Minimum Inhibitory Concentration (MIC) of Myrtus Communis Extract and Nystatin on Clinical Isolated and Standard Strains of Candida Albicans.

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ABSTRACT

Myrtle family is perennial, densely branched, evergreen plant which is described either as a shrub or tree depending how large it grows. In this survey, In vitro inhibitory effect of Myrtus communis extract and nystatin on clinical isolates of Candida albicans from vulvovaginal candidiasis and standard strains of C. albicans were studied. The plant extract was obtained from Barij essence co. The type strains of C. albicans were prepared from (PTCC 90028) collections. 45 clinical isolates of Calbicans that were confirmed by microbiological methods used in the tests. Inhibitory effects of the Extract analyzed by serial dilution broth technique. Based on the data analysis the best MIC of M. communis extract on clinical isolates and type strain of C. albicans were 25 mg/ml and 2.5 mg/ml, respectively. Also the best MIC of nystatin on clinical isolates and type strain of Calbicans were 36 mg/ml. The obtained results showed that Myrtle extract has inhibitory effect on clinical isolates and type Strain of Calbicans in lower concentrations than Nystatin drug. The present study suggest consideration of the plants extract with the highest antimicrobial activity and forms the basis for further investigations to isolate active components, elucidated the structures and evaluate them against wider range of microbial strains with the goal to find new the therapeutic principles. Substitution of commonly used antifungal and inhibiting chemicals by natural extracts such as Myrtle is recommended.

KEYWORDS: Myrtus communis, Nystatin, MIC, Candida albicans

1. INTRODUCTION

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance [1]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds [2]. Rural communities, depend on plant resources mainly for herbal medicines, food, forage, construction of dwellings, making household implements, sleeping mats, and for fire and shade. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries [3, 5]. Traditional healers claim that their medicine is cheaper and more effective than modern medicine. In developing countries, low-income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections [6]. We chose myrtle communis used in folk medicine to determine their antimicrobial activity higher plants. The research based on ethno pharmacological information’s is generally considered an effective approach in the discovery of new anti-infective agents from higher plants ([7, 4, 8, and 12]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants [10]. Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use. This paper reports the results of a survey that was done based on folk uses by traditional practitioners in north of IRAN (city of Lamia) along with bioassay test for antimicrobial activity. This plant can grow up to a 5m tree, but the wild ones in Malta are smaller - often shrub-like specimens. After fertilization, the stamens and petals drop off, followed by the style later. The developed fruit is a berry which is usually pale green, then turns deep red and finally becomes dark-indigo when fully mature. The glabrous berry can reach 1 cm in length and has a rounded (vase-like) shape with a swollen central part and remnants of the persistent calyx teeth (=sepals) at the outer part. Berries are edible with a sweet taste hence their widespread cultivation from ancient times in the Mediterranean region [6, 14]. It is reported that, on average, two or three antibiotics derived from microorganisms are launched each year. After a downturn in that pace in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited. Worldwide spending on finding new anti-infective agents (including vaccines) is expected to increase 60% from the spending levels in 1993. New sources, especially plant sources, are also being investigated. Second, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. So, the major focus for antifungal susceptibility testing has centered on Candida sp. This emphasis is due to. Candida albicans is the most common species implicated in Candida vaginitis worldwide. In recent years there has been an increasing interest

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in the use of natural substances, and some questions concerning the safety of synthetic compounds have encouraged more detailed studies of plant resources. Essential oils, odorous and volatile products of plant secondary metabolism, have a wide application in folk medicine, food flavoring and preservation as well as in fragrance industries. The antimicrobial properties of essential oils have been known for many centuries.

2-MATERIAL AND METHODS

**Preparation of extract:** Alcoholic extract of *Myrtus communis:* it was prepared from Barij essence in IRAN (KASHAN) [13].

**Test Organisms: Preparation of standard strains:** The type strains of *C.albicans* (ATCC90028) were prepared from in Iran. Yeasts were grown on Sabouraud dextrose agar over night at 37°C

**Preparation of clinical isolates:** 45 clinical Isolates of *C.albicans* that were confirmed by microbiological methods used in the tests.

**Preparation of inoculums:** Stock cultures were maintained at 4°C on slopes of Sabouraud dextrose agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of broth (SDB) for yeasts that were incubated without agitation for 24 hrs at 25°C. The cultures were diluted with fresh Sabouraud dextrose broth to achieve optical densities corresponding to (2.0-10³ spore/ml for yeast strains.

**Inoculation of drug containing tubes:** The semisolid agar tubes containing known concentrations of drug as well as drug-free controls, prepared in duplicate, were inoculated with one loopful of 0.5 McFarland adjusted culture by inserting the loop deep within the semisolid agar. One set of tubes was overlaid with 0.5ml of sterile oil. The tubes were incubated at 37°C for 48 hours. A loopful of the inoculums suspension was streaked onto Sabouraud dextrose agar to check for purity and viability.

2-1 **Antifungal susceptibility testing**

**Determination of MIC:** The minimum inhibitory concentration (MIC) of the extracts was estimated for each of the test organisms in triplicates. To 0.5ml of varying concentrations of the extracts (20.0, 18.0, 15.0, 10.0, 8.0, 5.0, 1.0 0.5, 0.05 and 0.005mg/ml), 2ml of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard (10⁵ cfu/ml) (for fungal isolates) was introduced to the tubes. The procedure was repeated on the test organisms using the standard antibiotics (nystatin for fungal isolates). A tube containing nutrient broth only was seeded with the test organisms as described above to serve as control. Tubes containing fungal spore cultures were incubated for 48 h at room temperature (30 – 32°C). After incubation the tubes were then examined for microbial growth by observing for turbidity. To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile sabouraud dextrose agar (for fungi) by streaking. Sabouraud agar only was streaked with the test organisms respectively to serve as control. Plates inoculated with inoculated with fungi were incubated at room temperature (30 – 32°C) for 48 h.

After incubation the concentration at which no visible growth was seen was noted as the minimum bactericidal concentration (8).

3-CONCLUSION AND DISCUSSION

The results of the control of the growth of the fragment for both extracts of *Myrtle communis* and nystatin as a drug, showed that the minimum inhibitory concentenction of extract and drug on clinical isolates is 25 microgram per milliliter and 2.5 microgram per milliliter respectively, and effect of the extract and nystatin on the clinical samples was 36 microgram per milliliter. Based on the statistics, the effect of the extract of *M. communis* on the isolated fungus and the standard strain in all of the three samples showed a kind of normal distrubution .Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is Very likely that these phytochemicals will find their way into the arsenal of antimicrobial drug...

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