

Potent Synergism of the Combination of Natural Honey and *Peganum harmala* Seeds against *Candida albicans* ATCC 10231

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Abstract

The research on natural products and compounds derived from natural products has accelerated in recent years because of their importance in antifungal drug discovery. The aim of the present study was to compare antifungal activity of *Peganum harmala* (*P. harmala*) alone and in combination with 6 honeys from different regions of Algeria against *Candida albicans* (*C. albicans*). The combination action of honey with *P. harmala* was assayed by the well agar diffusion. The results indicate that the powder of *P. harmala* and honey are efficient against the tested yeast. The diameter of the zone of inhibition ranged from 5 to 9.1 mm for honey and 1 to 5.6 mm for *P. harmala*. The diameter of the zone of inhibition ranged from 1.33 to 17 mm for honey and *P. harmala*. The combinations of *P. harmala* with six honeys samples were always more efficient. Thus, the mixture of *P. harmala* and honey could lead to the development of new combination antibiotics against yeast infection.

Keywords: Antifungal activity; Honey; *Peganum harmala*; *C. albicans*

Introduction

Invasive fungal infections have emerged as a major cause of morbidity and mortality in severely immuno-compromised patients such as those undergoing chemotherapy or haematopoietic stem cell transplantation [1]. *Candida albicans* (*C. albicans*) is a major fungal pathogen of humans and a commensal organism of the gastrointestinal tract [2]. In severely immunocompromised patients this fungus causes high morbidity and mortality. *C. albicans* is also the etiological agent of vulvovaginal candidiasis, a common pathological condition, afflicting normal women of fertile age, which frequently develops into a chronic, substantially incurable, disease [3,4]. In recent years, there has been an increasing search for new antifungal agents. However, since many of the available antifungal drugs have undesirable side effects or are very toxic (amphotericin B), produce recurrence, show drug-drug interactions (azoles) or lead to the development of resistance (fluconazole, 5-flucytosine), some shows ineffectiveness [5] and have become therefore less successful in therapeutic strategies. Therefore it is necessary to search for more effective and less toxic novel antifungal agents that would overcome these disadvantages. Natural products have been traditionally used in the treatment of diseases because they are sources of many active compounds.

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. *P. harmala* is a wild growing flowering plant belonging to the Zygophyllaceae family and is considered an important medicinal plant. The seeds are known to possess Antifungal properties [6]. Honey is the natural product obtained by honeybees from the nectar of flowers or from secretions of living parts of plants or excretions of plant sucking insects, which the bees collect and transform by combining with specific substances of their own and store in the honeycomb to ripen and mature [7]. Honey is regarded as an excellent food and as an elixir or medicine [8]. Although several *In vitro* studies have demonstrated the antibacterial activity of honey [9,10]. Limited numbers of studies have examined the activity of honey against fungi. The importance of *P. harmala* seeds and honey cannot be overemphasized as regards their rule in health remedy. However, no documented evidence has been found to show that the mixture of *P. harmala* seeds and honey can be used as a

natural remedy against vulvovaginal candidiasis, following a thorough review of related literature. The aim of this study was to investigate the effects of the mixture of natural honey and *P. harmala* seeds against *C. albicans*.

Materials and Methods

Plant materials and preparation

P. harmala seeds were obtained from the local seed supplier. The seeds were crushed manually in a mortar with a pestle and 100 ml of distilled water was added to 20 g of dry powder. It was vortexed continuously until there was no further change in color of the solution. This solution was centrifuged at 800 rpm for 15 min. The supernatant was filtered through Whatman filter N°4 and stored at 4°C in sterile tubes until use.

Honey samples

Six honey samples produced in different regions of Algeria. The samples were taken directly from the containers that the beekeepers use for the storage of honey. All samples were collected in their original packages and were transferred to the laboratory and kept at 4–5°C until analysis.

In vitro antifungal assay

Preparation of inoculum: The fungal culture of *C. albicans* strain (ATCC 10231) was grown on Sabouraud Dextrose Agar (SDA; Merck, Germany) plates at 28 ± 2°C for two weeks. The conidia fungi were collected using sterile normal saline solution on the agar surface

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followed by gentle shaking. The suspension was vortexed and heavy particles were allowed to settle for 3-5 min. The inoculum was prepared spectrophotometrically to give a final concentration of 1.0×10^6 CFU/mL [11].

Antifungal assay using well diffusion method: Antifungal activity was measured using a well diffusion method according to the National Committee for Clinical Laboratory Standard [11]. Briefly, Petri plates (90 mm) containing 20 ml of nutritive agar medium were inoculated with a 28 h culture of the yeast strains. Wells (8 mm diameter) were punched in the agar and filled with 50 μ l of *P. harmala* seed extract or honey and in case of synergism 50 μ l of each into the well. The plates were incubated at 37°C for 48 h. The antifungal activity was assessed by measuring the diameter of the area in which yeast growth was inhibited around the well. Each experiment was repeated at least twice. The controls were set up with equivalent quantities of water.

Results

The results of the assays of antifungal activity of the *P. harmala* with six concentrations (100, 50, 25, 12.5, 6.25 and 3.12%) in this study are shown in decreasing order of activity, the inhibition zones were ranked as follows: $5.6 > 1.13 > 1.1 > 1 > 0$ (Figure 1).

The antifungal assay indicated that the diameter of the zone of inhibition values ranged from 1 to 5.6 mm and varied with the concentrations. The highest zone of inhibition obtained was by 12.5% (5.6 mm), while the lowest zone of inhibition obtained for the five concentrations, respectively were 0, 1, 1, 1.1 and 1.3 mm (Figure 1). The zone of inhibition obtained also varies at different concentration used.

The results of the assays of antifungal activity of six honeys in this study are shown in ascending order of activity, the inhibition zones were ranked as follows: $H5 < H4 < H1 < H6 < H3 < H2$. The zone of inhibition values were between 5 to 9.1 mm (Figure 2).

The combination effects of anti-*Candida* activity of honey and *P. harmala* are summarized in (Figure 3). The zone of inhibition values were between 1.3 to 17 mm. The highest zone of inhibition obtained was by 100% (H3: *P. harmala*; 17 mm). While the lowest zone of inhibition obtained for 100% concentration was (H6: *P. harmala*; 1.13 mm). Results indicate a considerable antifungal activity of *P. harmala* and honey.

Discussion

The development of drug resistance in human pathogens against

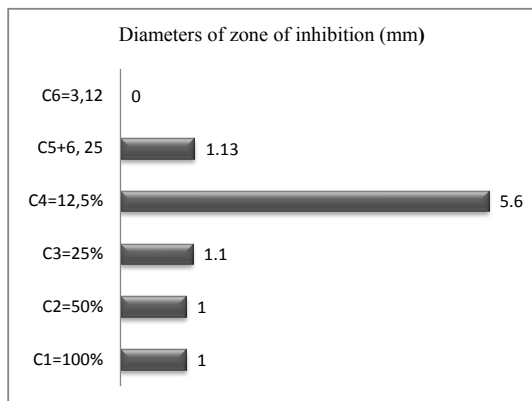


Figure 1: The diameters of zones of inhibition in (mm) of *P. harmala* seeds against *C. albicans* ATCC 10231.

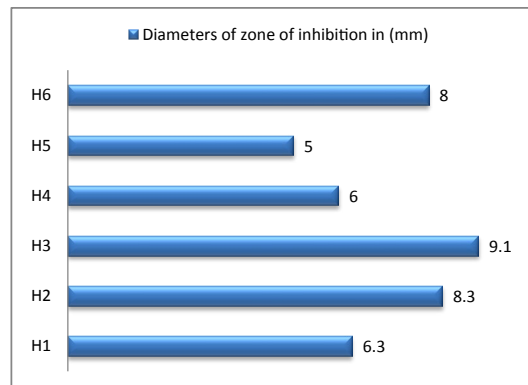


Figure 2: The diameters of zones of inhibition in (mm) of 6 honeys against *C. albicans* ATCC 10231.

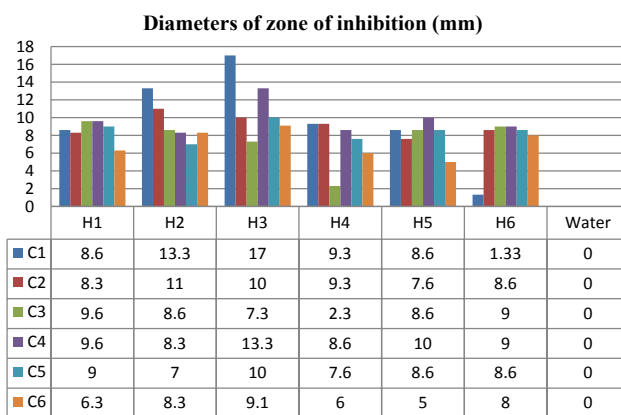


Figure 3: Mixture of honey and *P. harmala* seeds against *C. albicans* ATCC 10231.

commonly used antifungals has necessitated a search for new antimicrobial substances from plants [12,13]. Natural products have been traditionally used as therapeutic agents and about half of the drugs that we use today are derived from natural sources [14,15]. In recent years, interest in the application of honey and medicinal plants (MPs) in the treatment of infectious diseases has notably increased. In this study, we evaluated the anti-*C. albicans* activity of Honey and *P. harmala* seeds. Several publications on antifungal activity of honey and MPs are reported in literature. In the present paper powered obtained from *P. harmala* mixed with honey showed a good antifungal action against *C. albicans*. The antifungal activity of a number of alkaloids isolated from different plant species has been reported [16]. *P. harmala* seeds have been considered from ancient time to date as a plant with drug usages regarding to some alkaloids compounds such as harmalin and harmalol. Honey is a natural food produced by honey bees from the nectar of a variety of plants. Recently, the potential antifungal effects of honey have attracted serious attention within the scientific community [17-19].

The mechanism of action of honey has not been definitely proven though acidity, osmolality, and hydrogen peroxide production have been proposed as important factors [20]. Research on combination is very limited, and few studies have been reported. The secondary metabolites from plants are good sources for combination therapy. The results of this study showed that adding honey to *P. harmala* increases the antifungal effect against *C. albicans*.

It seems that there is an over-additive action between honey and the tested medicinal plants; this action is also called synergism [21]. The combination of honey plus some natural additives has superior results in its antibacterial, antifungal, and wound-healing promotion properties compared with pure bee honey and some other topical wound agents alone [22]. The exact mechanism of synergy between medicinal plants and honey is unclear.

Conclusion

Further studies are necessary to elucidate the mechanism of action of the synergistic combinations reported here. The shown potential of honey to enhance *P. harmala* and especially antifungal action is of potential medical interest especially for topical application.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgement

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