CYTOTOXIC ACTIVITY OF MEDICINAL PLANTS USED IN IRANIAN TRADITIONAL MEDICINE ON TWO STRAINS OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

The effects of methanolic crude extracts of some medicinal plants, which are used in Iranian traditional medicine on RS322N (rad52) of the yeast strians of *Saccaromyces cerevisiae* were investigated, using agar diffusion method and the concentration which produced 12 mm inhibition zone (IC_{12}) was determined. The RS322N IC_{12} for *Dorema ammoniacum* (oleogum resin), *Bunium persicum* (fruit) and *Illicium verum* (fruit) were 3.14, 5.58 and 5.27 mg/ml respectively. Based on these findings, oleogum resin of *Dorema ammoniacum* is a potential cytotoxic agent and a good candidate for further studies.

Keywords: Cytotoxic, Screening, Medicinal Plants, *Saccharomyces cerevisiae,* Topoisomerase I.

INTRODUCTION

Screening of medicinal plants to find new cytotoxic agents is a fruitful way for the discovery of anticancer drugs.

Camptothecin (cpt), an anticancer natural product which was isolated from *Camptotheca acuminata* (1) showed strong antitumor and anticancer activity in several studies, but its mechanism of action had not been identified until 1985 (2). It was found that cpt inhibits DNA topoisomerase I (top I) by trapping a reversible topI-drug-DNA ternary complex. Top I makes a transient single-strand break in DNA supercoil (3) and its inhibition is mechnism for drugs which show anticancer activity. Recently, the lower eukaryote, *Saccharomyces cerevisiae*, has been used for screening of top I inhibitors (4,5,6,7). This assay depends on the fact that yeast strains lacking the gene for the rad 52 DNA repair pathway are sensitive to agents which acts like camptothecin. The assay is carried out by measuring the growth inhibition of repair-deficient yeast in comparison to a wild type yeast strain which has the same permeability mutation (4,6,7). In the present investigation cytotoxic activity of three medicinal plants used in Iranian traditional medicine has been investigated by this method.

MATERIAL AND METHODS

Fresh oleo-gum-resin of *Dorema ammoniacum* D.Don was collected from Larestan , Fars province (south of Iran). Fruits of *Illicium verum* Mill. and *Bunium persicum* (Boiss.) B.Fedtsch were purchased from medicinal plants store and samples were identified by Department of Pharmacognosy, School of Pharmacy, Tehran University of Medical Sciences. RS188N (RAD^+) and RS322N (rad52) were from Laboratory of Biotechnology, Department of Plant Protection, School of Agriculture, Kerman University, Kerman. The fresh material was dried in air under shade and then ground to a fine powder. The powder plant materials (5gm) were extracted with 80% methanol (3×50 ml). The alcohol extract was filtered and the solvent was removed under reduced pressure at 40°C and the resulting extract was stored in refrigerator for biological studies. For performing bioaasay (6) YPD agar medium was prepared by following constituents per liter; yeast extract 10g, peptone 20g, dextrose 20g and agar 15 g. RS 188N (RAD^+) and RS322N (rad52) were plated on YPDA in 9×9 cm plates. Wells of 6 mm apart were made in the plates (9 wells in each plate) with sterilized cork borer. Methanolic extract was dissolved in DMSO- methanol (1:1 V/V)

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Table-1. Ethnobotanical information and cytotoxic activity of three medicinal plants used in Iranian traditional medicine.

Family	Umbelliferae	Umbelliferae	Magnoliaceae
Botanical name	*Dorema ammoniacum* D. Don	*Bunium persicum* (Boiss) B. Fedtsch	*Illicium verum* Mill
Common name Persian/English	Vashâ/ Gum Amoniacum	Zireh Kuhi/ Wild Caraway	Bâdeyân-e Katâi/ Star Anise
Plantpart	Latex	Fruit	Fruit
Traditional uses	Gasteric Problems antihelminitic	Anti-septic Carminative	Anti-septic Carminative
IC_{12} RS332	3.14	5.58	5.97
IC_{12} RS188	>20	>20	>20

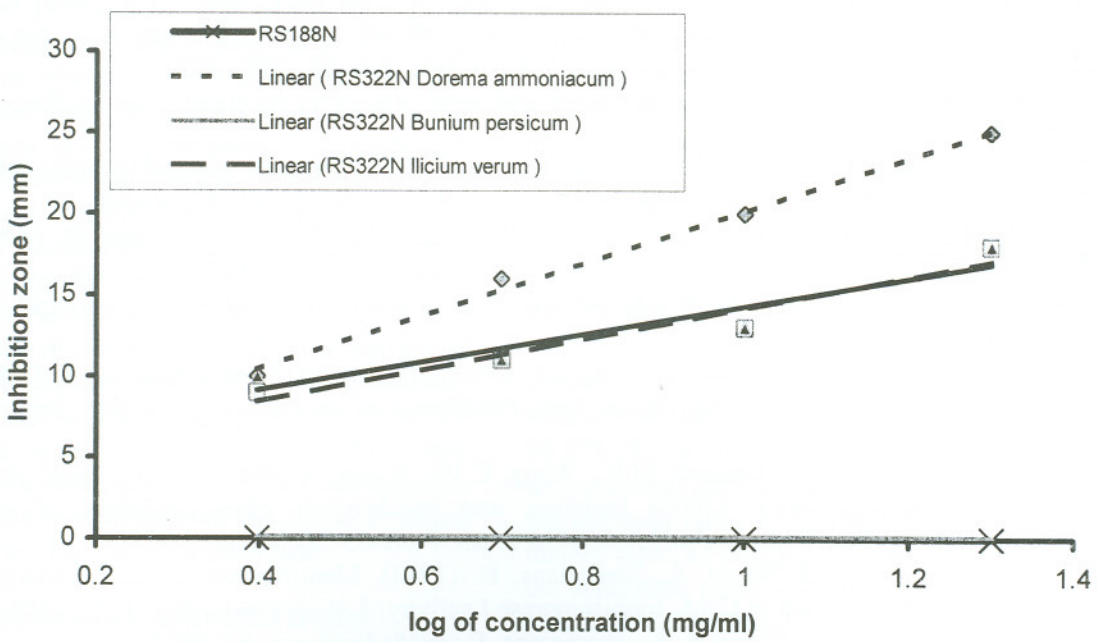

Figure 1: Linear regression analysis of log concentration vs zone size in two strains of *Saccharomycess cerevisiae*

and a 100 µl of 2.5, 5, 10 and 20 mg/ml samples were placed in each well. Cpt was used as positive control (3.125 µg/ml). Plates were incubated at 30°C for 72 h and the diameter of inhibition zone was measured. Activity was based on size differences of inhibition zones between the two strains. IC_{12} (dose that produces a 12mm zone) was determined by linear regression analysis of log concentration versus zone size (Figure 1).

RESULTS AND DISCUSSION

Yeast rad 52 mutant (RS322N) is hypersensitive to cpt (IC_{12} of 4 µg/ml) while RS188N strain is not (IC_{12} of 800 µg/ml). It has been shown that the presence of DNA top I is necessary for growth inhibition of cpt (9). Cpt is thought to increase the life time of top I-DNA covalent intermediate and apparently this intermediate is recognized as DNA damage by a DNA repair system. In *Saccharomyces cerevisiae* the Rad52

DNA repair system must be the predominate pathway for the repairment the cpt-stabilized enzyme-DNA covalent complex. Rad 52 mutants are much more sensitive to cpt than other repair-proficient strains because they are unable to complete the DNA repair process (4,9). In this study these strains were used for screening of natural products with cytotoxic potencies similar to cpt (6,7). It has been reported that an extract is active if it shows selective activity against one or more repair-deficient yeasts (IC_{12} less than one-third that of the wild type yeast) with IC_{12} less than 2000 µg/ml (6). In another report it has been indicated that 12-hydroxy-chiloscyphone, a sesquiterpenes from *Chiloscyphus rivularis* (Schard.) Hazlinsky (Hepaticaceae, Lophocoleaceae), showed selective bioactivity in yeast-based DNA-damaging assay (IC_{12} RS322=88 µg/ml , IC_{12} RS188>1000) (11). In the present study *Dorema ammonicum D.Don* showed the most selective cytotoxicity (IC_{12} RS322N: 3.14mg/ml and IC_{12} RS188N>20 mg/ml) (Table 1). An unpublished work has shown that *Ferula assa-foetida* L. and *F. gumosa* Boiss. (two Iranian endemic plants belonging to Umbelliferae) have cytotoxic components responsible for topI inhibition. The compounds responsible for the cytotoxic activity remain to be investigated.

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