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Evaluation the Bioactivity of Some Medicinal Plants and Arabic perfumes Extracts on Selected Pathogenic Fungi

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ABSTRACT

The antimicrobial effect of aqueous extracts of Six medicinal plants *Cinnamomum zylanicum* (Bark), *Dianthus caryophyllus* (Fruit), *Zingiber officinale*, *Allium sativum* (Fruit), *Curcuma longa* and *Elettaria cardamomum*), three kinds of musk (Red musk, China& Indian musk), Propris and Teab were studied against four fungi namely, *Aspergillus flavus*, *Epidermophyton floccosum*, *Microsporum canis* and one yeast *Candida albicans* that are had a medicinal importance and cause skin infections. The inhibition zones were determined by using agar well diffusion method. The results revealed that *Dianthus caryophyllus* was had the best antimicrobial properties (Fruit), followed in order by *Allium sativum* (Fruit), *Cinnamomum zylanicum* (Bark), then Red musk and Teab. The minimum inhibitory concentration (MIC) for the aqueous extract was between (1:6) and (1:1) dilutions for fungi under study. The results therefore established a good support for the use of *Dianthus caryophyllus, Allium sativum* and *Cinnamomum zylanicum*, in traditional medicine

INTRODUCTION

Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants (1). Many efforts have been done to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants (2). The active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (3).

In spite of the existence of potent antibiotic and antifungal agents, resistant strains are continuously appearing, so the antibiotic resistance has become a global concern (4). Imposing the need for a permanent search and development of new drugs (5). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (6). Numerous studies have identified compounds within herbal plants that are effective antibiotics (7). Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics (8). Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (9) others only use aquatic , alcoholic or organic extractions for the same purpose (10)

The aims of this study is to investigate the aqueous extracts of some natural compound and medicinal plants that may be used as therapeutic potential to control fungal diseases.

MATERIAL AND METHODS

Collection of Plants and other natural compound:

Six fresh or dried plant parts were collected randomly from Sana'a market, Yemen. Whole medicinal plants (*Cinnamomum zylanicum* (Bark), *Dianthus caryophyllus* (Fruit), *Zingiber officinale*, *Allium sativum* (Fruit), *Curcuma longa* and *Elettaria cardamomum*), in addition to three kinds of musk (Red musk, China musk& Indian musk), Propris and Teab were taken for investigation of antimicrobial property. The Fresh plant material were washed under running by sterilized distilled water, air dried and then homogenized to fine powder and stored in airtight bottles.

Preparation of extracts:

Aqueous extraction, 10 g of air-dried powder was added to10 ml distilled water and extracted by shaking at room temperature, for 30 minute and stored at 4 ^{o}C (11).

Microorganisms:

In vitro antimicrobial activity was examined for aqueous extracts from six medicinal plants and three natural products that used by traditional healers. The well identification microorganisms were obtained from Central Public Health Laboratory (CPHL) (20 specimens) and Modern International Laboratory (52 specimens), Sana'a, Yemen. Microorganisms were maintained at 4°C on Sabouraud dextrose agar.

Antimicrobial assay:

The antimicrobial assay was performed by well diffusion method (12). The Sabouraud dextrose agar was inoculated with the 100 µl of the inoculum (1 x 10^8 Cfu) and poured into the Petri plate. With the help of a cork-borer (0.8cm). 100 µl of the extract was introduced into the well. The plates were incubated overnight at 37 °C. Control was maintained where pure sterile distilled were used instead of the extract. The result was obtained by measuring the inhibition zone diameter (mm). The experiment was done three times and the mean values are presented.

Determination of minimum inhibition concentration (MIC).

Different concentration of the extracted antimicrobial agent were carried to obtain the lowest concentration that give antimicrobial effect. Dilution series was by using sterile distilled water (Gul, *et al.*,2004). Seven fold dilutions were prepared in the water serially. One ml of each dilution was added in to the holes of the seeded plates with target organisms. The MIC was determinate by agar well method; the inhibition zones that appeared around the wells determinate the effect of the extract. The highest dilution preventing the organism growth was represented as MIC (13**;** 14).

RESULTS AND DISCUSSION

The data reported in Table (1) presents the antimicrobial activity of the aqueous extracts agonist *Candida albican*s. The results indicate that the extracts from the medicinal plant studied showed the largest inhibition zone around *Dianthus caryophyllus* (40mm), and the MIC was at (1:6), follows by *Cinnamomum zylanicum* (35mm), and the MIC was at (1:4), *Allium sativum* (28mm), (see Fig 1), then *Red musk* and the MIC was at (1:2) dilution after one day. However the rest extracts were shown no effect.

Table (1): Antimicrobial activity of aqueous extracts at different dilutions on *Candida albican*s

Dilutions / Extracts	Zone of inhibition (mm)						
	1:1	1:2	1:3	1:4	1:5	1:6	1:7
Allium sativum	28	16	16	15	-	-	-
Curcuma longa	-	-	-	-	-	-	-
Cinnamomum zylanicum	35	20	17	16	-	11	-
Dianthus caryophyllus	40	29	25	23	12	11	-
Zingiber officinale	-	-	-	-	-	-	-
Elettaria cardamomum	-	-	-	-	-	-	-
Teab	-	-	-	-	-	-	-
Red musk	18	15	-	-	-	-	-
China musk	-	-	-	-	-	-	-
Indian musk	-	-	-	-	-	-	-
Propris	-	-	-	-	-	-	-

Values are mean of three replicates; -: no inhibition zone; cork-borer diameter 8 mm, the extract diluted by sterile distilled water

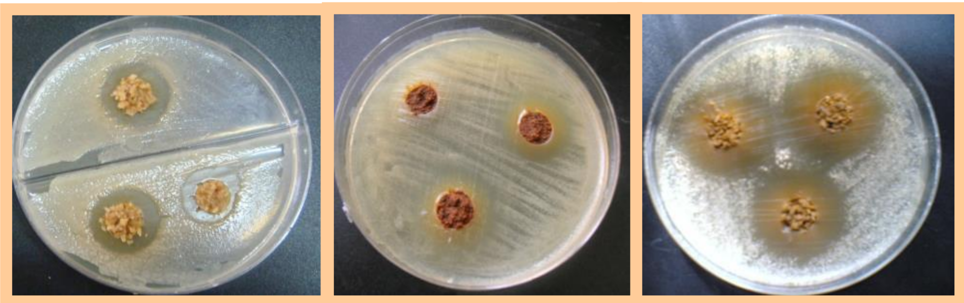

Fig (1) Inhibition zones of *Allium sativum* (left) *Cinnamomum zylanicum*(mid) *Dianthus caryophyllus* (right) on *Candida albican***s** at (1:2) dilution , after (1) day

Table (2) shows the antimicrobial activity of the aqueous extracts on *Aspergillus flavus*. The results of the medicinal plant extracts studied showed the largest inhibition zone around *Dianthus caryophyllus* (45mm), and the MIC was at (1:6), follows by *Cinnamomum zylanicum* (39mm), and the MIC was at (1:6), *Allium sativum* (28mm) , *Red musk* at (22mm) and the MIC was at (1:2), then Teab (19) and MIC was at (1:1) dilution after one day (Fig 2,3)**.** Although the other extracts had no effect.

Table (2): Antimicrobial activity of aqueous extracts at different dilutions on *Aspergillus flavus*

Dilutions	**Zone of inhibition (mm)**						
Extracts	1:1	1:2	1:3	1:4	1:5	1:6	1:7
Allium sativum	28	21	19	17	14	14	-
Curcuma longa	-	10	-	-	-	-	-
Cinnamomum zylanicum	39	23	17	16	15	15	-
Dianthus caryophyllus	45	31	26	15	13	10	-
Zingiber officinale	-	-	-	-	-	-	-
Elettaria cardamomum	-	-	-	-	-	-	-
Teab	19	-	-	-	-	-	-
Red musk	22	15	-	-	-	-	-
China musk	-	-	-	-	-	-	-
Indian musk	-	-	-	-	-	-	-
Propris	-	-	-	-	-	-	-

Values are mean of three replicates; -: no inhibition zone; cork-borer diameter 8 mm, the extract diluted by sterile distilled water

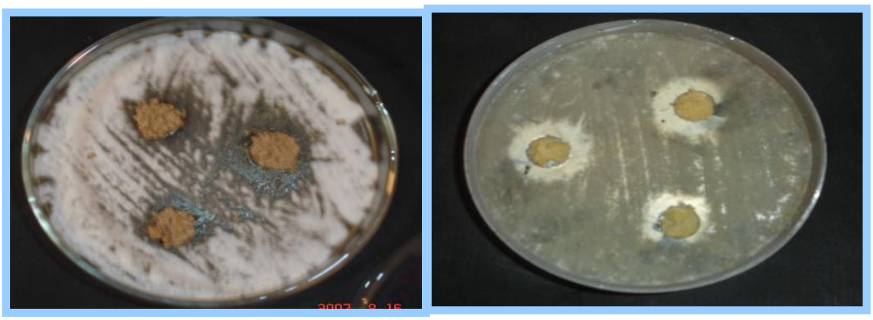

Fig (2) Inhibition zones of the teab (left) and Red musk (right) on *Aspergillus flavus* at (1:1) dilution, after (1) day

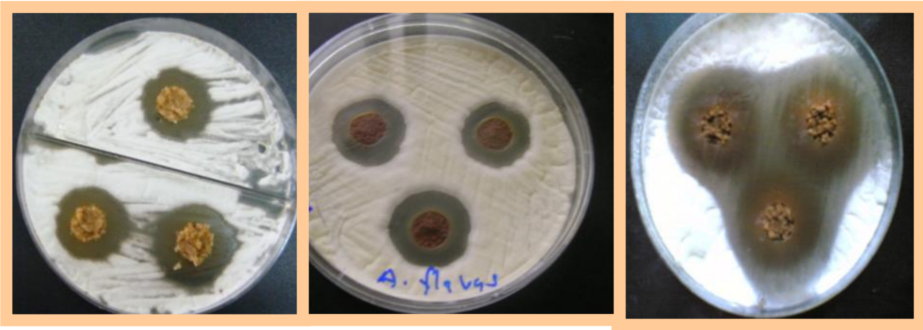

Fig (3): Inhibition zones of *Allium sativum* (left) *Cinnamomum zylanicum*(mid) *Dianthus caryophyllus* (right) on *Aspergillus flavus* at (1:2) dilution , after (1) day

All most all of studied extracts had no bioactivity on *Microsporum canis* except of the Teab aqueous extracts that shows in Fig (4)**,** the inhibition zone was (17mm) at (1:1) dilution

Fig (4): Inhibition zones of Teab on Microsporum canis at(1:1) dilution, after (10) days

Table (3): Antimicrobial activity of aqueous extracts at different dilutions on *Epidermophyton floccosum*

Dilutions / **Extracts**	**Zone of inhibition (mm)**						
	1:1	1:2	1:3	1:4	1:5	1:6	1:7
Allium sativum	-	35	-	-	-	-	-
Curcuma longa	-	-	-	-	-	-	-
Cinnamomum zylanicum	-	12	-	-	-	-	--

Dianthus caryophyllus	CI	CI	CI				-
Zingiber officinale	-	-	-	-	-	-	-
Elettaria cardamomum	-	-	-	-	-	-	-
Teab	-	-	-	-	-	-	-
Red musk	-	-	-	-	-	-	-
China musk	-	-	-	-	-	-	-
Indian musk	-	-	-	-	-	-	-
Propris	-	-	-	-	-	-	-

Values are mean of three replicates; - no inhibition zone; CI: Completely inhibited, cork-borer diameter 8 mm, extracts diluted by sterile distilled water

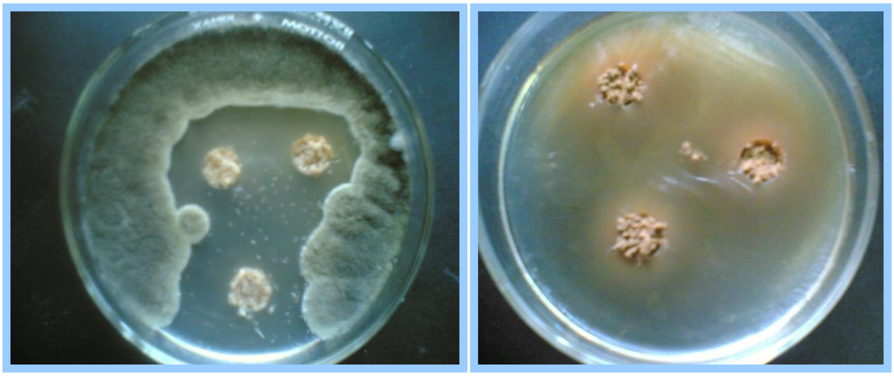

Fig (5): Inhibition zones of *Allium sativum* (left) *Dianthus caryophyllus* (right) on *Epidermophyton floccosum* at (1:2) dilution , after (5) days

The present study has shown that *Dianthus caryophyllus* (Fruit), *Allium sativum* (Fruit), *Cinnamomum zylanicum* (Bark) possesses remarkable fungi toxic activity against many human skin pathogens. Thus, there is a possibility of developing these plants sources of antifungal agent and further investigations are necessary to identify the bioactive principles. The antimicrobial effect of powerful extracts may be due to their characteristic, strongly aromatic taste, furthermore the effect of some essential oil and antimicrobial substances found in these plants. Afterward we can talk over researches that similar or related to our research, also had analogues result by similar or different microorganisms. Certain researches studied the antimicrobial agent *in vitro* or *in vivo*

Dianthus caryophyllus major chemical constituents is an essential oil, which is characterized by the presence of eugenol (60-95%), eugenol acetate (2-27%), and α- and β-caryophyllene (5-10%) (15, 16, 17, 18, 19), and use in pharmacopoeias and in traditional systems as external or local applications for the treatment of toothache, and minor infections of the mouth and skin. Also used as an antiseptic for dressing of minor wounds, and, in the form of lozenges, for sore throats and coughs associated with the common cold. The essential oil (1-5%) is used in mouthwashes (20, 18, 21)

In vitro ethanol (95%) or aqueous extracts of Flos Caryophylli (*Dianthus caryophyllus*) inhibited the growth *Staphylococcus aureus* (21). The juice of the flower bud inhibited the growth of *Mycobacterium tuberculosis* (minimal inhibitory concentration [MIC] 1:160) (22). The powdered crude drug inhibited the growth of *Yersinia enterolitica* when added to the medium at a concentration of 1-3% (w/w) (23, 24). An aqueous extract of the flower buds inhibited the growth of *Bacillus subtilis* (25). A chloroform extract of the flower buds inhibited the growth of *Cladosporium werneckii* (26). A 50% ethanol extract of the flower buds inhibited the growth of *Aspergillus fumigatus*, *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum*, *Penicillium digitatum*, *Rhizopus nigricans*, *Trichophyton mentagrophytes*, *Candida albicans* and *Saccharomyces pastorianus* at a concentration of 500mg/ml (27).

Eugenol, of *Dianthus caryophyllus* is one of the active constituents of the flower buds, inhibited the growth in vitro of *Staphylococcus aureus*, *Propionibacterium acnes* and *Pseudomonas aeruginosa*, with an MIC of 0.05, 0.05 and 0.80mg/ml, respectively (29, 28). In other studies, eugenol had a broad spectrum of antibacterial activity in vitro, inhibiting the growth of *Clostridium sporogenes*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella pullorum*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Comamonas terrigena* at various concentrations (30, 31). Eugenol also had a broad spectrum of antifungal activity in vitro, inhibiting the growth of *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium werneckii*, *Cladosporium cucumerinum*, *Colletotrichum capsici*, *Helminthosporium oryzae*, *Microsporum canis*, *Penicillium expansum*, *Phytophthora parasitica*, *Rhizopus nodosus*, *Trichophyton mentagrophytes* and *T. rubum* at various concentrations (32-33).

An aqueous extract of *Dianthus caryophyllus* flower buds suppressed the replication of herpes simplex virus (HSV) in vitro at a concentration of 50µg/ml (34). An aqueous extract of the flower buds had antiviral activity against HSV-1 in vitro (IC_{50} 60µg/ml), and in mice (250mg/kg body weight by gastric lavage) (35). A hot aqueous extract of the flower buds suppressed the replication of HSV-1, measles virus and poliovirus-1 in Vero cells in vitro at a concentration of 0.5mg/ml (36). Intragastric administration of a decoction of the flower buds (750mg/kg body weight) decreased HSV-1 genome titres and the severity of HSV infection in mice with recurring herpetic lesions induced by ultraviolet light (37). Eugenol at a concentration of 0.1-10µg/ml demonstrated antiviral activity against HSV and adenovirus-6 in vitro (38). Eugeniin isolated from the flower buds exhibited anti-HSV-1 activity in mice (39).

In vitro studies, *Allium sativum* (garlic) has been found to have antibacterial, antiviral, and antifungal activity. However, these actions are less clear in vivo. It is also claimed to help prevent heart disease (including atherosclerosis, high cholesterol, and high blood pressure) and certain types of cancer, including stomach and colon cancers.(40)

Garlic cloves(*Allium sativum*) are used as an antiseptic to prevent gangrene during World War I and World War II.(41) More recently, it has been found from a clinical trial that a mouthwash containing 2.5% fresh garlic shows good antimicrobial activity, although the majority of the participants reported an unpleasant taste and halitosis.(42]) and remedy for infections (especially chest problems), digestive disorders, and fungal infections such as thrush.(43) Garlic can be used as a disinfectant because of its bacteriostatic and bacteriocidal properties.(44) Likewise, has been used reasonably successfully in AIDS patients to treat Cryptosporidium in an uncontrolled study in China.(45)It has also been used by at least one AIDS patient to treat toxoplasmosis, another protozoal disease. (46)

When crushed, *Allium sativum* yields allicin, an antibiotic (47) and antifungal compound (phytoncide). Fresh or crushed garlic also affords the sulfur-containing compounds alliin, ajoene, diallyl polysulfides, vinyldithiins, S-allylcysteine, and enzymes, B vitamins, proteins, minerals, saponins, flavonoids, and Maillard reaction products, which are not sulfur-containing compounds. Furthermore, a phytoalexin (allixin) was found, a nonsulfur compound with a γ-pyrone skeleton structure with antioxidant effects, antimicrobial effects, (48) antitumor promoting effects, inhibition of aflatoxin B2 DNA binding, and neurotrophic effects. Allixin showed an antitumor promoting effect in vivo, inhibiting skin tumor formation by TPA and DMBA initiated mice. Analogs of this compound have exhibited antitumor promoting effects in in vitro experimental conditions. Herein, allixin and/or its analogs may be expected useful compounds for cancer prevention or chemotherapy agents for other diseases.(49)

The researches of *Cinnamomum zylanicum*, had explained that; the external treated as a poultice can treat minor bacterial and fungal infections on the skin. Some of the plant constituents have proven value against bacteria and fungi, including the fungi that produce the carcinogenic aflatoxins. Its essential oil contains both antifungal and antibacterial principles that can be used to prevent food spoilage due to bacterial contamination. (50,51,52)Certain uses of cinnamon as a germicide, that used internally in typhoid fever and in the treatment of cancer and other microbial diseases. It is also said to be used as an antiseptic. Further studies with regard to cinnamon extracts against food borne pathogens and foliage microorganisms by Lopez, p, et al, (2009) has proven that the highest antibacterial activity was found for cloves and cinnamon against most of the bacterial cocktails and they have further concluded that use of cinnamon essential oil can provide an adequate degree of protection against food borne pathogens to a certain extent due to its anti-bacterial quality(50,51)

A number of plants such as *Vitis vinifera* L. extract (Vitaceae) is used in conditions like burning sensations, hemorrhages, anemia, leprosy, skin diseases, syphilis, asthma, jaundice, bronchitis (Anjaria et al., 2002; Sriram et al., 2004). Similarly, an essential oil in the tubers has antibiotic activity and has been shown to arrest the growth of *Micrococcus pyrogenes* (53)

A similar study of screening the natural plant extracts against different fungal and bacterial pathogens was well recorded in literature (54,55). The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. In particular, the search for components with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms (56).

Consequently plants are potent biochemists and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (57) i.e. any part of the plant may contain active components. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct. Since plants have co- evolved with pathogens, it is reasonable to expect a variety of such compounds with specific as well as general antifungal activity (58).

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تقدير النشاط الحيوي للمستخلصات المائية لبعض النباتات الطبية وبعض الطيوب العربية على فطريات ممرضة منتقاة

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ملخص

درس النشاط الضد ميكروبي للمستخلصات المائية لستة نباتات طبية بالإضافة الى المسك الهندي، الصيني والاحمر وكذلك الطيب على اربعة اجناس مختلفة من الفطريات ذات الاهمية الطبية والتي تسبب العدوي الجلدية وهذه الفطريات هي: *Aspergillus flavus, Epidermophyton floccosum, Microsporum canis* بالإضافة الى نوع واحد من الخمائر هو *Candida albicans*. و استخدمت طريقة الانتشار عبر الاجار المثقوب لتحديد منطقة التثبيط وكانت النتيجة ان القرنفل *Dianthus caryophyllus* حاز على اكبر منطقة تثبيط يليه الثوم *Allium sativum* ويأتي بعدهما نبات القرفة *Cinnamomum zylanicum* ويليه المسك الاحمر والطيب. وكان اقل تركيز مثبط يتراوح بين التخفيفات (1:6) و (1:1) للفطريات قيد الدراسة وهذه الدراسة تدعم استخدام هذه النباتات في الطب الشعبي .