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ANTIMICROBIAL POTENCY OF *RAUVOLFIA TETRAPHYLLA* AND *JATROPHA CURCAS*

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In-vitro antibacterial activity of aqueous, alcoholic, and chloroform extracts of leaves of *Rauvolfia tetraphylla* L. and *Jatropha curcas* L. against various bacteria along with their photochemical analysis was studied. The antibacterial activities of different extracts were evaluated by disc diffusion assay with standard antibacterial drugs as a positive control in the present study. Methanol extract of both plants have shown good and comparable antimicrobial activity against most gram positive and gram negative bacteria tested which was in the range of except against S. typhimurim and E. Coli . Chloroform extract of Rauvolfia tetraphylla was also effective against majority of bacteria (12.00± 0.58 to 14.67± 0.88 mm) but Chloroform extract of Jatropha curcas had activity against S. aureus, K. pneumonia and P. vulgaris only with zone of inhibition in the range of (12.67± 1.20 to 14.33± 2.03 mm). Water extracts of both the plants did not show any antibacterial activity. Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, saponins, diterpenes, triterpenes, and carbohydrates in the methanol extracts of both the plants. The potency of the crude extracts of Rauvolfia tetraphylla and Jatropha *curcas* to inhibit the growth of bacteria is an indication of its antimicrobial potential which may be useful to explore its efficacy against bacterial infections.

Keywords: Antimicrobial activity, *Rauvolfia tetraphylla, Jatropha curcas,* disc diffusion assay

Medicinal plants are good source of natural novel antimicrobial compounds that can be employed in controlling some infections in human and animals (Preeti, *et al.,*2010). In recent era, more emphasis is placed on evaluation of herbal active principles in the control and treatment of various infectious diseases. Isolation and identification of active compounds from medicinal plants permits synthesis of newer drug with reduced toxicity in body and resistance amongst bacteria (Manna and Abalaka, 2000). The content of active principles of medicinal plants may vary according to nature of soil and climate (Kriker *et al.*, 2013). It is an essential to evaluate the medicinal properties of plants collected from different source or country. The action of these plants on microorganisms have been found to be due to the presence of certain substances such as alkaloids, glycosides, volatile oils, gums, tannins, steroids, saponins, phlobatannins, flavonoids and a host of other chemical compounds referred to as secondary metabolites that are present in them (Sofowora, 1993).

Rauvolfia tetraphylla is a plant of Apocynaceae family, growing as a bush or small tree which is also known as the devil-pepper or Be still tree. The plant is native to the tropical Americas. The plant is widely used as both an ornamental and a source of herbal medicine and is now naturalised throughout the tropics including India, Indochina and Australasia. Antimicrobial activity of some extracts from fruit and leaves of *Rauvolfia tetraphylla* against few gram-positive and gram-negative bacteria have been investigated (Shariff *et al.*, 2006; Suresh *et al.*, 2008; Alagesaboopathi, 2009; Kavitha *et al.*, 2012). *Jatropha curcas* is commonly called physic nut, purging nut or pig nut. Jatropha species belong to the family Euphorbiaceae and are used in

traditional folklore medicine to cure various ailments in Africa, Asia and Latin America. Previous studies have reported that the Jatropha plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm sores and joint rheumatism (Irvine, 1961; Oliver-Bever, 1986). The seeds and leaves extracts of *J. curcas*, have shown molluscidal and insecticidal properties (Rug and Ruppel, 2000)

The information on *in vitro* antimicrobial activity of aqueous, methanol and chloroform extracts of *Rauvolfia tetraphylla* and *Jatropha curcas* against various gram-positive and gram-negative bacteria like *Bacillus cereus, Bacillus subtilis, Salmonella typhimurium, Staphylococcus aureus, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Streptococcus agalactiae, Lactobacillus acidophilus* and *Escherichia coli* are not available. Thus, the present study was carried out to evaluate the presence of phytochemical constituents and *in vitro* antimicrobial activity of various crude extracts of *Rauvolfia tetraphylla* and *Jatropha curcas* against many gram-positive and gram-negative bacteria as part of searching new bio-active compounds.

MATERIALS AND METHODS

Plant material and extracts preparation

The leaves of *Rauvolfia tetraphylla* and *Jatropha curcas* were collected from premises of Department of Botany and authenticated by Botanist, from Junagadh Agricultural University, Junagadh, Gujarat. The collected leaves were washed thoroughly with running tap water and finally with sterile distilled water. The leaves were chopped into small pieces and put under shade to dry completely for few days. The completely shade dried plant leaves were powdered finely using grinder and stored in airtight container for further use. Soxhlet extraction apparatus was used for multiple extractions with water, methanol and chloroform. The obtained liquid extracts were subjected to rotary vacuum evaporator and subsequently concentrated under reduced pressure. The residues obtained were designed as crude extracts and labelled and stored at -20°C for further studies as the method described by Chhabra *et al.,* (1992). The residue was transferred to previously weighed petri dish and evaporated till it was free from the solvent. The yield in percentage was estimated by weighing the petri dish again. The test extracts in sufficient quantities were prepared and stored at -20C° for testing its antimicrobial properties. The percent extractability of plants (with respect to the powdered material) was calculated as total amount of extract obtained X 100/ total weight of powder taken for extraction. Extractability in per cent was recorded for all extracts. Crude extract residues were re-dissolved either in water or in 1% dimethyl sulfoxide (DMSO) to get 100 mg/ml concentration and filtration through a 0.45 µm membrane filter and stored in sterile brown bottles at -20°C until bioassay was carried out.

Preliminary phytochemical analysis

All chemical of analytical grades (Merck Pvt. Ltd. or SD fine Pvt. Ltd., India) were used for qualitative analysis for presence of different phytochemical constituents present in different solvent extracts as per previous published methods (Tiwari et al., 2011; Harbone, 1984).

Test microorganisms

Typed cultures of *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC12432), *Salmonella typhimurium* (ATCC23564), *Staphylococcus aureus* (ATCC9144), *Klebsiella pneumonia* (NCIM 5082), *Proteus vulgaris* (ATCC13315), *Pseudomonas aeruginosa* (ATCC19429), *Streptococcus agalactiae* (NCIM 2401), *Lactobacillus acidophilus* (ATCC 11975) and *Escherichia coli* (ATCC 25922) were procured from National Chemical Laboratory (NCL), Pune. Nutrient broth, Nutrient agar, MRS agar, MRS broth, TSA agar, TSA both, Muller Hinton Agar, Sterile blank disc and antibacterial discs were procured from Himedia Lab, India and media were prepared for sub-culturing and to test antimicrobial activity of plant extracts.

Determination of antibacterial activity

Extracts were reconstituted either in sterile water or in dimethyl sulphoxide (DMSO) at concentration of 100 mg/ml. Solution of extracts (50 µl) were dispensed on blank

sterile discs (Himedia Ltd., Mumbai) and sterilized under exposure to UV for 24 h. Antibacterial activity of different extracts was evaluated using Muller Hinton agar (MHA) plate by disc diffusion assay (Murray *et al.*, 1999). Antibacterial activity was evaluated by inhibition zones of bacterial growth surrounding the plant extracts. The entire antibacterial assay was carried out under strict aseptic conditions. Turbidity of broth which corrected by adding sterile saline until 0.5 McFarland turbidity standard of 10^6 Colony Forming Unit (CFU) per ml was achieved. These inoculums were used for seeding on the agar. Standard antibacterial discs like gentamicin, ampicillin, ceftriaxone, moxifloxacin, penicillin and tetracycline were used to compare the zone of inhibition (mm) of plant extracts against various gram-positive and gram-negative bacteria. Gentamicin was considered as positive drug control in the study. The assay was carried out in triplicates and the result thus obtained is taken as the mean of the three readings.

RESULTS AND DISCUSSION

The percent extractability and phytochemical constituents of different extracts of *R. tetraphylla* and *J. curcas* plants are depicted in Table 1. Mean zone of inhibition (mm) of different extracts of both plants and different antibacterial agents against various bacteria are shown in Table 2. Graphical presentation of zone of inhibition of methanol and chloroform extracts of *R. tetraphylla* and *J. curcus* and different antibacterial agents against various bacteria are depicted illustrated in Figure 1 & 2. Inhibition of bacterial growth by extract of *R. tetraphylla* and three antibacterial agents against *B. subtilis* were shown in Figure 3 and 4.

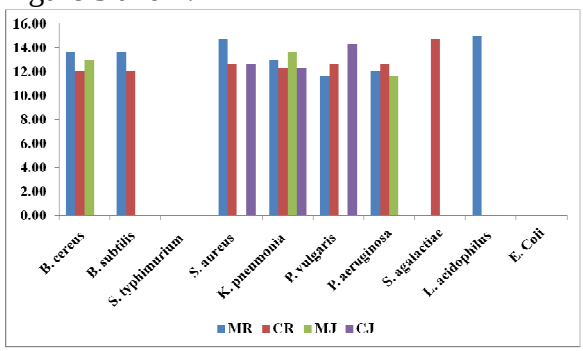

Figure 1: Mean zone of inhibition (mm) of different extracts of *R. tetraphylla* and *J. curcas* against different bacteria (MR: Methanolic extract of *R. tetraphylla*; CR: Chloroform extract of *R. tetraphylla*; MJ: Methanolic extract of *J. curcas*; CJ: Chloroform extract of *J. curcas*)

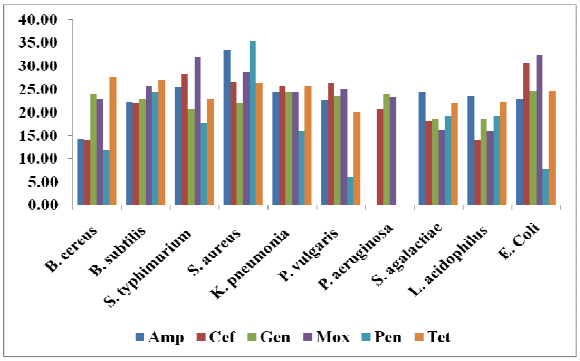

Figure 2: Mean zone of inhibition (mm) of different antibacterial agents against different bacteria (Amp: Ampicillin; Cef: Ceftriaxone; Gen: Gentamicin; Mox: Moxifloxacin; Pen: Penicillin; Tet: Tetracycline)

Table 1: Per cent extractability and phytochemical constituents of different extracts of *R. tetraphylla* and *J. curcas.*

Parameters	***R. tetraphylla***			***J. curcas***		
	Aqueous	**Alcoholic**	**Chloroform**	**Aqueous**	**Alcoholic**	**Chloroform**
Extractability (%)	32.65	30.61	16.33	16.75	20.31	5.45
Phytochemical constituents						
Alkaloids	-	+	+	-	+	+
Tannins	+	+	-	+	+	-
Flavonoids	+	+	-	-	+	-
Saponins	+	+	-	-	+	-
Diterpenes	+	+	+	-	+	+
Triterpenes	+	+	+	+	+	+
Carbohydrates	+	+	-	+	+	-

Figure 3: Zone of Inhibition of extracts of *R.. tetraphylla* against *B. subtilis*

Figure 4: Zone of Inhibition of antibacterial agents against *B. subtilis*

The chloroform extract showed good to moderate whereas aqueous extract showed negligible antibacterial activity. Highest and comparable antimicrobial activity of methanol and chloroform extract of *R. tetraphylla* have been observed against *B. cereus, B. subtilis, S. aureus, K. pneumonea , P. vulgaris, P. aeruginosa, L. acidophilus* in the range of (11.67± 0.33 to 15.00± 0.58 mm) except *E. Coli.* Kavitha R. et al. (2012) also reported that *R. Tetraphylla* did not show activity against *E. Coli*. Methanol extract of *R. tetraphylla* has highest growth inhibitory effect against *L. acidophillus* (15.00± 0.58 mm) similar with standard antibiotics like gentamicin, ceftriaxone, moxifloxacin and penicillin with (18.33± 1.67, 14.00± 0.58, 16.00± 1.15, 19.33± 0.67 mm, respectively). *Staphylococcus aureus* is second most sensitive organism against methanol extract of *R. tetraphylla* (14.67± 0.67 mm) which is in agreement with the observation reported previously by Alagesaboopathi (2009). Chloroform extract of *R. tetraphylla* is only extract which

Table 2: Mean ± S.E. zone of inhibition (mm) of different extracts of *R. tetraphylla* and *J. curcas* against different bacteria.

Organisms	Gen (C)	MR	CR	MJ	CJ	Amp	Cef	Mox	Pen	Tet
B. cereus	24.00± 0.58	13.67± 1.20**	12.00± 0.58**	13.00± 0.58**	---	14.33± 0.33**	14.00± 1.15**	23.00± 0.58	12.00± 0.58**	27.67± 0.33
B. subtilis	23.00± 1.00	13.67± 0.88**	12.00± 1.00**	---	---	22.33± 3.67	22.00± 3.61	25.67± 3.84**	24.33± 2.33	27.00± 1.00**
S. typhimuriu	20.67± 0.33	---	---	---	---	25.33± 2.33	28.33± 0.88**	32.00± 1.73**	17.67± 1.86**	23.00± 0.58
S. aureus	22.00± 1.00	14.67± 0.67**	12.67± 0.88**	---	12.67± 1.20**	33.67± 3.18**	26.67± 0.88*	28.67± 0.67**	35.33± 2.40**	26.33± 2.33
K. pneumo	24.33± 0.33	13.00± 1.53**	12.33± 0.88**	13.67± 2.19**	12.33± 0.88**	24.33± 4.63	25.67± 4.48	24.33± 2.33	16.00± 3.79	25.67± 0.67
P. vulgaris	23.67± 0.88	11.67± 0.33**	12.67± 0.88**	---	14.33± 2.03*	22.67± 1.76	26.33± 2.73	25.00± 2.08	6.00± 6.00	20.00± 1.15
P. aerugin	24.00± 0.58	12.00± 0.58**	12.67± 1.20**	11.67± 0.33**	---	---	20.67± 0.88*	23.33± 0.88	---	---
S. agalacti	18.67± 1.86	---	14.67± 0.88	---	---	24.33± 0.67*	18.00± 2.08	16.33± 1.20	19.33± 0.67	22.00± 3.06
L. acidoph	18.33± 1.67	15.00± 0.58	---	---	---	23.67± 0.88**	14.00± 0.58	16.00± 1.15	19.33± 0.67	22.33± 2.85
E. Coli	24.67± 0.33	---	---	---	---	23.00± 0.00**	30.67± 0.33**	32.33± 0.33**	7.67± 0.33**	24.67± 0.33

C: Control; Gen: Gentamicin; MR: Methanolic extract of *R. tetraphylla*; CR: Chloroform extract of *R. tetraphylla*; MJ: Methanolic extract of *J. curcas*; CJ: Chloroform extract of *J. curcas*; Amp: Ampicillin; Cef: Ceftriaxone; Mox: Moxifloxacin; Pen: Penicillin; Tet: Tetracycline (* Significant (P<0.05); ** Highly Significant (P<0.01) compared to Gentamicin)

suppressed growth of *S. agalactiae* (14.67± 0.88 mm) which was comparable with antibiotics like gentamicin, ceftriaxone, moxifloxacin, penicillin and tetracycline (18.67± 1.86, 18.00± 2.08, 16.33± 1.20, 19.33± 0.67 and 22.00± 3.06 mm, respectively). Methanol and chloroform extract of *J. curcus* have also shown antimicrobial activity against *B. cereus*, *S. aureus*, *K. pneumonea*, *P. vulgaris*, *P. aeruginosa*, in the range of 11.67± 0.33 to 15.00± 0.58 mm except *L. acidophilus*, *S. agalactiae* and *E. Coli*. which is in agreement with previous report (Kalimuthu et al., 2010). Methanol extract of *J. curcus* showed good inhibitory effect on *B. cereus* (13.00± 0.58 mm) which was comparable with some antibiotics like ampicillin, ceftriaxone, and penicillin. However, both the plants did not shown activity against *S. typhimurium*.

In conclusion, alcoholic and chloroform extracts of *Rauvolfia tetraphylla* and *Jatropha curcas* possess active phytochemical compounds which shown good *in vitro* antibacterial activity against several gram-positive and gram-negative bacteria. Further investigation is needed to explore the pharmacological value of both plants in the therapy of infectious diseases in human and animals.

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