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Antimicrobial studies of *Ricinus communis* seeds extracts

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Abstract

The bioactive molecules produced by medicinal plant react with microorganism and obstruct their growth and destroy their cells. Different polarity solvents were used for the extraction of *Ricinus communis* seeds. Antimicrobial activity of various *Ricinus communis* seeds extracts were screened against *Rhodococcus spp, Bacillus subtilis, Escherichia coli***,** *Aspergillus niger, Aspergillus flavus* and *Trichoderma harzianum.* Chloroform and Methanol extracts showed maximum inhibition zone of bacterial and fungal strains, while Acetone extracts showed significant antifungal activity than antibacterial activity and n-Hexane extracts were found unable to show any significant antimicrobial activity.

Keywords: Medicinal Plants; microorganism; *Ricinus communis;* Antifungal; Antimicrobial

1 Introduction

Many plants, spices and herbs are sources of various bioactive substances and therapeutic compounds, which are essential for maintaining the human health. These substances provide defensive mechanism to plants against predation by microorganisms, pests, insects and herbivores [1].

Ricinus communis (Euphorbiceae*)*, comprising the small, soft wooden trees that are widely distributed in tropics and warm temperature regions of world [2]. Its seeds are called castor beans, which are not true beans[3]. Castor oil plant have ability to tolerate bacteria, fungi, virus, insects, mycobacterium, nematodes, diseases, poor soil, heat, low and high pH, drought, salts, SO_2, weed, wind and wilt[4].

1.1 Chemistry of Castor Oil

Castor oil is a mixture of many triglycerides which contain several different fatty acids. It is a mono-unsaturated fat having one C-C double bond on each arm of the triglycerides. The main components of oil are ricin and ricinoleic acid. Ricinoleic acid also has a low quantity of oleic acid, linoleic acid, stearic acid and many other organic acids with it. Different percentage of all the acids present in castor oil has been shown in Table 1.

Table 1: Average composition of Castor oil/Fatty acids chain

Fatty acids in Castor oil	**Average Percentage Range**
Ricinoleic acid	95-85
Oleic acid	6-2
Linoleic acid	5-1
Linolenic acid	1-0.5
Stearic acid	1-0.5
Palmitic acid	1-0.5
Dihydroxystearic acid	0.5-0.3
Others fatty acids	0.5-0.2

Castor oil has been broadly used and very well known as laxative. It is considered to be a fast, safe and mild laxative and can promote a bowel movement in 2-3 hours, making it highly recommended for both children and aged. Castor oil is considered more effective when used regularly to clear the digestive track in case of poisoning [5].

2 Materials and Methods

2.1 Extraction of *Ricinus communis* seeds

In four different glass jars, 500 g of the *Ricinus communis* seeds (powder form) were taken and then dipped in 1000 ml of three different solvents i.e. methanol, *n*-hexane, chloroform and acetone for a week separately.

2.2 Preparation of Samples used for Inoculation

250 mg of each were taken in eppendrofs and dissolved in 1 ml of DMSO (Dimethyl sulfoxide). Then homogenized sample on vortex machine and centrifuged the sample for 10 minutes at 4 ^{0}C at 13000 rpm for inoculation on the Petri plates.

2.3 Antibacterial Assay

The Antibacterial activity of plant was checked against selected culture of both gram-positive and gram-negative bacteria. These bacterial strains i.e. (a) *Rhodococcus spp.* (Gram-positive) (b) *Bacillus subtilis (*Gram-positive) (c) *Escherichia coli* (Gram-negative) were used.

2.4 Preparation and Composition of Nutrient Agar Media

28 gram of nutrient agar was dissolved in 1000 ml distilled water in flasks, mixed by magnetic stirring to homogenize the solution and autoclaved at 15 minutes at 121 ^{0}C[6]. Composition of Nutrient agar media is given in Table 2.

Table 2: Composition of Nutrient Agar Media for bacterial strains

Chemicals	Amount
Peptone	5.0 g
Yeast extract	1.5 g
Agar-Agar	15 g
NaCl	5 g
pH	7.4
Distilled water	1000 ml

2.5 Preparation of Petri plates

200 ml autoclaved solution was poured in autoclaved Petri plates and let the medium to solidify over night. Next day, in Laminar flow burner the covers of plates were heated at burner to remove the moisture and then plates were kept in refrigerator for 30 minutes. Then 100 µl bacterial cultures were injected with micropipettes on three Petri plates and culture was spread on whole medium surface with the help of sterilized spreader.

2.6 Well Diffusion Method

Well diffusion method depends on the diffusion of various extracts from cavity through solidified agar layer of Petri plates to an extent, so that growth of inoculated microorganisms was prevented entirely in circular area or zone around the cavity containing the extracts.

Six wells were made in medium with the back side of 200 µl tip of micropipette. Centrifuged samples of methanol, *n*-hexane, chloroform, acetone, DMSO (control) and antibacterial drug, Kanamycine sulfate were poured in wells. All plates were kept in incubator over night. The incubator provides favorable temperature for microorganism's growth and next day inhibition zone were checked and measured in millimeter.

2.7 Antifungal Assay

Antifungal activity of *Ricinus communis* seeds extracts was checked against three fungal strains on Vogel Media [7].

2.8 Fungal Strains

These three fungal stains i.e. (a) *Aspergillus niger* (b) *Aspergillus flavus* (c) *Trichoderma harzianum* were used. Vogel media was used for antifungal assay which composition has described in Table 3.

Table 3: Composition of Vogel's media for fungal strains

Chemicals	Amount
Tri-Sodium citrate	2.5 g
KH_2PO_4	5.0 g
NH_4NO_3	2.0 g
$(NH_4)_2SO_4$	4.0 g
$MgSO_4.7H_2O$	0.2 g
$CaCl_2.7H_2O$	0.1 g
Peptone	2.0 g
Glucose	5.0 g
Agar	12 g
pH	5.5
Distilled water	1000 ml

2.9 Sterilization of Vogel's Media

Vogel Media was sterilized in Autoclave machine. The temperature and pressure were adjusted to 121 C^0 and 15 LD/sq for 15 minutes. Then flasks containing sterilized media were removed from Autoclave and kept at 50 C^0 for further use [8].

Solution prepared and washed plates were autoclaved and after the solution became cooled about 200 ml solution was poured in three plates. The plates were kept overnight till the medium was solidified. Then 100 µl fungal cultures were injected on all plates through micropipettes and spread on all plates.

2.10 Well Diffusion Method

Six wells were made on plates with the back side of 200 µl tip of micropipette, and 100 µl samples were injected in wells respectively. Dimethyl Sulfoxide (control) and antifungal drug (Terbinafine hydrochloride 125 mg) were also inoculated. All plates were kept in incubator providing suitable environment. Then next day plates were checked and inhibition zones were measured in millimeter (mm).

3 RESULTS AND DISCUSSION

3.1 Percentage Yield of Extracts

Percentage yield of *Ricinus communis* seeds extracts was determined by following formula, and has mentioned in Table 4.

Table 4: Percentage yield of plant extracts obtained.

Solvents used	Weight of plant material (g)	Weight of extracts obtained (g)	Percentage yield (%)
Methanol	500	3.5	2.33

n-hexane	500	2.5	1.66
Chloroform	500	4.4	3
Acetone	500	3.0	2

Percentage yield= Weight of extracts obtained/ Weight of the plant material×100

3.2 Antibacterial Activities

Antibacterial activity was investigated by applying methanol, *n*-hexane, chloroform and acetone extracts of plant seeds against three bacterial strains i.e. *Escherichia coli, Bacillus subtilis* and *Rhodococcus spp.* The results of antibacterial activity are mentioned in Table 5.

Table 5: Antibacterial activity of *Ricinus communis* seeds extracts against bacterial strains

Plant extracts	***Rhodococcus spp.*** Mean ± S.D.	***Bacillus subtilis*** Mean ± S.D.	***Escherichia coli*** Mean ± S.D.
Methanol	19.1 ±0.52	8.3 ±0.38	-
***n*-hexane**	-	-	-
Chloroform	14.9 ±0.30	12.1±0.59	14.0±0.68
Acetone	-	-	-
Kanamycine sulfate(Drug)	26.9 ±0.60	25.1 ±0.60	26.02 ±0.59

The extracts of seeds had showed (Table 5) effective antibacterial activity against *Rhodococcus spp*. The methanol extracts showed highest zone of inhibition of 19.1 mm and chloroform extracts showed inhibition zone of 14.9 mm, while *n*-hexane and acetone extracts didn't show any antibacterial activity against this bacterium. The antibacterial drug, kanamycine sulfate showed 26.9 mm inhibition zone. Comparison of antibacterial activity of *Ricinus communis* seeds extracts against *Rhodococcus spp.* has shown in figure 1(a).

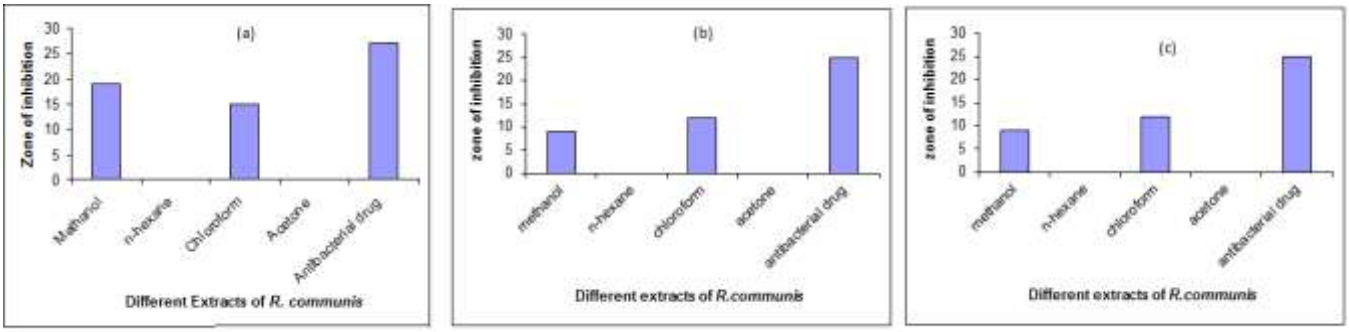

Fig. 1: Comparison of antibacterial activity of *Ricinus communis* seeds extracts against **(a)** *Rhodococcus spp* (b) *Bacillus subtilis* (c) *Escherichia coli***.**

Chloroform extracts of seeds showed maximum antibacterial activity of 12.1 mm and methanol extracts showed antibacterial activity of 8.3 mm while *n*-hexane and acetone extracts didn't show any activity against *Bacillus subtilis* while the drug used, kanamycine sulfate showed 25.1 mm inhibition zone as showed in Table 5. Comparison of antibacterial activity of *Ricinus communis* seeds extracts against *Bacillus subtilis* has shown in figure 1(b).

The chloroform extracts of seeds showed maximum inhibition zone of 14 mm while other extracts didn't show any antibacterial activity against *E.coli*. The antibacterial drug (kanamycine sulfate) used, showed inhibition zone of 26.02 mm as mentioned in Table 5. Comparison of antibacterial activity of *Ricinus communis* seeds extracts against *Escherichia coli* has shown in figure 1(c).

3.3 Antifungal Activity

Antifungal activity of plant seeds extracts was checked against three fungal strains *Aspergillus niger, Aspergillus flavus* and *Trichoderma harzianum.* Chloroform extracts of seeds showed greater inhibition zone of 18.8 mm than methanol extracts which is 17.1 mm, while extracts of acetone showed 14.0 mm zone and *n*-hexane extracts didn't show any activity against *Aspergillus niger*. The standard drug, Terbinafine hydrochloride showed inhibition zone of 14.1 mm while methanol, chloroform and acetone extracts have shown greater antifungal activity than the antifungal drug used as mention in Table 6. Comparision of antifungal activity of *Ricinus communis* seeds extracts against *Aspergillus niger* has also shown in figure 2(a).

Table 6: Antifungal activity of *Ricinus communis* seeds extracts against different strains of fungi

Plant extracts	***Aspergillus niger*** Mean ± S.D.	***Aspergillus flavus*** Mean ± S.D.	***Trichoderma harzianum*** Mean ± S.D.
Methanol	17.1 ±0.36	19.1 ±0.52	-

***n*-hexane**	-	-	-
Chloroform	18.86 ±0.47	16 ±0.35	-
Acetone	14.0 ±0.68	17.1 ±0.28	-
(Terbinafine hydrochloride)	14.1 ±0.40	14.4 ±0.30	13.9 ±0.35

Methanol extracts have shown greater antifungal activity of 19.1 mm and acetone extracts showed 17.1 mm inhibition zone while inhibition zone of chloroform extracts was 16 mm and *n*-hexane extracts didn't show any activity against *Aspergillus flavus***.** Standard drug used, Terbinafine hydrochloride has shown 14.4 mm inhibition zone, as showed in Table 6.

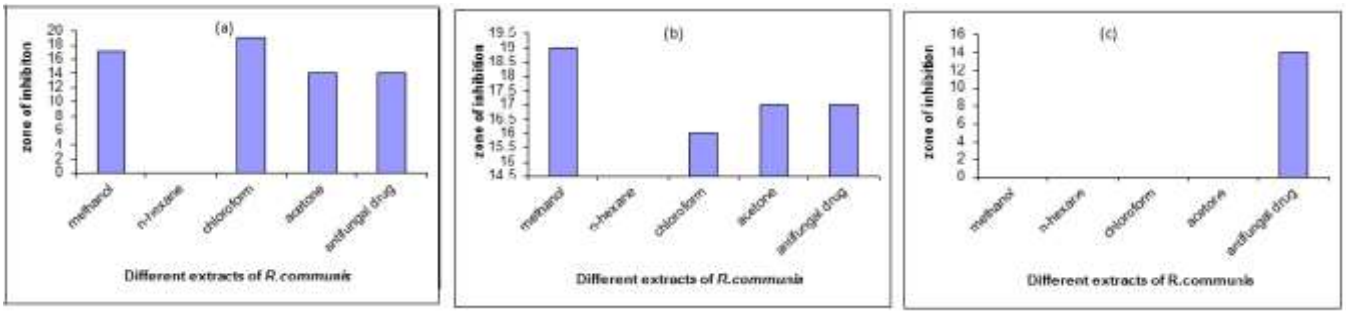

Figure. 2: Comparision of antifungal activity of *Ricinus communis* seeds extracts against **(a)** *Aspergillus niger*(b) *Aspergillus flavus* (c) *Trichoderma harzianum*

The data analysis exposed that all the extracts of plants seeds didn't show any inhibition zone against *Trichoderma harzianum* while standard drug (Terbinafine hydrochloride) has shown inhibition zone of 14 mm (Table 6), which revealed that *Trichoderma harzianum* was resistant to all plant extracts used. Comparision of antifungal activity of *Ricinus communis* seeds extracts against *Trichoderma harzianum* has shown in figure 2(c).

Conclusion

Most of the plant extracts contain such ingredients, which show inhibitory action against the different microorganisms like fungi and bacteria. The methanol extracts had adequate antimicrobial activity. Against some strains, chloroform extracts showed maximum activity than methanol extracts i.e. against *Aspergillus nige*r. Acetone extracts also showed activity against many strains. Nevertheless *n*-hexane

extracts seemed to have neither a synergistic nor additive antimicrobial activity on the tested microorganism.

Against the strains of bacteria much activity was showed by methanol and chloroform extracts while *n*-hexane and acetone extracts didn't show any activity.

Against the strain of *Trichoderma harzianum* none of the extract showed any activity. The methanol, chloroform and acetone have showed much activity against *Aspergillus niger* and *Aspergillus flavus,* even their activities are greater than the activity of standard antifungal drug used.

It is concluded that herbal drugs are very important source for discovery of new agents for treating various diseases related to bacterial and fungal infections.

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