ANTIFUNGAL ACTIVITIES OF SOME THERAPEUTIC DRUGS AND EXTRACTS OF ZIZIPHUS NUMMAULARIA AND CURCUMA DOMESTICA AGAINST DERMATOPHYTES

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Abstract

Dermatophytes are the most common causes of superficial or cutaneous fungal infections around the world and remain a major public health problem in spite of the presence of some numbers of antifungal drugs. The difficulties associated with the treatment of dermatophytosis and antifungal drugs resistance remain challenges to select an effective antifungal agents. This study aimed to investigate the activity of three antidermatophytic drugs in vitro (Clotrimazole, Itraconazole and Fluconazole) and the activity of the ethanol and chloroform extracts of two medicinal plant (Ziziphus nummaularia and Curcuma domestica). Fourteen species of dermatophytes were examined by disk diffusion method using Sabouraud's Dextrose Agar. The study revealed that, from the collected specimens, 180 (60%) of cases were positive for isolating of dermatophytes. Clotrimazole was the most effective antidermatophytic drug against the tested dermatophytes, it was clear that ethanol and chloroform extracts of Ziziphus nummaularia had the same activity of Clotrimazole against E. floccosum. Similarly the chloroform extract of Ziziphus nummaularia has the same activity of Itraconazole against M. nanum. The ethanol and chloroform extracts of both Ziziphus numularia and Curcuma domestica showed more activity towards T. violaceum comparing with the three tested antifugal drugs. This study concluded that the antifungal activity of Ziziphus nummaularia and Curcuma domestica against tested dermatophytes correlates well with the claims of their traditional uses for skin infections. However, further studies are needed to demonstrate the active ingredients that responsible for their inhibition of dermatophytes.

Keywords: Dermatophytes, Antidermatophytic drugs, Medicinal plants, Ziziphus nummaularia, Curcuma domestica.

1. Introduction:

There are an increasing prevalence of dermatophytic infections across the world, especially in tropical countries [1]. According to WHO, dermatophotoses affect about 25% of the world population [2] and 30-70% of adults are asymptomatic carriers of these diseases [3]. Although the skin lesions of dermatophytosis usually respond well to the routine treatments with topical antifungal drugs, sometimes dermatophytes cause general health problems and some of the species express various susceptibility to antifungal drugs and they might be often chronic and do not respond well to the usual therapeutic procedure, on the other hands, patients tend to stop using medications after the symptoms resolve which leads to disease recurrence and they also be motivated to apply an excessive quantity of topical medication [4-6]. Treatment not only improves the symptoms of dermatophotoses, but also prevents the risk of spread to other regions of the body, thus reducing local and systemic complications [7]. Clotrimazole is a medication used in the management and treatment of fungal infections [8], it is in the imidazole class of drugs which has a broad-spectrum against dermatophyte species and even some bacteria [9]. Other antifungal agent such as triazoles have been reported to have broad
spectrum activity against dermatophytosis. Itraconazole and Fluconazole are some of the first triazoles synthesized but limitations associated with their use have been reported [10]. The same authors added that growing number of invasive fungal species becoming resistant to current antifungal medication is of appreciable concern. The in vitro antifungal susceptibility tests could help to optimize the therapy and to select an effective antifungal agent for this mycosis [11]. In the last few decades, there has been a lot of interest in the investigation of natural materials as sources of new antimicrobial agents and has attracted a lot of attentions due to they are rich in a wide variety of secondary metabolites also many extract of plants have antidermatophytic activities in vitro and some exhibited synergy with conventionalazole antifunge [12]. Plants herbal remedies play a dominant role in the primary health care of about 80% of the world’s population [13]. A standard method for susceptibility testing of dermatophytes is lacking, but good results of minimal inhibitory concentration have been obtained in several reports [14]. Ziziphus sp. contains various important bioactive compounds, belonging to the different phytochemical classes, which justifies their use as a medicinal plants [15]. Ziziphus contains various functional compounds such as vitamin C, amino acids, triterpene acids, polysaccharides, polyphenols [16], derivatives and fatty acids. In fact, saponins, flavonoid C-glycosides and fatty acids in this genus were responsible of plants sedative and hypnotic effects [17]. Recent pharmacological results have also revealed that polysaccharides, flavonoids, triterpene and betulinic acids are the main active ingredients within this genus Ziziphus contributing respectively to its immune-modulating and hematopoietic functions, antioxidative effect, anti-inflammatory, anticancer activities, and beneficial effects on cardiovascular system [15]. Ziziphus can be used against pathogenic fungi and oxidative damage. The leaves extracts of Ziziphus have antifungal activities against yeast, filamentous fungi and dermatophyte like Trichophyton and Microsporum [18]. Many polyphenols, such as simple phenols, flavonoids and tannins have antifungal activity, which depends in part on the number and position of the hydroxyl groups. They can inhibit the enzymes of microorganisms by reacting with sulphhydryl groups or form complex with extracellular and soluble proteins ; they could also disrupt cell membranes [19, 20]. Another studies showed that tannins are toxic to filamentous fungi and yeast. Saponins are stored in plant cells as inactive precursors, that tannins are toxic to filamentous fungi and yeast. Disrupt cell membranes extracellular and soluble proteins ; reacting with sulfhydryl groups or form complex with extracellular and soluble proteins. They can inhibit the enzymes of microorganisms by reacting with sulphhydryl groups or form complex with extracellular and soluble proteins ; they could also disrupt cell membranes [19, 20]. Another studies showed that tannins are toxic to filamentous fungi and yeast. Saponins are stored in plant cells as inactive precursors, that tannins are toxic to filamentous fungi and yeast. Disrupt cell membranes extracellular and soluble proteins ; reacting with sulfhydryl groups or form complex with extracellular and soluble proteins. They can inhibit the enzymes of microorganisms by reacting with sulphhydryl groups or form complex with extracellular and soluble proteins ; they could also disrupt cell membranes [19, 20]. Another studies showed that tannins are toxic to filamentous fungi and yeast. They appear to act by disrupting the membrane integrity of fungal cells [20]. The alkaloids can exhibit antimicrobial activity by many mechanisms, DNA intercalation, targeting RNA polymerase, gyrase and topoisomerase IV, inhibition of cell division [21] perturbations in the biosynthesis or metabolism of heme [22], and inhibiting enzyme activity [23]. Curcuma is acts as a scavenger of oxygen free radicals and also helps to protect the oxidations of hemoglobin [24]. Tumeric will help to destroy the growth of cancer cells and helps to cure prostate and breast cancer [25]. Curcuma is rich in different phytochemicals and shows the antifungals activities. It is having more antifungal potential as compare to other plants [24]. The therapeutic properties of Curcuma sp. include insecticidal [26], antimicrobial [27], antifungal [28], antimalarial [29], antiviral [30], and antioxidant properties [31]. There are many chemical components of Curcuma sp. have been reported like curcumenol, curdione, curcumin, isocurcumenol,curcumol, stigmasterol, zingiberene and curcumene. Many ethnic groups use this plant’s rhizomes to cure all kinds of skin diseases and for many other therapeutic purposes. The GC-MS analysis revealed the presence of 83 components, of which ß-Myrcene, epicurzeronone, squalene, α-acardial, β-pinene, 2,6,11,15-Tetramethyl hexadeca -2,6,8,10,14-pentaene and aromadandrene were the major components. The essential oil of the rhizome exhibited a wide range of antifungal activity against dermatophytes and yeasts with MIC ranging from 8-128µg. [32]. The present research was designed to study in vitro the susceptibility of some species of dermatophytes towards three different antidermatophytic drugs including Clotrimazone, Itraconazole and Fluconazole and the effect of ethanol and chloroform extracts of Ziziphus nummularia and Curcuma domestica against some species of dermatophyte.

2. Materials and Methods:

2.1. Collection & Culturing of specimens

Three hundred specimens were collected from patients suffering from dermatophytosis during one year (April 2018 - April 2019). Patients were attending dermatology department in The National Laboratories and Khartoum hospital. The specimens (skin, nails and hair) were collected depending on clinical examination of the lesions as suspected infected patients with dermatophytosis [33]. Samples were inoculated on Sabouraud’s Dextrose Agar plates enriched with cyclohexamide and chloramphenicol then the inoculated plates were incubated at 30°C for up to 4-6 weeks and were daily examined for the colony formation [34].

2.2. Identification of the isolated dermatophytes

The recovered dermatophyte were identified based on macroscopic (duration of growth, surface morphology and pigment on the reverse) and microscopic examination a small portion of the mycelium was mixed with a drop of Lactophenol cotton blue on a slide then under high power objective seen the hyphae, sprores and conidia shapes [35].
2.3. Antifungal susceptibility test (in vitro)

The isolated dermatophytes were examined for their susceptibility to three antifungal drugs (Clotrimazole, Itraconazole and Fluconazole). Each drug was dissolved in distilled water. The Kirby- Baured disk diffusion method was performed [36].

2.4. Plants collection, preparation and extraction

Rhizomes of Curcuma domestica and leaves of Ziziphus nummularia were collected and cut into small pieces and shade dried at room temperature for 15 days. Each selected part of plant was ground separately into powder then extracted using ethanol and chloroform as different solvents [37]. The plants extracts were prepared by soaking 100 g of dried powdered for each plants in 200 ml of solvent ethanol or chloroform separately for 12 h of duration and subjected to soxhlet extraction [38]. The concentrations of extractions that applied for the tests were 100%.

2.5. Antidermatophytes assay (in vitro)

Using a sterile cotton swab, suspension of each tested isolate was inoculated and distributed onto the surface of Sabouraud's Dextrose Agar medium plates. Then plates have been kept to dry. Disks of 6 mm in diameter were performed then soaking in the plant extraction and put on the inoculated plates. The plates were incubated at 30°C for 2-4 weeks. The inhibition zones were measured [39]. The activity of antifungal drugs was performed by the same method using the antifungal drugs disks instead of plant extracts disks.

3. Results:

Out of 300 samples analyzed for culturing examination, 180 were positive culture and the remaining 120 samples were negative culture. Fourteen species of dermatophyte were isolated from the positive samples Figures (1-3). *E. floccosum* was the most frequently isolated dermatophyte (29=16%) following by *T. mentagrophytes* (26=14.4%) then *M. canis* (22=12.2%) Table (1).

The obtained results revealed that Clotrimazole was the most effective antidermatophyte among the three tested antidermatophytic drugs against the tested dermatophytes, followed by Itraconazole. On the other hand, Fluconazole showed low effect against dermatophytes among the three tested antidermatophytic drugs, although this drug showed similar activity as Clotrimazole and Itraconazole towards *M. ferrugineum*, *T. mentagrophytes*, *T. tonsurans* and *T. verrucosum*, otherwise clear resistance for this drug was shown by *E. floccosum* and *T. schoenilii* and *M. gypseum*. Low activity was shown against *M. audouini*, *T. soudanense* and *T. violaceum*. It is worth to mention that *M. ferrugineum*, *T. mentagrophytes*, *T. tonsurans* and *T. verrucosum* showed the highest sensitivity towards the three antidermatophyte drugs applied and the inhibitory zone was 50 mm for each species, while *M. audouini*, *M. gypseum* and *T. violaceum* showed the least sensitivity towards the three antidermatophyte drugs applied in which the range of inhibitory zone was 40 - 10 mm Table (2).

On the other hand, obtained data showed that, in general, the plant extracts exhibited high anti-dermatophytes
activity against the dermatophyte isolates. However, the Ziziphus chloroform extracts showed less anti-
dermatophytic activity against almost half of the tested
dermatophytes while Ziziphus ethanolic extracts exhibited more activity against T. concentricum T.
mentagrophytes T. soudanense and T. tonsurans Table (3). Both Curcuma and Ziziphus extracts exhibited the
highest antidermatophytic activity against T. violaceum Figs.(4-6). However; the two tested plants extracts were
inactive against M. audouini, M. gypseum, T. verrucosum and M. canis while Curcuma extracts had no
activity with M. nanum and M. audouini. It was clear that ethanol and chloroform extracts of Ziziphus
nummaularia had the same activity of Clotrimazole against E. floccosum Figs. (7&8). Similarly the
chloroform extract of Ziziphus nummaularia has the same activity of Itraconazole against M. nanum
Figs.(9&10). The ethanol and chloroform extracts of both Ziziphus nummaularia and Curcuma domectica showed
more activity towards T. violaceum comparing with the three tested antifungal drugs. These can be noticed in the
activity of plants extracts against many of dermatophytes as shown in tables (2 & 3). On the other hands,
Trichophyton species were more sensitive to plant extracts than Microsporum species.

Table (2): Susceptibility of dermatophytes towards commonly used antifungal drugs

<table>
<thead>
<tr>
<th>Dermatophytes</th>
<th>Clotrimazole</th>
<th>Itraconazole</th>
<th>Fluconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. floccosum</td>
<td>50</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>M. audouini</td>
<td>40</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>M. canis</td>
<td>50</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>M. ferrugineum</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>M. gypseum</td>
<td>40</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>M. nanum</td>
<td>50</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>T. concentricum</td>
<td>50</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>T. mentagrophytes</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>T. rubrum</td>
<td>50</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>T. schoenlinii</td>
<td>40</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>T. soudanense</td>
<td>50</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>T. tonsurans</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>T. verrucosum</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>T. violaceum</td>
<td>40</td>
<td>30</td>
<td>20</td>
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</table>

Table (3): The effect of ethanolic and chloroformic extracts of Curcuma sp. and Ziziphus sp. against
dermatophytes

<table>
<thead>
<tr>
<th>Plant Extracts</th>
<th>Ziziphus nummaularia</th>
<th>Curcuma domestica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>Chloroform</td>
</tr>
<tr>
<td>E. floccosum</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>M. audouini</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M. canis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M. ferrugineum</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>M. gypseum</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M. nanum</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>T. concentricum</td>
<td>20</td>
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<tr>
<td>T. mentagrophytes</td>
<td>50</td>
<td>0</td>
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<tr>
<td>T. rubrum</td>
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<tr>
<td>T. schoenlinii</td>
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<td>30</td>
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<tr>
<td>T. soudanense</td>
<td>50</td>
<td>0</td>
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<tr>
<td>T. tonsurans</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>T. verrucosum</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T. violaceum</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Fig.4: High antifungal activity of both Ziziphus sp. extracts against T. violaceum
1. Chloroform extract
2. Ethanol extract

Fig.5: High antifungal activity of both Curcuma sp. extracts against T. violaceum
1. Chloroform extract
2. Ethanol extract

Fig.6: Antifungal activity of antidermatophyte drugs against T. violaceum
1. Clotrimazole
2. Itraconazole
3. Fluconazole
4. Discussion:

In our results, the most frequent species were *E. floccosum, T. mentagrophytes* and *M. canis*. Different results about the etiological agents of dermatophytoses were recorded by different researchers. In this respect, *T. rubrum* was found to be the most common organism causing human dermatophytoses in India [40]. However, [41, 42] found that *T. violaceum* was the most common dermatophyte in Tripoli, Libya and Addis Ababa, Ethiopia. [43] found that *M. canis* and *T. violaceum* were the most frequent dermatophytes isolated in Yemen. [44] reported that *T. rubrum* was the predominant species in Rajkot. [45] and [46] reported that *T. mentagrophytes* as the predominant species in India. [47] found that *T. tonsurans* was the most frequent dermatophytes followed by *M. canis* in Egypt. [48] found that the most common species was *T. mentagrophytes* followed by *T. tonsurans* then *E. floccosum*. while [49] recorded that *T. soudanense* and *M. cookie* were the most frequent isolated dermatophytes. In this respect, it was clear that the most prevalent species of dermatophytose differ strikingly from one geographic locality to another and change often from decade to decade [50].

The dermatophytosis is the most common infectious dermatologic condition throughout the world [51]. Antifungal susceptibility testing is a dynamic field of medical mycology [52]. The disk diffusion method for determine the susceptibility of dermatophytes is simple, inexpensive and does not require specialized equipment. The disk diffusion method has a good correlation with reference dilution assay [53]. In the present study, the evaluation of in vitro susceptibility of dermatophytes isolates towards the antifungal drugs test display a good activity against dermatophytes. Clotrimazole was the most effective antifungal followed by Itraconazole while Fluconazole exhibited the least effective antifungal among the three tested drugs. These findings agree with those reported by [54] who noted that high activity of antifungal against dermatophytes was exhibited by Clotrimazole and ketoconazole. However, [55] reported that Clotrimazole found to be less effective drugs comparing with the other antifungal drugs, they also found that Fluconazole was showed the least activity than the other tested antifungal drugs. Also [14] their results showed that the tested antifungal drugs displayed a good activity against dermatophytes with the exception of Fluconazole and Itraconazole had low minimal inhibitory concentration value. In the present study, *M. ferrugineum, T. mentagrophytes, T.tonsurans* and *T. verrucosum* showed the highest sensitivity towards antidermatophyte drugs, while *M. audouini, M. gypseum* and *T.violaceum* showed the least sensitivity. [14] revealed that in vitro the different antifungal agents are...
active against dermatophytes independent of species. In this respect, they found that T. rubrum strains were more susceptible to Fluconazole than T. mentagrophytes and M. canis strains. The same results were previously demonstrated by [5] who reported that T. rubrum has high susceptibility than T. mentagrophytes, M. canis and M. gypseum towards tested antifungal agent.

A number of extracts from medicinal plants are being investigated as possible additional therapeutic agents for treatment of dermatophytes infections. Many plants have antidermatophytic activity in vitro and some exhibited synergy with conventionalazole antifungals [12]. During this study, Ziziphus sp and Curcuma sp ethanol and chloroform extracts exhibited a high antidermatophyte activity against the tested dermatophytes. These results showed equal activity of these plants comparing with some of dermatophytes commonly used antifungal drugs. On the other hand, some dermatophytes showed more susceptibility towards these plants extracts than the antifungal drugs such as in T. violaceum. In similar study [13] observed that Ziziphus nummaularia ethanolic extract exhibited high activity against dermatophytes compared to that of the antifungal drug Ketoconazole. Also, [56] recorded that all dermatophytes were markedly inhibited by extractions of Curcuma in different concentrations. This agree with [57] and [58] who reported that the medicinal plants form antifungal activity against some types of dermatophytes. [59, 60] found that medicinal plants extract showed broad fungitoxic spectrum when tested against ringworm fungi. Similarly, ethanolic extracts of Solanum melongena, Lawsonia inermis and Justicia gendarussa showed significant antifungal effect against phytopathogenic fungi. Hence, it could be used as alternative source of antifungal agents against fungal infection [61]. Previous study carried by [13] revealed that methanol extract of Ziziphus which collected from Nigeria, showed antifungal activity when tested by agar diffusion method against T. mentagrophytes, T. rubrum and M. canis.

Conclusion:

The results demonstrate that Clotrimazole has high activity as antidermatophyte drug. Antifungal activity of Ziziphus nummaularia and Curcuma domestica against dermatophytes correlates well with the claims of traditional uses for skin infections. Since some of these plants appeared to have broad spectrum activity and are cheap, they could be useful in antiseptic or disinfectant formulations. However, further studies for Ziziphus nummaularia and Curcuma domestica are needed including in vivo investigations and toxicity evaluation.

References:


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