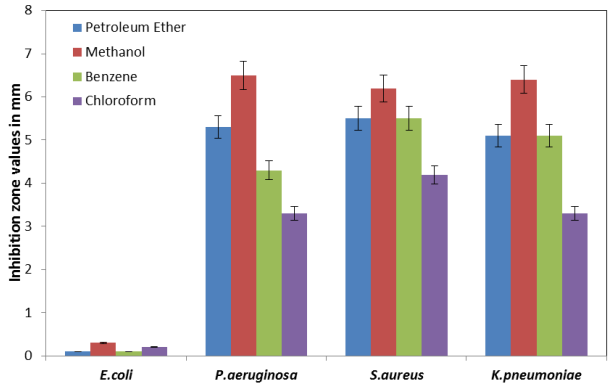

Fig. 7: Antibacterial activity of NaCl stressed root callus extracts of *R. tetraphylla* in different solvent systems, measured by average inhibition zone values in mm

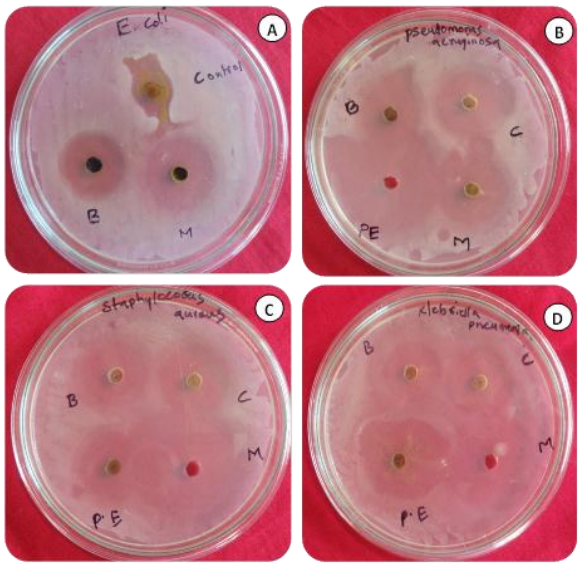

Fig. 8: Antibacterial activity of leaf extracts of *R. tetraphylla* in different solvent systems against (A) *Escherichia coli* (B) *Pseudomonas aeruginosa* (C) *Staphylococcus aureus* (D) *Klebsiella pneumoniae*

antihypertensive as well as for intestinal disorders. *R. tetraphylla* contains a number of bioactive phytochemicals (alkaloids) like reserpine in roots, yohimbine in the leaves (Kumar *et al.*, 2011).

Conclusion

Alkaloids are major phytochemicals in leaf, stem and root extracts of *R. tetraphylla* (24 months old). Antibacterial and antifungal activities were expressed in leaf, stem and root extracts of *R. tetraphylla* (24 months old) and their activities in leaf, stem and root callus (NaCl stressed) was not comparable to natural extracts.

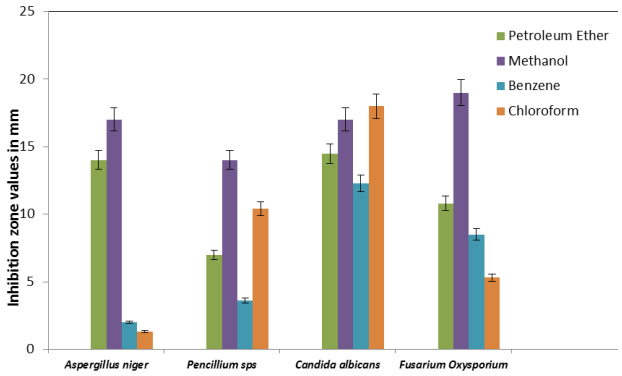

Fig. 9: Antifungal activity of leaf extracts of *R. tetraphylla* in different solvent systems, measured by average inhibition zone values in mm

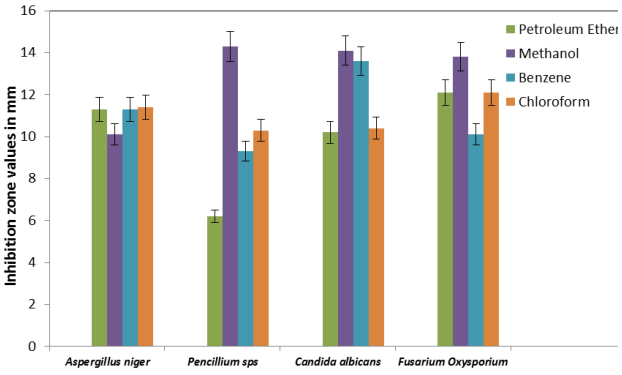

Fig. 10: Antifungal activity of stem extracts of *R. tetraphylla* in different solvent systems, measured by average inhibition zone values in mm

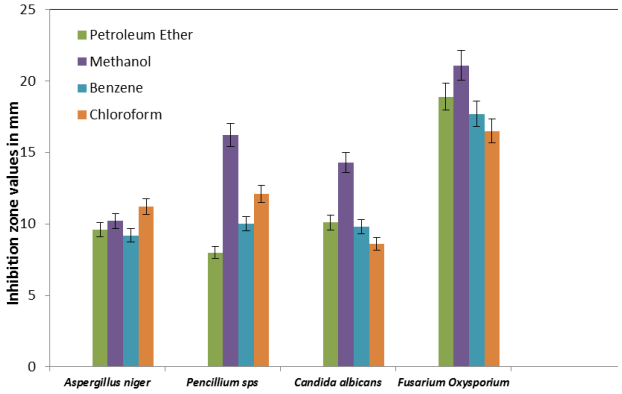

Fig. 11: Antifungal activity of root extracts of *R. tetraphylla* in different solvent systems, measured by average inhibition zone values in mm

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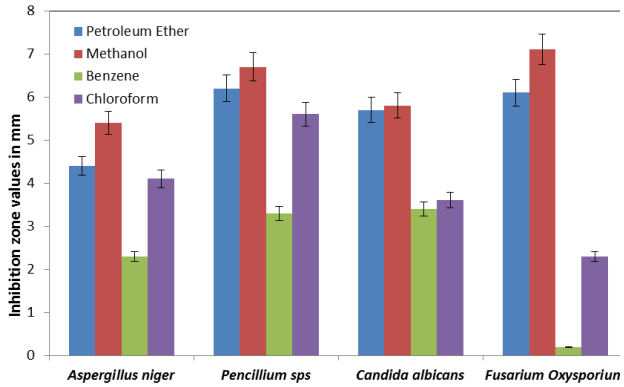

Fig. 12: Antifungal activity of NaCl stressed leaf callus extracts of *R. tetraphylla* in different solvent systems, measured by average inhibition zone values in mm

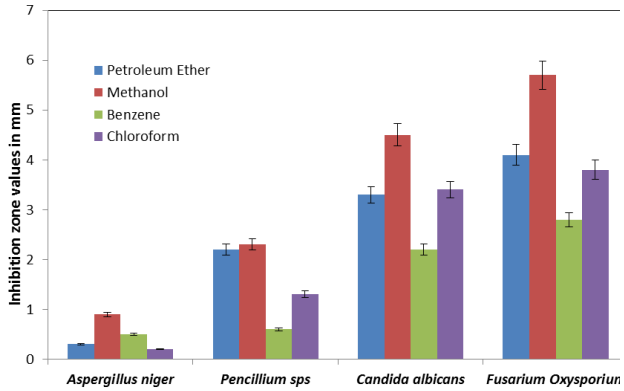

Fig. 13: Antifungal activity of NaCl stressed stem callus extracts of *R. tetraphylla* in different solvent systems, measured by average inhibition zone values in mm

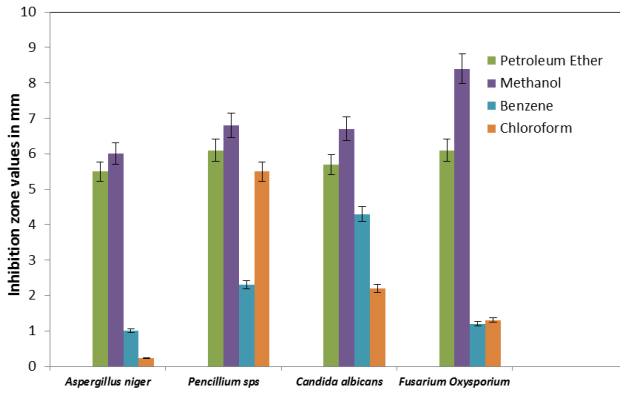

Fig. 14: Antifungal activity of NaCl stressed root callus extracts of *R. tetraphylla* in different solvent systems, measured by average inhibition zone values in mm

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References

Amelia, M., A. Rakesh, R. Dash Shilpa and N. Shrotriya, 2011. Recent investigations of plant based natural gums, mucilages and resins in novel drug delivery systems. *Ind. J. Pharm. Edu. Res.*, 45: 86–99

Anitha, S. and B.D.R. Kumari, 2006b. Reserpine accumulation in NaCl treated calli of *Rauvolfia tetraphylla Life Sci. Asia*, 32: 417–419

Bagde, S., M. Khare, R.K. Patidar and V. Singh, 2013. Antimicrobial Properties and Characterization of Phytoconstituents of the Leaf Extracts of Some Medicinal Plants. *J. Pharmacognosy Phytochem.*, 1: 127–132

Bauer, A.W., W. Kirby, J.C. Sheriss, and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. Clinic. Pathol*., 45:493–495

Cragg, G.M. and D.J. Newman, 2005. Biodiversity: A continuing source of novel drug leads. *Pure Appl. Chem.*, 7: 7–24

Doss, A., V. Parivuguna, M. Vijayasanthi and S. Surendran, 2011. Antibacterial evaluation and phytochemical analysis of *Medicago sativa* L. against some microbial pathogens. *Ind. J. Sci. Technol*., 4: 550–552

De Britto, J.A., S. SteenaRoshan and R.M. Sujin, 2011. Phytochemical and antibacterial screening of seven apocynaceae species against human pathogens. *Int. J. Pharm. Pharmaceut. Sci.*, 3: 278–281

Deshmukh, S.R., Dhanashree S. Ashrit and Bhausaheb A Patil, 2012. Extraction and evaluation of indole alkaloids from *Rauwolfia serpentine* for their antimicrobial and anti proliferative activities. *Int. J. Pharm. Pharmaceut. Sci*., 4: 329–334

Faisal, M., N. Ahmad and M. Anis, 2005. Shoot multiplication in *Rauvolfia tetraphylla* L. using thidiazuron. *Plant Cell Tiss. Org. Cult.*, 80: 187–190

Ghani, A., 1998. *Monographs in Medical Plants of Bangladesh*, 2nd edition, p: 276. Chemical Constituents and Uses, Asiatic, Soc. Bangladesh

Green, R.A. and G.G. Larks, 1955. A quick method for the detection of gelatin liquefying bacteria. *J. Bacteriol*., 69: 224

Greshoff, M., 1890. *Ber. Dtsch. Chem. Ges.*, 23: 35–37

Gupta, R., 1989. Genetic resources of medicinal plants. *Ind. J. Plant Genet. Resour*., 1: 98–102

Haack, E., A. Popekak, H. Springler and F. Kaiser, 1954. Isolation of yohimbine and serpentine from *Rauwolfia canescens. Naturwissenshaften*, 41: 479–482

Harisharanraj, R., K. Suresh and S. Saravanababu, 2009. Rapid Clonal Propagation *Rauvolfia tetraphylla* L. *Acad. J. Plant Sci*., 2: 195–198

Hasan, M.M., A.O. Oyewale, J.O. Amupitan, M.S. Abdullahi and E.M. Okonkwo, 2004. Preliminary phytochemical and antibacterial investigation of crude extracts of the root bark of *Detarium microcarpum. J. Chem. Soc. Nig*., 29: 26–29

Iqbal, A.M., A. Firoz, K. Khan and M. Khan, 2013. Ethno-Phyto-Pharmacological Overview on *Rauwolfia tetraphylla L. Int. J. Pharm. Phytopharmacol. Res.*, 2: 247–251

Japanese Pharmacopoeia, Society of Japanese Pharmacopoeia, 2007. Amended Chapters 35.1, 35.2, 7. The Minister of Health, Labor, and Welfare

Jyothi, T., K. Brijesh Hari and K.R. Venkatesh, 2012. Pharmacognostic evaluation of *Rauwolfia tetraphylla* L. *J. Pharmaceut. Sci. Innovation*, 1: 57–60

Kapoor, L.D., A. Singh, L. Kapoor and S.N. Srinivastava, 1969. Survey of Indian plants for saponins, alkaloids and flavonoids. *Lloydia*, 32: 297–304

Kumar, H.K., K.K. Kullatti, P. Sharanappa and P. Sharma, 2010. Comparative antimicrobial activity and TLC-bioautographic analysis of root and aerial parts of *Andrographis serphyllifolia*. *Int. J. Pharmacy Pharmaceutical Sci.*, 2: 52–54

Kumar, A.C., S. Bindu, C.R. Chitra and P.J. Mathew, 2011. Taxonomic significance of fruit and seed morphology in identification of South Indian *Rauwolfia*. *Rheedea*, 21: 160–166

Ling, W.H. and P.J.H. Jones, 1955. Dietary phytosterols: a review of metabolism, benefits and side effects. *Life Sci.*, 57: 195–206

Mazid, M., T.A. Khan and F. Mohammad, 2011. Role of secondary metabolites in defense mechanisms of plants. *Biol. Med.*, 3: 232–249

Mukherjee, A.J., 1961. Alkaloids of *Rauwolfia canescens. J. Ind. Chem. Soc.*, 18: 33–35

Ng, F.S.P., 2006. *Tropical Horticulture and Gardening: Clearwater Publications*. Kuala Lumpur, Malaysia

Ojha, J., U. Mishra and D. Nighantuh, 1985. Dhanvantari Nighantuh, With Hindi Translation and Commentary, 1st edition, p: 204. Dept. of Dravyaguna Institute of Medical Sciences, BHU, Varanasi, Malaysia

Panda, S.K., D. Debajoyti N. Bichitra and T.L. Nayak, 2012. Phyto-pharmacognostical studies and quantitative determination of reserpine in different parts of *Rauwolfia* (Spp) of Eastern Odisha by UV Spectrocsopy Method. *Asian J. Plant Sci. Res*., 2: 151–162

Pullaiah, T., 2002. *Medicinal Plants in India*, pp: 441–443. Regency Publications, New Delhi, India

Ramchandran, K.R. and Kashyapa, 1986. *The Useful Plants of India*, pp: 516–517. Ramesh, C.K. (ed.). Publications and Information Directorate, CSIR, New Delhi, India

Rajakaruna, K.M., H.K. Sainju and G.D. Bhatta, 2002. *In vitro* culture of *Rauwolfia serpentine* L. benth. ex. Kurz. *Proceedings of the Nepal Japan Joint Symposium*, pp: 232–234

Rao, G.B., P. Umamaheshwara Rao, E. Sambasiva Rao, T. Mallikarjuna Rao and V.S. Pranitha, 2012. Evaluation of *in vitro* antibacterial activity and anti-inflammatory activity for different extracts of *Rauwolfia tetraphylla* L. root bark. *Asian Pacific J. Trop. Biomed.*, 2: 818–821

Rohela, G.K., P. Bylla, S. Kota, S. Abbagani, R. Chithakari and T. Christopher Reuben, 2013. *In vitro* plantlet regeneration from leaf and stem calluses of *Rauwolfia tetraphylla* (*R. canescens*) and Confirmation of Genetic Fidelity of Plantlets Using the ISSR-PCR Method. *J. Herbs Spices Med. Plants*, 19: 66–75

Rizk, A.M., 1982. Constituents of Plants Growing in Qatar: A Chemical Survey of Sixty Plants. *Fiototerapia*, 52: 35–44

Salehi, S.M.H., Y. Aynehchi, G.H. Amin and Z. Mahmoodi, 1992. Survey of Iranian plants for saponins, alkaloids, flavonoids and tannins IV. *Daru*, 2: 281–291

Segleman, A.B. and N.R. Farnsworth, 1969. Biological and Photochemical Screening of Plants IV. A New Rapid Procedure for the Simultaneous Determination of Saponins and Tannins. *Lloydia*, 21: 53–56

Shariff, N.S., M.S. Sudharshana, S. Umesha and P. Hari Prasad, 2006. Antimicrobial activity of *Rauwolfia tetraphylla* and *Physalis minima* leaf and callus extracts. *Afr. J. Biotechnol.*, 5: 946–950

Somolenskin, S.J., H. Slilins and N.R. Farnsworth, 1972. Alkaloid screening. *Llyodia*, 35: 1–34

Srivastava, A., A.K. Tripathi, R. Pandey, R.K. Verma and M.M. Gupta, 2006. Quantitative determination of reserpine, ajmaline and ajmalicine in *Rauwolfia serpentina* by reversed-phase high-performance liquid chromatography. *J. Chromatogr. Sci.*, 44: 557–560

Stoll, A., A. Hofmann and R. Brunner, 1955. Alkaloideaus den Blatternvo *Rauvolfia tetraphylla*. *Helvetica Chim. Acta*, 38: 270

Suresh, K., S. SaravanaBabu and R. Harisaranraj, 2008. Studies on *in vitro* antimicrobial activity of ethanol extract of *Rauwolfia tetraphylla*. *Ethnobot. Leaflets*, 12: 586–590

Tona, L., K. Kambu, K. Mesia, K. Cimanga, S. Apers, T. De Bruyne, L. Pieters, J. Totte and A.J. Vlietinck, 1999. Biological screening of traditional preparations from medicinal plants used as antidiarrheal in Kinshasa, Congo. *Phytomedicine*, 6: 59–66

Usman, H. and J.C. Osuji, 2007. Phytochemical and in vitro anti microbialassay of the leaf extract of *Newbouldialeavis. Afr. J. Trad. CAM*, 4: 476–480

Vakil, R.J., 1949. A clinical trial of *Rauwolfia serpentina* in essential hypertension: *Brit. Heart J.*, 9: 350

Varuna, K.M., M.U. Khan and P.K. Sharma, 2010. Review on *Achyranthes aspera*. *J. Pharm. Res.*, 3: 714–717

Villar, R., J.M. Calleja, C. Morales and A. Caceres, 1998. Screening of Guatemalan medicinal Plants for platelet antisegregant activity. *Phytol. Res.*, 11: 441–445

Wiart, C., 2006. *Medicinal Plants of Asia and the Pacific*. CRC Press, Taylor & Francis, Boca Raton, Florida, USA

Wilkins, T.D., J.J. Holdeman, J.J. Abramson and W.E.C. Moore. 1972. Standardized single-disc method for antibiotic susceptibility testing of anaerobic bacteria. *Antimicrob. Agents Chemother*., 1: 451–455

Yusuf, T.E., S. Ho and D.A. Pavey, 2009. Retrospective analysis of the utility of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) in pancreatic masses, using a 22-gauge or 25-gauge needle system: a multicenter experience. *Endoscopy*, 41: 445–448

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