**In vitro Study on Antifungal Activity of Achillea wilhelmsii Flower Essential Oil Against Twenty Strains of Candida albicans**

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**Received:** 22 April 2013
**Accepted:** 30 May 2013

**ABSTRACT**

Infection diseases always were important worry. In the past decade numerous reports of treatment failures are reported. It is important to increase effective therapy. The aim of this study was in vitro studing of antifungal effects of the flowers essential oil of Achillea wilhelmsii C. Koch. In this study essential oil was obtained by hydrodistillation using Clevenger type apparatus for 3h. It was tested in vitro against 20 fungi strains. Several dilution of essential oil (10%, 15%, and 20%) was prepared. Antifungal activity was tested by using microdilution MIC determination, disk diffusion assay and agar well diffusion assay. Data were analyzed by ANOVA test in the P-Value< 0.05. The results illustrated that essential oil in dilution 20% has more inhibitor activity against 20 Strains of Candida albicans. The results of the present project indicate that the Achillea wilhelmsii flowers inhibited fungi growth but their effectiveness varied. Therefore we will be able perform researches with extraction of this plant effective compound for the treament of infectious disease.

**Keywords:** Achillea wilhelmsii, Candida albicans, essential oil

**1. INTRODUCTION**

Candida species are innocuous saprophyte yeasts that found in the gastrointestinal tract, oral and vaginal mucosae[1, 2, 3]. Nevertheless, if the immune defense of the host becomes compromised, Candida can cause intensive systemic infections in human immunodeficiency virus (HIV), anticancer therapy, organ transplantation, abdominal surgery, catheters, diabetes and the use of broad-spectrum antibiotics [1, 4].

The most popular way for controlling Candida infection is using limited synthetic drugs and fungicides. They are limited on the eukaryotic nature of fungal cells, which are similar to host cells. Beside, structures of synthetic drugs create resistant strains. Accordingly, researchers focus on natural therapy such as essential oils and extracts [5].

Medicinal herbs illustrate one of the
important fields of traditional medicine all over the world. Over the past 20 years, there has been an increased interest in the research of natural materials as sources of new antimicrobial factors. Various extracts from traditional medicinal plants have been experimented to determine the source of the therapeutic effects. The most important of plant’s bioactive compounds are tannins, alkaloids, flavonoids and phenolic compounds [6].

The Achillea, which belongs to botanical family Asteraceae, comprises more than 120 species. In the recently years, the Achillea has been the subject of much scientific research which have showed its anti-inflammatory, antihypertensive, anti-hyperlipidemia and antitumor [7]. Achillea is employed in man medicine for the treatment of various diseases such as gastrointestinal disorders, antispasmodic, choleretic, antiulcer, antibacterial (Helicobacter pylori) and hepatoprotective on the gastrointestinal tract [8].

Achillea wilhelmsii C. Koch (Asteraceae) is widely found in different parts of Iran. This plant is full of flavonoids and sesquiterpene lactones, which have been shown to be effective in lowering blood lipids and hypertension [9]. It has chemical components, including flavonoids, alkaloids (achilleine), cineol, borneol, α- and β-pinen, camphor, caryophyllene, thujene, rutin, sesquiterpenoids and monoterpenoids [8]. The chemical composition of the essential oil of flowers Achillea wilhelmsii have determined by GC/MS analysis. Among the fifty-three components representing %86.53 composition of the oils. The major constituents of the essential oil were: camphor, berbenone, α-pinene, chryphenone, 1-8 cineole [10]. Leaves and flowers methanolic extract and essential oil of Achillea wilhelmsii have shown antibacterial properties against Escherichia coli, Bacillus cereus and Staphylococcus aureus [11, 12, 13]. There have been some reports on the essential oils antimicrobial activity of Achillea biebersteinii Afn., Achillea toreyeta, Achillea frasii, Achillea setacea, Achillea clavennae and Achillea teretifolia [14, 15, 16, 17].

Hence, the main aim of this study was to determine antifungal properties of flowers essential oil Achillea wilhelmsii C. Koch. It was tested against twenty different strains of Candida albicans isolated from oral, vaginal, and nail candidacies patients.

2. MATERIALS AND METHODS
2.1 Plant Material
Achillea wilhelmsii C. Koch was collected from countryside of Isfahan, Iran in May 2011. The species was identified in herbarium of Research Institute of Isfahan Forests and Rangelands. They were air-dried in the shade at room temperature. The samples were homogenized in an electric grinder to fine powder.

2.2 Essential Oil Preparation
For essential oil’s preparation, 50 gram of flowers powder of Achillea wilhelmsii C. Koch was obtained from hydro-distillation (500 c.c distillate water) for 3 h using a Clevenger-type apparatus [18, 19]. The oil yield was 150 μl (50g flower powder). Oil sample was stored at 5°C in dark sealed glass vials until each experiment [5, 20, 21]. The essential oils were dissolved in dimethylsulfoxide (DMSO) [20, 21].

2.3 Strains and Growth Conditions
Microorganisms were obtained from the veterinary faculty of Tehran University, Iran. Twenty strains of Candida albicans, with names: SC-2-a; SC-2-a1; SC-2-a12; SC-2- a16; SC-2-a17; SC-2-b11; SC-2-b19; SC-2-b21; SC-2-b23; SC-2-b27; SC-2-b29; SC-2-c14; C.589; C.590; C.592; C.593; C.596; C.62061; C.1167; C.1677.
were used. They were maintained at 4°C on Sabouraud dextrose agar (SDA) plates and subcultured at 37°C in Sabouraud dextrose broth (SDB) before each experiment to ensure viability and purity [1].

2.4 Determination of MIC by Microdilution

Serial dilutions of the essential oil (10%, 5%, 2.5%, 1.25%, 0.625 %, 0.313 %, 0.156 %, 0.078 %, 0.039 %, 0.019 %) were loaded in a microdilution plate (96 wells). Then, the inoculums (20 μl 1.5×10^8 cfu/ml) were added to each well. The microplates were incubated at 37°C for 48 h. The MIC was defined as a lowest concentration which resulted in inhibition of visual growth absorbance was read in an ELISA plate reader at 492 nm. Minimal fungicidal concentrations (MFC) were determined by subculturing 10 μl of the culture from each negative well and from the positive control, measured as explained [1, 18]. Growth inhibition of each fungal strains was counted as the percentage of forbid of growth relative to the control, fluconazole. A standard antifungal agent, fluconazole served as a positive control and dimethysulfoxide was used as a negative control [19].

2.5 Disk-diffusion Assay

100 ml of 1.5×10^8 CFU/ml yeast suspension was spread monotonically onto the SDA plate using cotton swabs. Next, paper disks which arranged on the agar surface impregnated with 10%, 15%, and 20% dilution of essential oil were placed onto agar plates. Inhibition diameters (in mm) were measured after incubation at 37°C for 48 h.

2.6 Statistical Analysis

The experiments were performed three times to minimize the error and the mean values are presented. Data were analyzed using ANOVA test in the P<0.05.

3. RESULTS AND DISCUSSION

The antifungal activity of the essential oil of *Achillea wilhelmsii* C. Koch’s flower evaluated against twenty strains of *Candida albicans* isolated from patients with a MIC assay. The mean MICs and MFCs for the essential oil are given in Figure 1. It should be noted that, it in dilution 20% had strong effect. As well as, it showed strong activity with MICs of 0.039%.

![Figure 1. MICs and MFCs for the essential oil (%)](image-url)

Essential oil was tested for its power of antifungal activity using a disk-diffusion assay which disks were plated onto SDA plates. Maximum inhibition zone (31±1 mm) of essential oil was obtained against Sc-2-b11, in 20% dilution. In other, minimum inhibition zone (12.67±0.58 mm) of that was obtained against C.62061, in
10% dilution. Results showed that, there is direct relationship between dilution and inhibition zone Figure 2. It is worth mentioning that there are significant changes between the essential oil and positive control sample, fluconazole (P < 0.05). The reason for higher inhibition by the standard antibiotic is the purity of the drug [22], Thus, the results of Mar & Lumyong (2012) clearly demonstrate that growth media plays an important role in the determination of antibacterial and antifungal activities [23].

![Figure 2](image)

Figure 2. The antifungal activity of the flowers essential oil of *Achillea wilhelmsii* on different strains of *Candida albicans*.

Nowadays Candida species are one of the most frequent organisms isolated from blood of hospitalized patients. Despite the increasing need for efficient treatment, span of antifungal available is limited. Furthermore, some of the most impressive agents are toxic and some of them, such as azoles have numerous reports of remedy failures in new literature [1, 3].

Plant extracts have been used for many thousands of years in food preservation and pharmaceuticals. *Achillea wilhelmsii* employed in folk medicine for treatment of various diseases such as sedative, anti-inflammatory, analgesic, and to relieve symptoms in premenstrual syndrome (PMS) [24].

Herbs have a highly capability to synthesize aromatic products specially phenols which are secondary metabolites. Mostly, these products serve as plant defense mechanisms against versus predation by microorganisms, insects, and so on [7]. Universally, herbal products provide some changes in microorganism’s cell, such as cell wall collapse, cytoplasmic membrane disruption, cytoplasm granulation, and inactivation or inhibition of enzyme activity within cell and outside cell [25, 26, 27].

Several of these products, such as sesquiterpene lactones, terpenoids which are in charge of plant odours; others like quinones and tannins are responsible for plant pigment [6].

Lipophilic secondary metabolites, such as monoterpenes, diterpenes, triterpenes, steroids and tetraterpenes function as substrates for ABC transporter from fungi (Art B) or the Nor A efflux pump in bacteria [28]. Because, they function as inhibitors contesting for binding to the active side of the transporters [28].

Essential oils are non-polar solution and high antifungal potential of these due to terpenoids. They provide membrane disruption on microorganisms [29]. Essential oils are aggregate compounds characterized by a strong smell and are formed by aromatic plants as secondary metabolites. In nature, essential oils play an important role in the conservation of the plants. The chemical
profile of the essential oil products can modify in quality, quantity and in composition according to climate, soil composition, plant organ, age and vegetative cycle stage [30]. In the Davari (2012) studies showed that, the major compounds in the Achillea wilhelmsii flowers essential oil were camphor, verbenone, α-pinene, 1,8-cineole and camphene. Jose Abad et al., (2007) have shown that Rosmarinus officinalis essential oils were antifungal, because the major compounds in the essential oil were α-pinene, camphene, camphor, verbenone, borneol and bornyl acetate [31]. Thus, according to the findings of Jose Abad et al., (2007) 1, 8-cineole was already known as antifungal agent [31]. The studies of Deba et al., (2008) indicated that α-pinene detected in Bidens pilosa Linn. and Pistacia lentiscus essential oils play an important role in antifungal activities [32]. These findings are compatible with our finding, which showed the this plant with presence camphor, verbenone, α-pinene, 1,8-cineole and camphene compounds, inhibited fungi growth.

Possible synergistic and antagonistic properties of compounds also play an important role in fungi inhibition [32]. However, It is possible that the activity of the basic component is modulated by other minor molecules [30].

4. CONCLUSION
These results demonstrate antifungal potential of the essential oil of Achillea wilhelmsii C. Koch flower. In following, the results indicate backing idea that medicinal plants can be promising sources of potential antifungal agents. The authors suggested displaying the essential oil of Achillea wilhelmsii C. Koch against other important human pathogens. This supports its gaining popularity as an antifungal source.

ACKNOWLEDGEMENTS
This work was supported by Islamic Azad University, Falavarjan Branch; the authors also thank Dr. Ranjbar and Dr. Monajemi for their kindly aid.

REFERENCES


