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Assessment of Antifungal Properties of *Ricinus communis*

Jahir Alam Khan[1], *Krishna Pratap Yadav[2]

[1] R &D Division, MRD LifeSciences (P) Ltd., Lucknow (U.P), India.

[2]Department of Biotechnology, R.M.L. Awadh University, Faizabad (U.P). India.

Abstract:

Cold and hot water, methanol, ethanol, ethyl acetate, acetone, and hexane extracts of leaves, stem and roots of *Ricinus communis* in a final concentration of 500mg/ml were evaluated for their antifungal properties against pathogenic microorganisms such as *Trycophyton rubrum*, *Candida albicans*, *Microsporum Spp.* using agar well diffusion method. In case of leaf cold aqueous, methanolic and acetone extracts were effective. Only cold aqueous extract of stem was effective. Cold aqueous extract of root was most effective followed by acetone, ethyl acetate and hexane extracts. MIC was also calculated for the most effective extracts and it was found to be **31.25mg/ml**.

Key words : Antifungal properties, *Ricinus communis,* Pathogens, Solvent extraction, Agar well diffusion.

Introduction:

Dependence of human population on the available antibiotics has led to an increment in the resistance in the microorganisms towards them [1], however a large portion of the world is still working in the search of better drugs, but the problem is still the same after some time the new drugs become ineffective or have a large number of side effects associated with them. Herbal antibiotics can be a good answer to this challenge by the microorganisms, a large portion of the available plants have been used since time immemorial for the treatment of various diseases [2]. As per a report of WHO in 1993 nearly 80 % of world population is dependent on the tradional system of medication that is the use of plants and their parts as medicine [2].

The castor oil plant, *Ricinus communis*, is a species of flowering plant in the spurge family, *Euphorbiaceae*. It belongs to a monotypic genus, *Ricinus*, and subtribe, *Ricininae*. The evolution of castor and its relation to other species is currently being studied [3]. Although seeds of *Ricinus communis* which have been used as a medicine in traditional system for treatment of Lumbago, rheumatism and sciatica, boils and swellings etc.[4] are well known very less focus has been given to other plant parts say the leaves, stem, and root. A few researchers of the field include [2, 5-8]. who have contributed to the exploration of plant parts of *Ricinus communis* for their medicinal uses.

The present study was also designed to assess the antifungal properties of leaves, stem and roots of *Ricinus communis* in order to better understand its medicinal importance.

Material and methods:

Sample Collection

The castor plant (*Ricinus communis*) was collected from Jiamau, Lucknow after proper identification.

All the plant parts were washed with tap water then with distilled water, cut into small pieces, packed in envelops and kept for drying at 50°C in oven. After drying they were ground into fine powder by the help of grinder.

Test Microorganisms

Three different fungal strains namely *Trycophyton rubrum*, *Candida albicans*, *Microsporum Spp.* collected from Gaurang Clinic & Centre for Homoeopathic Research, Lucknow were sub cultured and used throughout the project work.

Extract Preparation

For hot aqueous extraction 5gm ground plant material was soaked in 50ml of hot water and kept in boiling water bath for 2 hours. After that it was filtered in a weighed petriplate and kept in hot air oven for drying, dried extract was dissolved in double volume of DMSO (DimethylSulfoxide) thus giving the final concentration of 500mg/ml. For rest of the extracts 5gm of ground powder was soaked in 50ml of the respective solvents for 3-4 days. Thereby filtered in weighed petriplate and dried in hot air oven, the dried extract was dissolved in double volume of DMSO thus giving the final concentration of extract to 500mg/ml.

Antibiogram Analysis

Antibiogram of the plant extract was performed by agar well diffusion method of [2] wherein sterile PDA (Potato dextrose agar) media was poured into sterile petriplates under sterile conditions and left for solidification. After solidification 25 µl of fungal pathogens were spread on different plates and 4 wells of 8mm diameter were bored by the help of sterile borer. 50 µl of different antimicrobial samples to be assayed were loaded in 1st three wells, and the 4th well with standard antifungal drug Flucan (1mg/ml). Plates were incubated at 28°C for 24 hours, and observed for zone of inhibition. The zone of

inhibition by the sample was compared with the standard antifungal drug Flucan. All the tests were performed in triplicates.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of the extracts showing good antifungal activity was determined by using broth dilution technique of [3], 12 test tube were taken and 3ml of PDB (potato dextrose broth) was filled in each test tube and autoclaved. Test tubes were left to cool down to room temperature. Two sets each containing 6 test tubes were made and 200 µl of the antimicrobial extract was serially diluted. Out of the two, one set was inoculated with the pathogen against which MIC is to be calculated. The inoculated set was kept for incubation at 120 rpm at 28°C for 24 hrs whereas the blank or uninoculated set was preserved at 4 °C. In case of fungal pathogens the test tubes were incubated at 28°C. Optical Density was read at 600nm for each inoculated test tube using the un-inoculated test tube as blank. All the experiments were performed in triplicates.

Results:

Antifungal activity of all the extracts was performed by agar well diffusion method and some of the extracts were found to be effective against used pathogens. Detailed results can be seen below in ***Table 1-3.***

Table 1: **Antibiogram analysis of *Ricinus communis* leaf extracts**

S.NO.	Extracts	Diameter of ZOI against *C. albicans*		Diameter of ZOI against *T. rubrum*		Diameter of ZOI against *Microsporum spp.*	
		By Extract	By Flucan	By Extract	By Flucan	By Extract	By Flucan
1.	**Cold aqueous**	**20mm**	18mm	**0**	22mm	0	15mm
2.	**Hot aqueous**	0	18mm	0	22mm	0	15mm
3.	**Methanolic**	**18mm**	18mm	0	22mm	0	15mm
4.	**Ethanolic**	15mm	18mm	0	20mm	0	18mm
5.	**Ethyl acetate**	0	18mm	0	20mm	0	18mm
6.	**Acetone**	0	18mm	**0**	20mm	0	18mm
7.	**Hexane**	15	18mm	**17mm**	20mm	**0**	17mm

Note: Well diameter= 8mm, ZOI in case of D/W in each case= 0mm.

Table 2: **Antibiogram analysis of *Ricinus communis* stem extracts**

S.NO.	Extracts	Diameter of ZOI against *C. albicans*		Diameter of ZOI against *T. rubrum*		Diameter of ZOI against *Microsporum spp.*	
		By Extract	By Flucan	By Extract	By Flucan	By Extract	By Flucan
1.	**Cold aqueous**	0	15mm	**15mm**	17mm	0	17mm
2.	**Hot aqueous**	0	15mm	0	17mm	0	17mm
3.	**Methanolic**	0	15mm	0	17mm	0	17mm
4.	**Ethanolic**	0	15mm	0	17mm	0	17mm
5.	**Ethyl acetate**	0	15mm	0	17mm	0	17mm
6.	**Acetone**	0	15mm	0	17mm	0	17mm
7.	**Hexane**	0	15mm	0	15mm	0	17mm

Note: Well diameter= 8mm, ZOI in case of D/W in each case= 0mm.

Table 3: **Antibiogram analysis of *Ricinus communis* root extracts**

S.NO.	Extracts	Diameter of ZOI against *C. albicans*		Diameter of ZOI against *T. rubrum*		Diameter of ZOI against *Microsporum spp.*	
		By Extract	By Flucan	By Extract	By Flucan	By Extract	By Flucan
1.	**Cold aqueous**	**15mm**	23mm	0	18mm	**17mm**	20mm
2.	**Hot aqueous**	0	23mm	0	18mm	0	20mm
3.	**Methanolic**	0	23mm	0	18mm	0	20mm
4.	**Ethanolic**	0	15mm	0	15mm	0	18mm
5.	**Ethyl acetate**	0	15mm	**15mm**	15mm	**15mm**	18mm
6.	**Acetone**	13mm	15mm	**16mm**	15mm	13mm	18mm
7.	**Hexane**	0	18mm	0	15mm	0	17mm

Note: Well diameter= 8mm, ZOI in case of D/W in each case= 0mm.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of the extracts effective against pathogens was determined by broth dilution technique and MIC in all the cases was found to be 31.25mg/ml.

Table 4: **MIC of cold aqueous extract of Leaf against *C. albicans***

S. No.	Concentration of Antimicrobial extract (mg/ml)	OD at 600nm
1.	**31.25**	0
2.	1.95	0.22
3.	0.12	0.40
4.	0.007	0.40
5.	0.0004	0.40
6.	0.00002	0.43

Table 5: **MIC of cold aqueous extract of root against microsporum spp**

S. No.	Concentration of Antimicrobial extract (mg/ml)	OD at 600nm
1.	**31.25**	0
2.	1.95	0.52
3.	0.12	0.42
4.	0.007	0.17
5.	0.0004	0.13
6.	0.00002	0.33

Discussion:

Some of the extracts were found to be effective against the used pathogens giving a good sign of the future herbal drugs without any side effects.

The cold aqueous, methanol and ethanol extracts of *Ricinus communis* leaves were effective against *Candida albicans* most effective being the cold aqueous extract. Even the standard antifungal Flucan was ineffective against *Candida albicans* but cold aqueous and methanol extracts gave good zones of inhibition of 20mm and 18mm respectively. Zone of inhibition given by ethanol extract was also comparable to the zone given by standard antifungal Flucan.

Extracts of stem did not give satisfactory results and only cold aqueous extract was found to be effective against *Trycophyton rubrum* giving a zone of inhibition of 15 mm which was nearly close to the zone of 17mm shown by the standard antifungal Flucan. So it can be said that cold aqueous extract is the only hope in case of stem being used as an effective drug.

Cold aqueous, ethyl acetate and acetone extracts of root were found to be effective against the fungal pathogens, cold aqueous extract gave a zone of inhibition of 15mm and 17mm against *Candida albicans and Microsporum spp.* The zones were comparable to the zones given by the standard antifungal used. Ethyl acetate extract gave a zone of inhibition of 15mm against *Trycophyton rubrum* which was equal to the zone shown by the standard antibiotic used in the study, giving an indication of the effectiveness of the extract. Ethyl acetate extract also gave a zone of inhibition of 15mm against *Microsporum spp.* which was comparable to the zone of 18mm shown by the standard antifungal used. Acetone extract was effective against the all the used pathogens and zones were also comparable to the standard antifungal used. Acetone extract gave a zone of 16mm against *Trycophyton rubrum* which was more than the zone of 15mm shown by the standard antifungal.

MIC of most effective extracts was also calculated and it was found to be 31.25mg/ml.

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Corresponding Author:-
Mr.Jahir Alam Khan.,
R &D Division, MRD Life Sciences (P) Ltd., Lucknow (U.P), India.

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