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Antimicrobial Activity of Castor Oil Plant (*Ricinus communis*) Seeds Extract Against Gram Positive Bacteria, Gram Negative Bacteria and Yeast

[1]Hashem Rahmati, [3]Saeid Salehi, [2]Abdorrasoul Malekpour and [4]Farzaneh Farhangi
[1]Community Based Psychiatric Care Research Center,
[2]Stem Cell and Transgenic Technology Research Center,
Shiraz University of Medical Sciences, Shiraz, Iran
[3]Qeshm International Branch of Azad University, Qeshm, Iran
[4]Islamic Azad University of Kazeroun, Kazeroun, Iran

Abstract: The methanolic extract of *Ricinus communis* seeds was studied for its *in vitro* antimicrobial activities through the Agar Dilution Method. The seed extract of the castor oil plant inhibited the growth of *Bacillus subtilis*, *Staphylococcus aureus* (ATCC15156), *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli*. Also growth of *Candida albicans* was inhibited by crude extract of *Ricinus communis* seeds. The Minimum Inhibitory Concentration (MIC) of the extract ranged from 0.723-9 μg mL^{-1}. The phytochemical analysis of the crude extract of this plant seeds revealed the presence of anthocyanin, vitamins A and C a well as a good source of calcium, iron and vitamin B6. It also contains ricin, sterols, tannins and essential oils and can readily be considered as a healthy food. However, more evidence is needed to really sustain any claim related to their medicinal values. The results of the present study suggest that *Ricinus communis* can be used in treating diseases caused by the test organisms.

Key words: Antimicrobial activity, castor oil plant, pathogenic microorganisms, inhibitory activity, food

INTRODUCTION

Nature is a valuable source of medicinal plants. In Iran, almost all plants have therapeutic properties and the application of medicinal plants, especially in traditional medicine is currently well acknowledged and is established as a viable profession (Amin, 1991). Preparation of extracts which contain bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacological studies which lead to the production of more potent drugs with reduced toxicity (Ebana *et al*., 2001; Pamplona-Roger, 1999; Manna and Abalaka, 2000). However, these complementary components give the plant as a whole, a safety and efficiency much superior to those of its isolated and pure active components (Shariff, 2001). There is therefore, the need to search much more for plants of medicinal value and their medicinal applications. The World Health Organization (WHO) along with other national authorities now recognizes the antimicrobial resistance in both medicine and agriculture as a major emerging problem of public health importance.

Ricin, a toxic protein in the seeds, acts as a blood coagulant. Oil used externally for dermatitis and eye ailments. Seeds which yield 45-50% of a fixed oil, also contain the alkaloids ricinine and toxalbumin ricin and considered purgative, counter-irritant in scorpion-sting and fish poison. Leaves applied to the head to relieve headache and as a poultice for boils (Duke and Wain, 1981).

Fat droplets isolated from maturing castor bean seeds contain high concentrations of fatty acid synthetase and triglyceride synthesizing enzymes (Harwood *et al*., 1971).

In one study the agglutinin consistently agglutinated transformed cells at much lower concentrations than those required to agglutinate normal cell lines unless the normal cells were first treated with low concentrations of trypsin (Nicolson and Blaustein, 1972). Schurr and Schulze (1995) concentrated the xylem sap constituents in root exudate and in sap from intact, transpiring castor bean plants (*Ricinus communis L.*) and Halling *et al*. (1985) characterized and cloned and the ricin gene from *Ricinus communis*. In one study, Targosz *et al*. (2002) reported suicidal poisoning with castor bean (*Ricinus communis*) extract injected subcutaneously.

Corresponding Author: Abdorrasoul Malekpour, Stem Cell and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Ricinus communis root extract has anti-inflammatory and free radical scavenging activity (Ilavarasan *et al*., 2006) and seed extract of *Ricinus communis* anticonceptive and estrogenic effects (Okwuasaba *et al*., 1991). Klapheck *et al*. (1990) showed that the scavenging of hydrogen peroxide in the endosperm of *Ricinus communis* is because of ascorbate peroxidase. Purified fractions of 50% ethanolic extract of *Ricinus communis* have antidiabetic activity (Shokeen *et al*., 2008) and antifertility effects on rats (Sandhyakumary *et al*., 2003).

The aim of this study is to determine the antimicrobial activity of *Ricinus communis* seed extract against some gram positive bacteria, gram negative bacteria and yeast.

MATERIALS AND METHODS

Collection of plant materials: Castor oil plant seeds were collected at the plains and jungles of Shiraz, Fars, Iran. The plant was duly authenticated at the College of Agriculture of Shiraz University, Shiraz, Iran.

Source of microorganisms: The organisms used in this study were *Bacillus subtilis*, *Escherichia coli* (RITCC 2310), *Pseudomonas aeruginosa*, *Staphylococcus aureus* (ATCC 15156), *Candida albicans* (clinical: isolated from oral lesions caused by candidiasis) and *Salmonella typhi* (CT18). The organisms were obtained from the Department of Pathobiology, College of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Standardization of microorganisms: Exactly 0.2 mL of overnight pure cultures of each organism was dispensed into 20 mL of sterile nutrient broth and was incubated for 3-5 h at 37°C to standardize the cultures to 10^6 cfu mL^{-1}. A loop full of the standard cultures was used for the antimicrobial assay.

Extract preparation: The method by Okogun (2000) was used to obtain the seed extract. The 50 g of seeds were extracted with 200 mL of the solvent (9:1 mL distilled methanol:water, respectively). Extraction was allowed to proceed for 48 h. The extract was decanted and the solvent was then removed by evaporation at the room temperature (28±2°C) in order to obtain the extract. The air dried extract was stored for 48 h in sterile universal bottles at the room temperature. The sterility of the extract was tested before use.

Phytochemical screening of crude extracts: The phytochemical components of the medicinal plants were screened for determining the presence and amounts of ricin, oils, triglycerides, anthocyanin, vitamins A and C and calcium, iron, vitamin B6, sterols, tannins, saponins, phenols, saponin glycosides and essential oils by standard methods.

Screening for antibacterial activity: The method utilized by Collins *et al*. (1995) was employed to test the antimicrobial activity of the obtained extract. The 0.2 g of the extract was reconstituted in 5 mL sterile distilled water and was vortexed for homogeneity. Then, 1 mL of the reconstituted extract was added to Petri dishes which have sterile molten nutrient agar (Oxoid), in order to make a final concentration of 2000 μg mL^{-1}. The plates were prepared in duplicates and were set at the room temperature. A loop full of the standardized culture of test organisms was streaked on the solidified medium and was incubated for 24 h at 37°C. The control plates comprising extract without inoculum and inoculum with extract were made in parallel.

Determination of Minimum Inhibitory Concentration (MIC) of the extract: The MIC of the castor oil plant seed extract was determined on a solid medium (Nutrient agar) using the method utilized by Collins *et al*. (1995). Different concentrations of the prepared extract ranging from 0.5- 5.0 μg mL^{-1} were incorporated into the nutrient agar medium followed by the application of a standardized number of cells to the surface of the agar plate. The growth was assessed after the incubation for a defined period of time (16-20 h) at the temperature of 37°C and then the MIC value was read.

RESULTS

Phytochemical screening of active fractions of the extracts: The results of the phytochemical screening of the active fractions in the extracts of *R. communis* revealed the presence of ricin toxin, anthocyanin, vitamins A and C and B6 and sterols. Also, calcium, iron and tannins were detected in active fractions of castor oil plantseed extract. There were alsosaponin, saponin glycosides and phenols were found in its extract (Table 1).

Table 1: Phytochemical components of castor oil plant seed extract

Phytochemical components	Presence in castor oil plant extract
Saponin glycosides	+
Ricin	+
Saponin	+
Anthocyanin	+
Vitamin A	+
Vitamin C	+
Vitamin B6	+
Phenol	+
Sterol	+
Alcium	+
Iron	+
Tannins	+

'-': Absent; '+': Present

Table 2: The Minimum Inhibitory Concentration (MIC) of *Ricinus communis* seed extract for Gram positive and negative bacteria

Bacteria/Yeast	MIC ($\mu g\ mL^{-1}$)
Staphylococcus aureus	9.000
Bacillus subtilis	9.000
Pseudomonas aeruginosa	1.450
Escherichia coli	2.470
Salmonella typhi	4.900
Candida albicans	0.723

Antimicrobial activity of the crude extracts: The results revealed that the crude extract of the *R. communis* seeds exhibited antimicrobial effects on all test organisms. The extract inhibited the growth of *B. subtilis* and *S. aureus*. Nevertheless, it proved to be more effective on inhibiting the growth of *Pseudomonas aeruginosa, Salmonella typhi* and *Escherichia coli*. However, it mostly inhibited strongly the growth of *Candida albicans*.

The Minimum Inhibitory Concentration (MIC) of the extract: The MIC of *Ricinus communis* seed extract for *S. aureus* and *B. subtilis* was 9 $\mu g\ mL^{-1}$ whereas it was 0.723 $\mu g\ mL^{-1}$ for *C. albicans*. The extract inhibited the growth of *P. aeruginosa E. coli* and *S. typhi* at the concentrations of 1.45, 2.47 and 4.9 $\mu g\ mL^{-1}$, respectively (Table 2).

DISCUSSION

The crude extract of *Ricinus communis* seeds studied was found to contain the following phytochemical compounds: anthocyanin, vitamins A and C, calcium, iron and vitamin B6. It also contains sterols, tannins and essential oils.

The inhibitory effects of this medicinal plant on the microorganisms may therefore be due to the presence of the above phytochemical components. The results of the present study showed that the crude seed extracts of *R. communis* inhibit the growth of *Candida albicans*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* very well. This means that the extract has strong effects on these organisms.

The Minimum Inhibitory Concentration (MIC) for *Bacillus subtilis* is the same as of *Staphylococcus aureus* meaning that equal doses of antimicrobial agents will be required in infections where *B. subtilis* is the etiologic agent. The MIC value for *Candida albicans* was 0.625 $\mu g\ mL^{-1}$ suggesting that a very small amount of the drug can inhibit the growth of the organism. Thus, *S. nigra* is highly potent against *C. albicans*.

The results of the present study also showed the presence of anthocyanin, vitamins A and C, calcium, iron and vitamin B6. Also, the occurrence of tannins in *Ricinus communis* seed extract shows that the plant may be useful in various industries. For example, tannin is useful in food, pharmaceutical and leather industries as well as agriculture (Nguji, 1988; Dalziel, 1995). It could also be used as expectorants and decongestants.

CONCLUSION

Ricinus communis seeds have been found to be effective against some important pathogenic microorganisms involved in wounds, burns, skin infections and some microorganisms involved in enteritis, typhoid and candidiasis. Thus, the extract of *Ricinus communis* seed can be used in order to treat of these ailments, of course after the ricin toxin was excluded from it. The extract proved to be active against *Staphylococcus aureus* and *Bacillus subtilis* at high concentration and against *C. albicans* at very low concentrations. It is, however, more effective against *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*.

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