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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 *C.albicans* 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 *C.albicans* were 36 mg/ml .The obtained results showed that Myrtle extract has inhibitory effect on clinical isolates and type Strain of *C.albicans* 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 initially 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 *Candidia 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 co 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 ($2.0 \cdot 10^5$ 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 (10^6 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^{o}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 saboraud dextrose agar (for fungi) by streaking. Saboraud 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^{o}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 concencentration 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 distribution .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 drugs prescribed by physicians and already being tested in humans. In recent years (1987-2001), a large number of essential oils and their constituents have been investigated for their antimicrobial properties against some bacteria and fungi in more than 500 reports. This paper reviews the classical methods commonly used for the evaluation of essential oil of antifungal activities of *Myrtus communis* [10, 11]. This study shows that myrtle extract has several acceptable inhibitory effects *on Candida albicans.* Shahidi and his colleagues made a similar study on the extract on the standard strain of *C.albicans* .The final report of it was based on distribution of disc in agar, while this study is done by the use of clinical strains and the determination of MIC was based on serial dilutions method in broth that has more sensitivity. Both clinical and standard strain of *C.albicans* in the same concentration had the same(similar) sensitivity of nystatin.Because of selective use of nystatin both virulent and standard strains and had the same results in relation to the drug .In result, we come to this point that extract has a less effect on the clinical strains in MIC rather than the standard strains, the same research is done on the native extract in kerman.It was shown that the effect of the extract was more in similar MICs on dermatophytes.In this study ,the leaf of the extract showed the most fungicidal effects ,but because of the variety and different climates in different regions, it is necessary to recognize the rate of effectiveness of the extract in different regions. To answer the question that if the results that are reached in the laboratory is successful In Vivo conditions or not, refer to the state of the invader strain, condition of host, immunological and pharmacological of the extract. So, it is necessary to do more studies and researches in future about the parameters such as Toxicity, allergic reactions and or microbial resistance to the extract [9, 10, and 13].

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