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Research Article

Effect of Phytochemical Constituents of *Ricinus Communis*, *Pterocarpus Santalinus*, *Terminalia Belerica* on Antibacterial, Antifungal and Cytotoxic Activity

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ABSTRACT

Three medicinal plants, *Ricinus communis, Pterocarpus santalinus, Terminalia belerica were* studied for their phytochemical constituents showing antibacterial activity, antifungal activity and cytotoxicity. In phytochemical analysis, results showed the presence of tannins, alkaloids, cardiac glycosides, terpenoids, flavonoids and steroids in the all three plants. Antibacterial activity was tested against human pathogenic micro-organisms like *B. subtilis, S. aureus, S. abony, E.coli, P. aeruginosa* by agar well diffusion method. Antifungal activity was tested against certain pathogenic fungi *A. niger, C. albicans, Rhizopus, Lasiodiploidia theobromeae* and *A. solani* by pour plate method. The cytotoxic effect of selected plants were tested against HEK293T (Human embryonic kidney cell line) and c2c12 (Mouse, Muscle cell line) by MTT assay. Results showed that selected plants contain important phytochemicals which can be used to investigate its potential use for developing new drugs.

Key Words - Eranda, Rakhtachandan, Behda, Phytochemical analysis, Antibacterial activity, Antifungal activity, cytotoxicity.

INTRODUCTION

Medicinal plants are the source of natural drug so use for treatment of various diseases. *Ricinus communis* L. (2n=20) (*R. communis*) belongs to family: Euphorbiaceae aiso known as 'castor oil plant' and Erandi[1]. The plant is reported to contain antioxidant, anti inflammatory, antidiabetic, antitumour, antiasthmatic activity and antibacterial activity and used for cure of jaundice. It is also used for the treatment of hepatitis and skin and breast cancer in initial phase[2]. *Pterocarpus santalinus* L. (2n=26) belongs to the family: Fabaceae[3,4] and commonly found along riverine forests in tropical South America and Africa[5]. It is used in treatment of fever, scorpion sting, diabetes headache, skin diseases,[6] and also used for treatment of diabetes mellitus and eye diseases, ulcers[6,7]. *Terminalia belerica (2n=48)* belongs to the family: Combretaceae, commonly known as myrobalan and Behda. The plant is reported to contain anti-diabetic, anti-cancer, anti-oxidant and anti-viral activity and also use for treatment of wound healing ulcers, local swelling, anemia, diabetes, and chronic recurrent fever. The fruits are purgative, laxative, gastro protective[8].

We studied the effect of the three plant extracts on different pathogenic bacteria like *B. subtilis, S. aureus, S. abony, E. coli, P. aeruginosa* and fungi, *A. niger, C. albicans, Rhizopus, Lasiodiploidia theobromeae* and *A. solani.* The cytotoxicity of the phytochemical components of the three plants were tested on c2c12 (Mouse, Muscle cell line)[9] and HEK293T (Human embryonic kidney cell line) using the MTT assay.

MATERIALS AND METHODS

Plant Collection: The leaves and seeds of *Ricinus communis were* collected from a herbal farm from Bedwa. Seeds and leaves of *Pterocarpus santalinus* and *Terminalia belerica* were collected and all three plants were identified from Directorate of Medicinal and Aromatic Plants Research Center at Boriavi, Gujarat.

Preparation of plant extract.

Ricinus communis and *Pterocarpus santalinus* (leaf extracts)-The leaves were washed, dried and powdered. 20gm powder was taken and was defatted with petroleum ether and allowed to stand at room temperature for 3 days with recurrent agitation. It was washed with distilled water. The extract was soaked in 50 ml distilled water for 48 hrs, filtered and used for phytochemical analysis[10].

Terminalia belerica (seeds extracts*)* - Seeds were coarsely powdered and 20gm powder was sequentially extracted with ethanol on a shaker for 48 hrs at room temperature. The filtered extracts were used for phytochemical analysis[11].

These three plant extracts were tested for tannins, terpenoids, alkaloids, flavonoids, cardiac glycosides and steroids

Test for tannins - Few drops of ferric chloride solution was added in filtrate . A greenish black precipitate indicates the presence of tannins.

Test for terpenoids - Chloroform and concentrated H2SO4 was added in filtrate. A reddish brown colour or ring indicates the presence of terpenoids.

Test for Alkaloids - Filtrate was dissolved in dilute HCL and saturated picric acid was added. A yellow precipitate indicates the presence of alkaloids.

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Table- 1 Phytochemical analysis of *Ricinus communis, Pterocarpus santalinus, Terminalia belerica* .

Test	*Ricinus communis*	*Pterocarpus santalinus*	*Terminalia belerica*
Tannin	+ve	+ve	+ve
Terpenoids	+ve	+ve	+ve
Alkaloid	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve
Phlobatannin	-ve	-ve	-ve
Cardiac glycosides	+ve	+ve	+ve
Steroid	+ve	+ve	+ve

+ve =Phytochemical present, -ve = Phytochemical absent PC= Phytochemical test

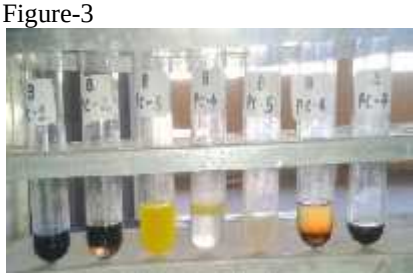
Figure-1

E= Eranda

Figure-2

R=Raktchandan

Figure-3



B = Behda

Table-2 Antibacterial activity of *Ricinus communis, Pterocarpus santalinus* and *Terminalia belerica.*

	Ricinus communis	*Pterocarpus santalinus*	*Terminalia belerica*
B. subtilis	6mm	18mm	8mm
S. aureus	16mm	10mm	8mm
S. abony	10mm	10mm	10mm
E.coli	18mm	10mm	8mm
P. aeruginosa	10mm	10mm	10mm

Test for Flavonoids - Few drops of lead acetate was added to the filtrate A yellow precipitate indicates the presence of flavonoids.

Test for phlobatannins - Aqueous HCL was added in filtrate and boiled. Red colour or precipitate indicates the presence of phlobatannin.

Test for cardiac glycosides – Glacial acetic acid, ferric chloride and concentrated H2SO4 was added to the filtrate. A brown coloured ring indicates the presence of cardiac glycoside.

Test for steroids - Concentrated H2SO4 was added in filtrate red colouration indicates the presence of steroids[12,13,14].

Antibacterial activity: Antibacterial activity of the test extracts was tested by agar well diffusion method using molten nutrient agar. After solidification of the medium, wells were made, and test extracts were added into the wells, separately. Plates were incubated at 37º C for 24 h. The antibacterial activity of the test samples was determined by measuring the diameter of clear zone around the well[15]. Methanolic extract of *Ricinus communis* and *Pterocarpus santalinus* leaves ,ethanolic exract of *Terminalia belerica* seeds were dried and finally dissolved in DMSO and used for testing antibacterial activity againt *B. subtilis* (ATCC6633), *S. aureus (*ATCC*6538), S. abony (*ATCC *6017), E. coli (*ATCC *8739), P. aeruginosa (*ATCC *9027)*

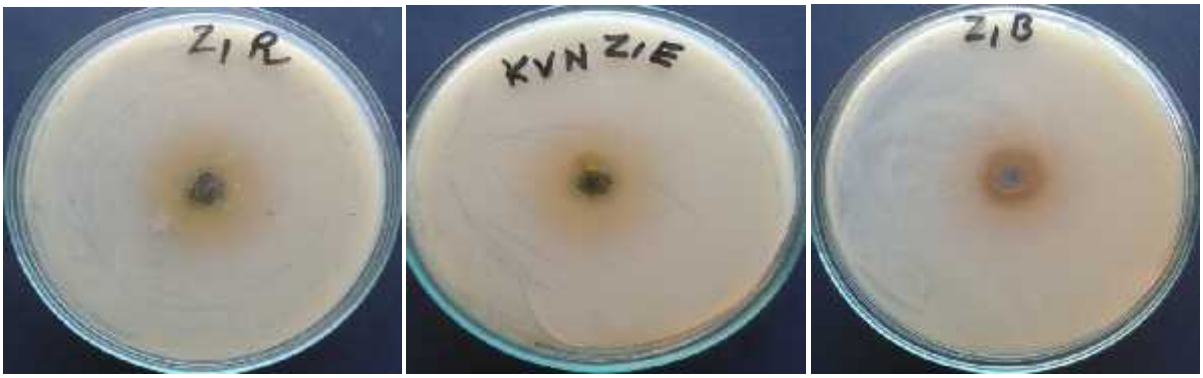
Antifungal activity: Antifungal activity of the test extracts was tested by pour plate method. The fungal spores were inoculated in PDB allowed it grow for 24 to 48 hrs. The spores were transferred to autoclaved PDA medium, mixed properly and poured in sterile petriplates and allowed to solidify. Then wells were made and test extracts were added into the wells. Plates were incubated at 28º C for 24 to 48 hrs. The antifungal activity of the test samples was determined by measuring the diameter of clear zone around the well[16]. Methanolic extract of *Ricinus* and *Pterocarpus* leaves and ethanolic etxract of *Terminalia belerica* seeds was dried and dissolved in DMSO and used for testing antifungal activity against *A. solani, A. niger, C. albicans, , Lasiodiploidia* and *Rhizopus.*

Determination of cell viability

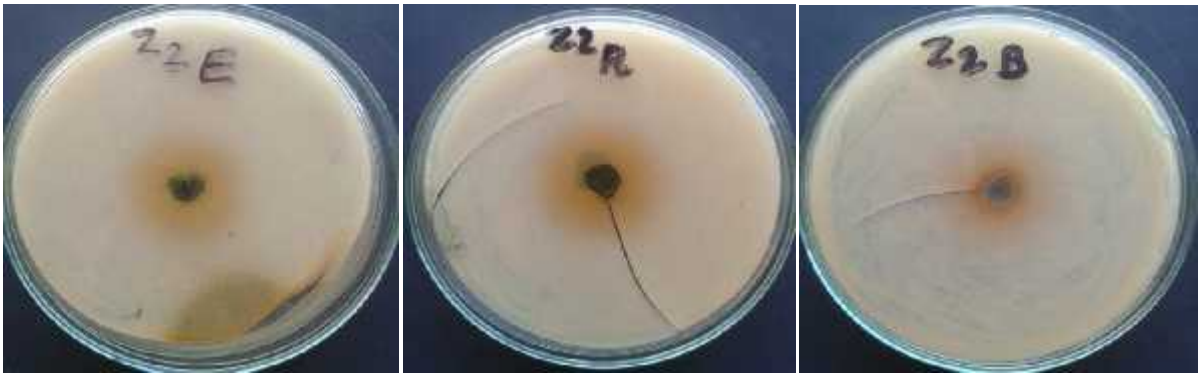
Preparation of plant extract: Methanolic extract of seeds and leaves of *Ricinus communis*, Methanolic etxract of leaves and 70% ehanolic extract of *Pterocarpus* seed, ethanolic extract of seeds and methanolic extract of *Terminalia belerica* leaves was used for analysis. All the extracts were poured into sterile dry petriplates and allowed to get evaporated. The sediments were scrapped off dissolved in DMSO and used for testing anticancer activity. Three concentrations, low dose (50µg/ml), mid dosage (250µg/ml), and high dosage (500µg/ml) were prepared from that and used along with DMSO as a negative control for all the plants used in the study.

Cell-lines used: The cytotoxic activity of the extract was evaluated on c2c12 (Mouse, Muscle cell line) and HEK293T (Human embryonic kidney cell line) which

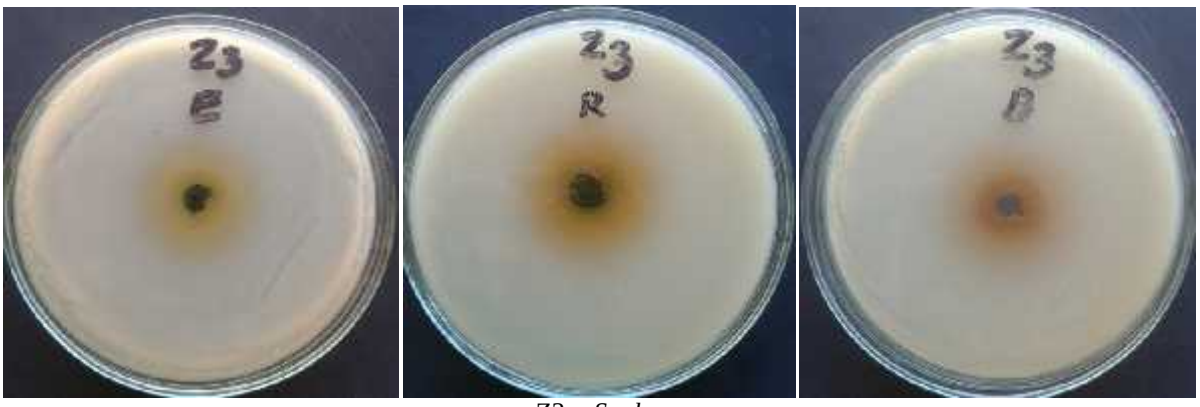
Antibacterial activity of selected plant against selected bacteria



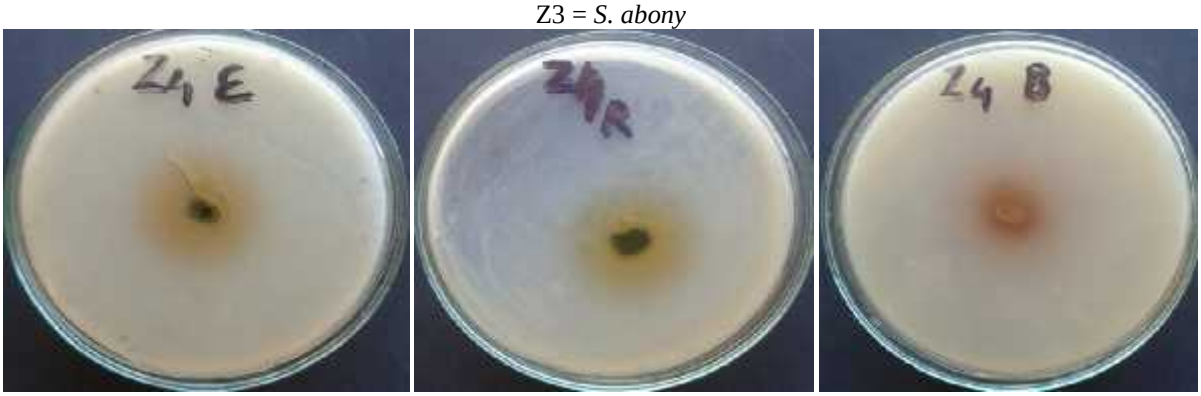
Z1 = *B. subtilis*



Z2 =*S. aureus*



Z3 = *S. abony*



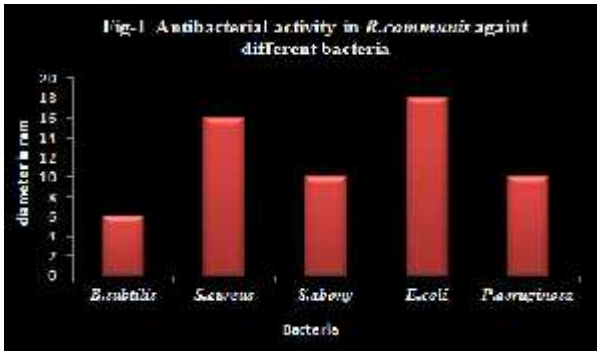
Z4 = *E. coli*

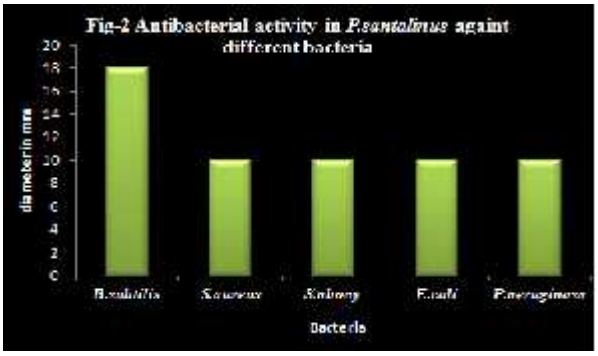
were procured as a kind gift from Dr. C.G. Joshi, Anand Agriculture University, Anand. Both the cell lines were grown in DMEM supplemented with 10% foetal bovine serum (FBS) and 1% penicillin, streptomycin, neomycin (PSN) at 37ºC in a 5% CO2, 95% humidified atmosphere.

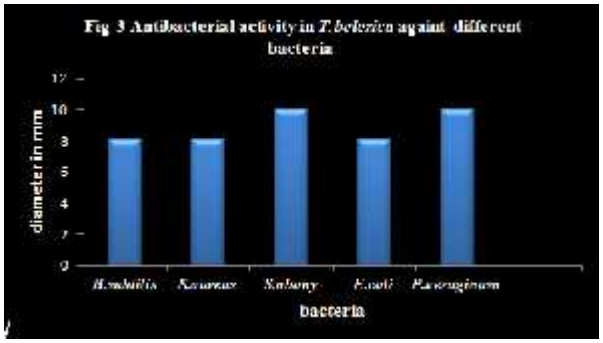
MTT cytotoxicity assay: Principle of the test is to convert the yellow tetrazolium salt MTT to purple formazan crystals by metabolically active cells. The amount of formazan produced is proportional to the number of viable cells. Cells were plated in 96-well flat bottom tissue culture plates at a density of approximately 10,000 cells/well and

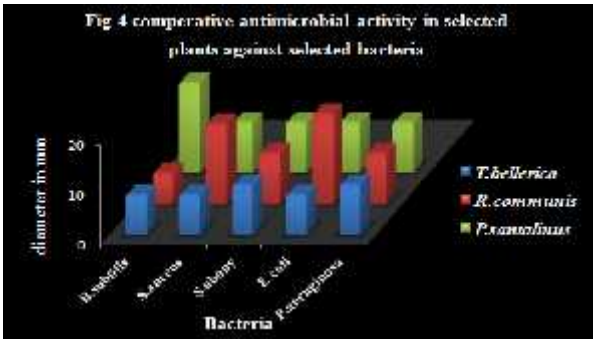
Z5 = *P. aeruginosa*

E= Eranda (Ricinus communis),
R = Raktchandan (Pterocarpus santalinus),
B=Behda (Terminalia belerica)









allowed to attach overnight at 37°C. The cells were then incubated with the extract at a concentration of 50 µg/mL, 250 µg/mL and 500 µg/mL for 24 hours. Untreated cultures and blank wells without cells received negative control for respective controls. After the drug exposure period, the cells were grown for an additional 24 hours in extract-free fresh medium. Next, 20 µL of the MTT (5mg/ml) reagent was added to each well, and the plate was incubated for 4 hours at 37°C. The MTT crystals were then solubilized in 200µl of DMSO. Absorbance measurements were made at 570 nm using a Biotek ELISA plate reader. Proliferation was expressed as the fraction of treated cells that survived relative to untreated cultures. Every experiment included a set of negative controls (untreated cultures). All experiments were performed in triplicate[10].

The percentage of cytotoxicity was calculated using the following formula[17].

% Cytotoxicity = (1-Abs test /Abs Control) × 100

RESULTS

Qualitative Phytochemical analysis

PC-1 = Tannin, PC-2 = terpenoids, PC-3 = Alkloids, PC-4 = Flavanoids, PC-5 = Phlobatannin, PC-6 = Cardiac glycosides, PC-7 = steroids

Antibacterial activity: The methanolic extract of *Ricinus communis* leaves showed the highest activity against *E.coli*. and lowest activity against *Bacillus subtilis.* (Fig-1) The methanolic extract of *Pterocarpus santalinus* leaves mostly inhibited *Bacillus subtilis* followed by *Staphylococcus aureus, Staphylococcus abony, Pseudomonas aeruginosa,* and *E.coli.* (Fig-2) The ethanolic extract of *Terminalia belerica* seeds gave highest response againt *S. abony and Pseudomonas aeruginosa* (Fig-3).

Antifungal activity: The methanolic extract of *Ricinus communis* leaves showed considerable activitiy against A. niger with a lowest activity against *Alternaria solani*.(Fig-

Antifungal activity of selected plant against selected fungi

A.N = A. niger

SDA and PDA^{+}= *C. albicans*

A = *A. solani*

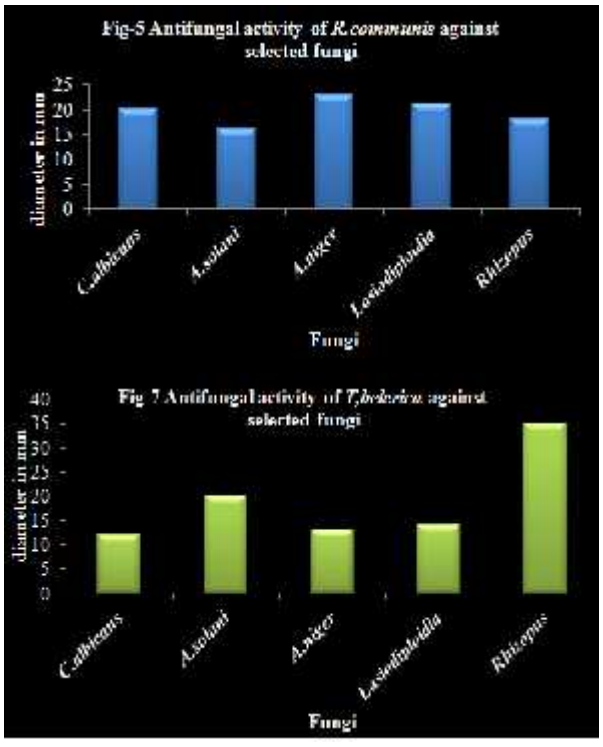
L = *Lasiodiploidia theobromeae*

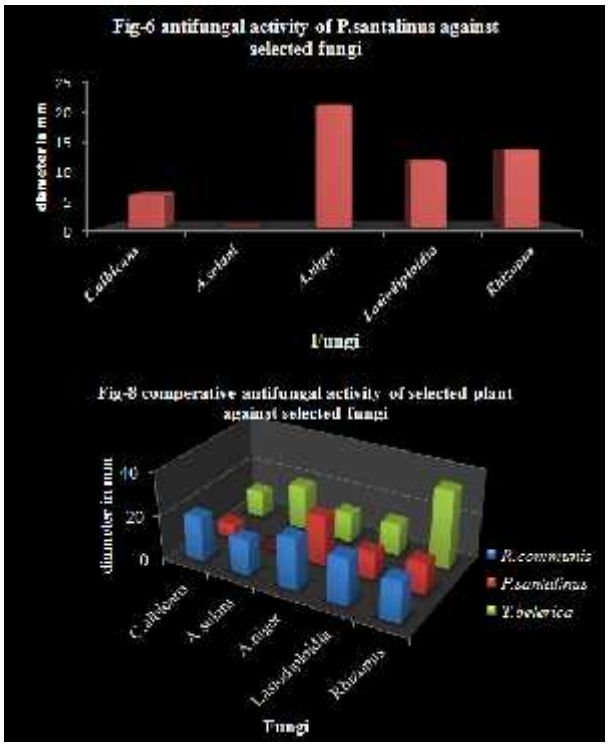
R = *Rhizopus*, E= Eranda (*Ricinus communis)*
R = Raktchandan (*Pterocarpus santalinus)*, B=Behda (*Terminalia belerica*)

Table-3 Antifungal activity of *Ricinus communis*, *Pterocarpus santalinus* and *Terminalia belerica.*

	Ricinus communis	*Pterocarpus santalinus*	*Terminalia belerica*
Candida albicans	20mm	6mm	12mm
Alternaria solani	16mm	NF	20mm
A.niger	23mm	22mm	13mm
Lasiodiploidia	21mm	12mm	14mm
Rhizopus	18mm	14mm	35mm

NF=NOT FOUND





5).The inhibition of *A. niger* was maximum in response to methanolic extract of *Pterocarpus santalinus* leaves (Fig-6).The ethanolic extract of *Terminalia belerica* fruit gave highest response againt *Rhizopus* and lowest activity against *A. niger* and *Candida albicans. (*Fig-7)

Cytotoxic activity: The highest Cytotoxic effect was found in *Ricinus communis* seeds and leaves but more effect was seen in seeds [236±0.016] against c2c12 and *Pterocarpus santalinus* seeds was more effective [87±0.02] against HEK293T.

DISCUSSION

The water extract of *Ricinus communis* leaves and the ethanolic extract of *Pterocarpus santalinus* and

Table -4 Cytotoxic effect of *Ricinus communis, Pterocarpus santalinus* and *Terminalia belerica.*

Plant Name	Plant part	Solvent	Concentration (ug/ml)	% Cytotoxicity		SD	
				HEK293T	c2c12	HEK293T	c2c12
Ricinus communis	Seeds	Methanol	50	17.27	170.11	0.26	0.007
			250	75.45	236.59	0.08	0.016
			500	64.54	223.18	0.02	0.024
	Leaves	Methanol	50	20.22	153.63	0.14	0.002
			250	69.66	229.88	0.02	0.01
			500	73.03	202.79	0.04	0.045
Pterocarpus santalinus	Seeds	70% ethanol	50	66.29	222.34	0.03	0.006
			250	78.65	210.33	0.07	0.03
			500	87.64	217.03	0.02	0.004
	Leaves	Methanol	50	22.47	174.30	0.07	0.008
			250	84.26	165.64	0.01	0.079
			500	70.78	184.63	0.01	0.008
Terminalia belerica	Seeds	Ethanol	50	-40.90	141.62	0.19	0.02
			250	8.18	156.42	0.13	0.008
			500	3.63	11.45	0.05	0.112
	Leaves	Methanol	50	19.09	91.34	0.02	0.022
			250	88.18	-472.06	0.04	0.098
			500	9.09	-295.81	0.02	0.129

Terminalia belerica seeds showed the presence of all important phytochemicals except Phlobatannin.

Methanolic leaf extracts of *Ricinus communis* were found to be active against all bacteria selected for the study. The plant exhibited highest zone of inhibition against *E.coli* (18mm) and *S. aureus* (16 mm), whereas it was comparatively lower but significant against other bacterial species (6-10mm). The results obtained are in agreement with the previous studies on *Ricinus communis*[18]. *Pterocarpus santalinus* leaves extract showed highest zone of inhibition against *Bacillus subtilis* (18mm). *Terminalia belerica* seed extract was more effective and gave highest zone of inhibition against *S. abony* (10mm), *P. aeruginosa* (10mm).

It is concluded that antifungal activity of *R. communis, Pterocarpus santalinus* leaves extracts and fruit extracts of *T. bellerica*, contain active constituents which would be helpful in treating various kinds of plant diseases and seed borne diseases. *R. communis* leaves extract exhibited more activity against *A.niger* (23mm), *lasiodiploidia* (21mm), *Candida albicans* (20mm). The results obtain are agreement to previous studied against *C. albians*[19]. *Pterocarpus santalinus* leaves extract gave highest zone of inhibition against *A.niger* (22mm). *Terminalia belerica* fruit extract showed highest activity *Rhizopus (35mm).*

Cytotoxic activity results showed that *Ricinus communis* and *Pterocarpus santalinus* seeds and leaves extract exhibited better cytotoxicity effects on c2c12 and HEK293T line compared to *Terminalia belerica* leaves and seeds after 24 hr incubation.

CONCLUSIONS

The present work emphasizes the importance of medicinal components of the selected plants as antibacterial, antifungal and anticancer agents. The results of the investigation therefore offer a scientific basis for exploitation and use of solvent extracts of *Ricinus communis, Pterocarpus santalinus, Terminalia belerica* in traditional medicine. This could be a possible source for developing medicines to treat infections caused by multi-drug resistant strains of microorganisms. Therefore, there is an urgent need to investigate the biological activity of its phytochemical components for designing drugs against several important diseases.

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REFERENCES

1. Jitendra Jena, Ashish Kumar Gupta (2012) *Ricinus communis* linn a phytopharmacological review I. Pharm Pharm Sci. Vol 4, Issue 4.
2. Manpreet Rana, Hitesh Dhamija, Bharat Prashar, Shivani Sharma (2012) *Ricinus communis L.* – A Review. Int.J.PharmTech, 4(4).
3. Naidu CV. Rajendrudu G (2001) Influence of kinetin and nitrogenous salts on seed germination of red sanders (*Pterocarpus santalinus* Linn.f.) Seed. Science and Technology 29, 669-72.
4. S. Vipranarayana, T.N.V.K.V. Prasad and T. Damodharam (2012) In Vitro Seed Germination and Induction of Enhanced Shoot Multiplication in *Pterocarpus santalinus* Linn.f: An Endemic Medicinal Plant of Seshachalam Hills, Tirumala. Int. J. Pure Appl. Sci. Technol., 9(2).

5. C.F. Anowi, B.O. Umeokoli, A.F. Onyegbule , C. Okonkwo and Chibeze (2012) Analgesic, phytochemical and acute toxicity evaluation of the methanol extract of the leaves. International journal of pharmaceutical science and Reserch. Vol. 3, Issue 07.
6. Chopra RN, Nayar SL, Chopra IC (1956) Glossory of Indian Medicinal Plants. New Delhi. C.S.I.R. Publications.
7. Latheef, S A, Prasad, B. Bavaji, M,. Subramanyam,G (2008) A database on endemic plants at Tirumala hills in India Bio information .2(6) 260-262.
8. Manohar V.R, Chandrashekar. R, Rao S.N (2012) Phytochemical analysis of *terminalia belerica* fruit pulp extracts. world journal of pharmacy and pharmaceutical sciences Volume 1, Issue 4.
9. Vijayabaskaran M, Venkateswaramurthy N, Arif pasha MD, Babu G, Sivakumar P, Sivakumar, Perumal P, Jayakar B (2010) In vitro cytotoxic effect of ethanolic extract of psedarthria viscid linn. International Journal of pharmacy and pharmaceutical sciences. Vol 2, Issue 3.
10. Mohd Nadeem Shah Syed Bukhari and Ajaz Ahmad Dar (2012) Treatment of Cancer on human Melanoma Cell lines by *Ricinus communis* Extract. Asian J. Plant Sci. Res. 2 (6):680-687.
11. Kannan P, Ramadevi S.R.and Waheeta Hopper (2009) Antibacterial activity of *Terminalia chebula* fruit extract. African Journal of Microbiology Research Vol. 3 (4).
12. Harbone, J. B. (1973) Phytochemical methods, A guide to Modern Techniques of Plants analysis. John Wiley and Son Inc. New York pp. 1 – 26.
13. Sofowora, A. (1984) Medical Plants and traditional medicines in Africa. Spectrum Book Ltd, Ibadan, Nigeria. P. 289.
14. M.K.Gafar, L.G. Hassan,S.M. Dangoggo and A.U.Itodo (2010) Amino acid estimation and Phytochemical screening of Indigofera astragolina leaves. J. Chem. Pharm. Res 2(5).
15. E. Christy Jeyaseelan, P.T. Justin Jashothan (2012) In vitro control of *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922) by *Ricinus communis* L. Asian Pacific Journal of Tropical Biomedicine .2(10).
16. S. Hyder Raza Naqvi, Mahayrookh Asif, Asif Bin Rehman And Mansoor Ahmad (2010) EVALUATION OF ANTIMICROBIAL PROPERTIES OF *TERMINALIA CHEBULA* Retz. Pakistan Journal of Pharmacology. Vol.27, No.1
17. Rajesh N. Gacche, Rafik U. Shaikh, Mahesh M. Pund (2011) In vitro evaluation of anticancer and antimicrobial activity of selected medicinal plants from Ayurveda. Asian Journal of Traditional Medicines, 6(3).
18. Rabia Naz, Asghari Bano (2012) Antimicrobial potential of *Ricinus communis* leaf extracts in different solvents against pathogenic bacterial and fungal strains. Asian Pac J Trop Biomed . 2(12).
19. Jahir Alam Khan, Krishna Pratap Yadav (2011) Assesment of antifungal avtivity of *Ricinus communis*. journal of pharmacehtical and biomedical science,11(02)