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Role of medicinal plants in the treatment of eumycetoma: A review

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ABSTRACT

Mycetoma is a resultant infection of either fungi (eumycetoma) or bacteria (actinomycetoma). It is categorized under neglected tropical diseases, as it typically affects the economically backward communities of the endemic regions. The infection occurs with the invasion of etiological agents through the open wounds of the body. The cutaneous and subcutaneous tissue is characterized by the sinus formation and discharge. Primarily affecting the foot, it further spreads to other parts of the body. Eumycetoma has a worldwide distribution yet considered endemic, as it is restricted only to few countries. Diagnosis and treatment of the disease has been burdensome since poor response and unavailability of medical support in endemic regions. Microbiological and serological diagnostic methods have been unreliable except molecular diagnostics. Treatment with antifungal agents has given a mixed response, with only itraconazole being effective. These techniques are relatively expensive and cannot be afforded by people with weak economic background. In the context, it becomes essential to introduce phytotherapy to relieve the health and economic burden. Herein, we discuss few medicinal plants with profound antifungal activity towards eumycetoma. We highlight the possible course of actions that needs to be put forward to deliver the plant-based drugs to cure eumycetoma.

1. INTRODUCTION

Mycetoma refers to a chronic, inflammatory, granulomatous, nodular infection of cutaneous and subcutaneous tissue that is caused by either bacteria or fungi [1,2]. Based on the causative agents, it is further classified as eumycetoma (fungi) and actinomycetes (aerobic bacteria). Both the diseases share similar medical complexities and predominantly affect the feet, and rarely infect the other parts of the body [3,4]. Actinomycetes account for the 60% of the worldwide cases, whereas the rest is linked with the eumycetoma. In case of eumycetoma, 90% of the infections caused by the fungi including *Madurella mycetomatis*, *Madurella grisea*, *Leptosphaeria senegalensis*, and *Pseudallescheria boydii*. In addition, there are several other fungi reported with rare infections, which are depicted in Table 1 [5,6]. These fungi are suspected to enter the host through localized trauma such as open wounds from woody plants and soil. This is followed by the progressive, painless formation of tumor projections on the feet in the subsequent time interval [7,8].

The morphological and histological examinations reveal the formation of abscesses comprising large and compact masses of fungal filaments termed as grains. Through the draining sinuses, these grains comprising the etiological agent are discharged [9]. This is considered as a unique character of this disease. The tumors thus caused on the cutaneous and subcutaneous tissue are often putrefying [10]. Destruction of deep tissues including muscles, tendons, bones, and joints is also observed [11]. This may further lead into the loss of function, deformity, and occasionally to death. The disease usually affects young adults and is prevalent in males aged between 15 and 30 years. However, early detection and treatment could reduce the morbidity and augment the treatment outcomes [7,12].

The disease is believed to possess several adverse impacts with respect to medical, health, and socio-economical aspects. Inaccurate and deficient amount of data on epidemiological details is available, though few of the studies depict some of the countries

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Table 1: Fungal species causing eumycetoma

Type of infection	Fungal species
Most common	*M. mycetomatis*
>90% of the infection	*M. mycetomatis*, *M. grisea*, *L. senegalensis*, and *P. boydii*
Rare infection	*Acremonium* spp. (*Acremonium falciforme*, *Acremonium kiliense*, and *Acremonium recifei*), *Aspergillus spp.* (*Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus nidulans*), *Biatriospora mackinnonii*, *Cladophialophora* spp. (*Cladophialophora bantiana* and *C. mycetomatis*), *Cladosporium carrionii, Cochliobolus lunatus, Curvularia geniculata, Cylindrocarpon cyanescens, E. jeanselmei*, *F. senegalensis, Fusarium* spp. (*F.* chlamydosporum, *Fusarium falciforme, Fusarium moniliforme, Fusarium solani,* and *Fusarium subglutinans*), *Geotrichum candidum*, *Hormonema* spp. *Leptosphaeria tompkinsii, M. romeroi*, *Microsporum* spp. *Neoscytalidium dimidiatum*, *Neotestudina rosatii, Paecilomyces lilacinus, Phialophora* spp. (*Phialophora jeanselmei* and *Phialophora verrucosa*), *Pleurostomophora ochracea, Pyrenochaeta* spp. (*Pyrenochaeta mackinnonii* and *Pyrenochaeta romeroi*), *Rhinocladiella atrovirens*, *S. boydii*, *T. grisea*, *Trichophyton* spp.

Adapted from Verma and Jha [5], Zijlstra *et al*. [6].

as endemic [12]. For example, omnipresence of collective mycetoma is observed in the region between 30° N and 15° N, that is known as "Mycetoma belt." Several countries fall in this region including Mexico, Venezuela, Argentina, Columbia, Senegal, Sudan, and India [11,13]. Being prevalent in such countries with low-low socioeconomic status, malnutrition, and sub-standard hygienic practices, it affects poor populations in remote areas that lack basic health facilities, trained medical staff, diagnostic tools, and treatment. These things make it to the list of neglected tropical diseases (NTDs), as recognized by the World Health OrganisationOz [8,11].

In comparison with actinomycetes, eumycetoma is reportedly less responsive to medical treatment, which is regarded as problematic and challenging task. Treatment of actinomycetes can result with a cure rate up to 90%, whereas eumycetoma is reported to exhibit resistance to antifungals like itraconazole and ketoconazole [8]. The entire landscape of treatment is based on the location, dissemination, and severity of the disease, which is completed either with the prolonged chemotherapy or surgical intervention [8,14]. In countries with sub-standard medical support, extension of the disease may lead to chronic progressive lesions, which in turn lead to amputation [8,15].

In general, the treatment outcome of eumycetoma is suboptimal and unsatisfactory in many patients. But this can be overturned with the utilization of herbal medications, which prove to be cost-effective and acceptable in terms of medical complications like adverse health effects. Several medicinal plants like *Moringa oleifera*, *Acacia nubica*, *Nigella sativa*, and *Boswellia papyrifera* have already been recognized for comprising several phytochemicals with potential antifungal properties [16,18]. Thus, advanced research could surely be able to deliver phytotherapeutic agents against eumycetoma. In this review, we emphasis on the eumycetoma, elements of its infection and transmission, extended pathogenicity and phytotherapy. Our object is to highlight the perspective on futuristic approaches that could aid the phytochemical-based drug discovery process.

2. GLOBAL PREVALENCE OF EUMYCETOMA

2.1. Distribution

Most of the studies do not report eumycetoma as a separate disease. The statistics gathered from the sources depict the status of collective mycetoma patients all around the globe. However, eumycetoma is prevalent in only few of the countries that comes under the "Mycetoma belt" [11,13]. One must notice that these countries belong either to the under-developed or developing category. Suboptimal socio-economic standards in these countries have surely affected the people living in rural areas, with most of them being economically weak. These aspects evenly affect the medical supporting, with deficient instrumentation and staff [19]. Such limitations could even affect the reporting of cases, further diminishing the data for research and development. For example, there are only 8,763 reported mycetoma cases hitherto which, are expected to be much higher escalating [20]. Yet there is no separate report of eumycetoma cases. Among these, most of the cases were reported from Sudan (>7,000), among which 70% of them are detected with the fungus *M. mycetomatis*. The country appears to be the most endemic country with a prevalence of 14.5% per 1,000 population. In Mexico, 3,933 cases have been reported in 54 years (mean 73 per year), where 3.5% of them were eumycetoma [6]. Another study reported 1,392 cases in India. Although these numbers seem to be outdated, efforts put by these have revealed the extensive pathogenesis, even in the countries outside the Mycetoma belt. Reports from Mali and Mauritania depict the rate as 5.4 and 69.7 cases per year, respectively. Apart from this, cases were also reported from Rumania, Nigeria, Uganda, Bulgaria, and Thailand. There is no overall study performed to determine the prevalence of the eumycetoma at world-wide scenario. Moreover, it is not considered as a reportable disease; thus, much remains unknown about the same [21].

2.2. Pathogenicity

Mycetoma affects more men than women. About 4,060 males were affected all over the world except Tunisia and Thailand, in comparison with 1,175 women. No occupation is exempt yet farmers and herdsmen are majorly affected. In Thailand, the ration is nearly 50:50, with 8 men and 9 women affected. Contrastingly, more women (16) were affected in Tunisia, compared to men (12). Only 5,240 cases reported with ages, where 70% of them (3,664 cases) found in the age group between 11 and 40. In all the studies, foot was the most affected organ (68.7%), followed by hands (15%), leg (9.9%), trunk (6.1%), and arm (4.0%) which have been shown in Figure 1. In South American patients, trunk was the most affected portion in comparison with patients from Sudan (1.4%) and Mexico (18.7%) [22,23]. The discrepancies exist in the reports; despite they are conducted on world-wide basis. This may be attributed to the differences in maintaining the actual medical records of the disease. Furthermore, mycetoma is believed to affect children unevenly with 3.0%–4.5% of cases in

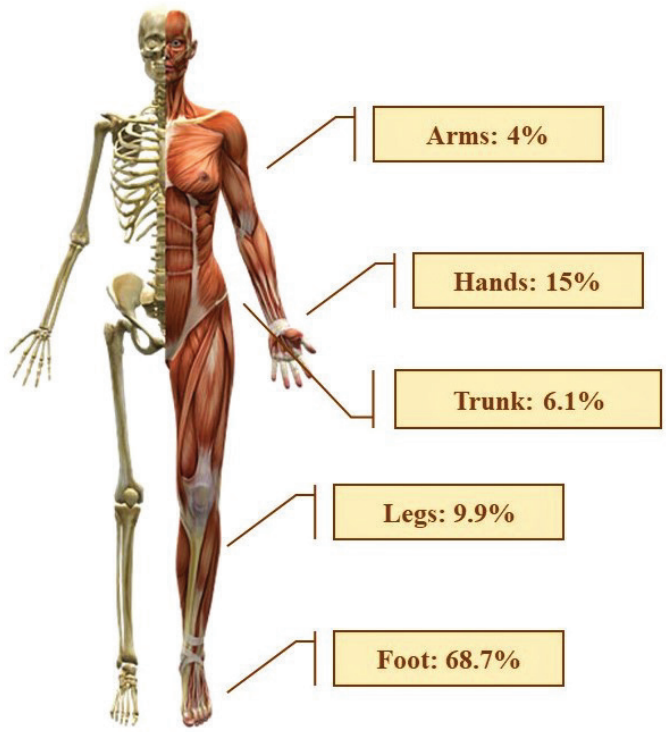

Figure 1: Body parts affected by mycetoma.

endemic regions. Children share similar clinical presentations and diagnostic revelations with the adults. However, shorter duration of infection and early diagnosis often result in lower amputation rates. But still there is a risk of children getting social outcasts, followed by the impact of disease on their personal and educational life [6].

2.3. Etiology

The predominant dematiaceous fungi differ from one to another region of the world, depending on the climatic factors, especially on annual rainfall. Diverse class of moulds have been involved in the pathogenesis, and it has been a difficult task tracking them [22]. For example, it is reported that 219 of the fungi have not been identified at the genus level. Presence of diverse class of fungi makes it even more difficult to treat, as the biological impacts vary based on differential pathogenesis of these fungi. Out of several such fungi, only *M. mycetomatis* is regarded as the most common etiological agent, which is responsible for about 24.3% of the cases studied. In addition, *M. grisea*, *L. senegalensis*, and *P. boydii* are responsible for 90% of the cases [5,21]. *Madurella mycetomatis* is prevalent in Africa, South America, and India. *L. senegalensis* is found commonly in West Africa, and *M. grisea* in South America. However, several fungal species represent very rare amount of infection around the world [5,11,22].

3. PATHOGENESIS AND CLINICAL MANIFESTATION

3.1. Host Factors

Pathogenesis of eumycetoma involves three types of tissue reactions that coexist in the host body. In type 1 reactions, neutrophils play the principle role by surrounding the grains formed. Further they invade the grains and dismantle them. Granular immune cells, mononuclear cells were reported to be oriented towards fibrous tissue. Hypertrophy and hyperplasia are also reported to occur in the sweat glands. In type 2 reactions, neutrophils are replaced by macrophages and multinucleated cells that engulf the grains. Type 3 reactions are like type 1 and 2, except the fact that remnant fungal material is surrounded by a well organized epithelioid granuloma [23]. These inflammatory responses do not eliminate the grains. Giant cells with hyphae are expected to escalate the grain formation further. Presence of neutrophils and grains in the regional yet normal lymph nodes indicates the presence of the eumycetoma. In advanced cases, fibrosis is reported to substitute the lymphoid tissue [2]. Apart from this, T-cell responses are also present in eumycetoma patients. Significantly higher levels of Th-1 cytokines including interleukins, interferon, and tumor necrosis factor (IL-1β, IL-2, IFN-γ, TNF-α), and lower levels of Th-2 cytokines (IL-4, IL-5, IL-6, IL-10) were found in patients undergone surgical excision, in comparison with those who did not undergone the same [24]. It is supported by the presence of Th-2 like responses including IL-10 and IL-4 were found in primary lesions and draining sinuses caused by the peripheral mononuclear cells by *M. mycetomatis* antigens. In addition, macrophages stimulated with *P. boydii* conidia also generated Th-2 response, whereas hyphae induced Th-1 response. The Th-1 responses are generally observed during acute phase of infection [6]. To this date, there is no report on the presence of humoral antibody response in case of eumycetoma.

3.2. Pathogenic Factors

Several pathogenic factors play a role in survival of the fungus in the host body against the defense mechanisms. *Madurella mycetomatis* is reported to produce melanin pigments that protect pathogens against antifungal drugs, ultraviolet radiation, alveolar macrophages, enzymatic lysis, and oxidative agents. The schematic diagram representing the role of melanin in pathogenesis has been given in Figure 2. Melanin is suspected to play a key role in the pathogenesis of the moulds. For example, an *in vitro* study using fungal strains *M. mycetomatis* showed that melanin production in the fungi could elevate the minimum inhibitory concentrations (MICs) of antifungal drugs like ketoconazole (32-fold) and itraconazole (64-fold) [25]. Fungi are believed to possess several melanin biosynthetic mechanisms like 1,8-dihydroxynaphthalene-melanin pathway, L-dopamine pathway, and pyomelanin pathway [25–27]. This further induces resistance to the antifungal drugs used by the host, where the efficacy of the drug is reduced by pathogen to survive in the host. Histological evidences claim that presence of lipids, proteins, and heavy metals, alongside melanin in the grain cement matrix avoids the entry of antifungal agents [28]. The fungus *M. mycetomatis* is also found resistant to different combinations of antifungal drugs, including amphotericin B, itraconazole, and terbinafine [29]. Further, the fungi tend to decrease the activity of chitotriosidase, which tends to eliminate the fungi by binding to chitin in the mycetoma grain [30].

3.3. Clinical Manifestation

Both actinomycetoma and eumycetoma share virtual similarities in terms of clinical symptoms, despite caused by different

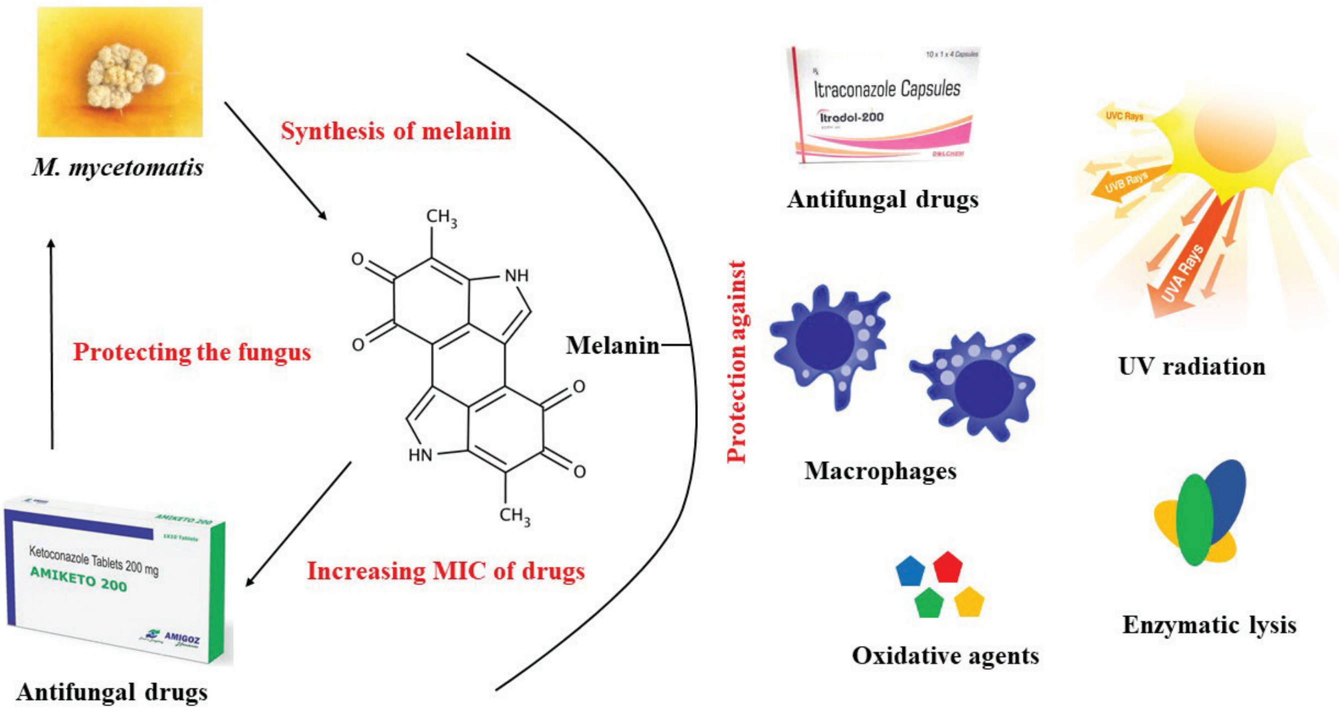

Figure 2: Role of melanin synthesized by *M. mycetomatis* in pathogenesis of eumycetoma.

etiological agents. However, eumycetoma appears to be less aggressive and destructive in comparison with actinomycetoma, where the latter is found invading the bones earlier than eumycetoma [31]. Asymptomatic infection leads to the development of pathogens to develop in the host undetected for several years. The time between initial infection and consultation could vary from 3 months to 50 years [6]. This is attributed to the poor health facilities, absence of economic aid, lack of health education, delayed and painless progression of lesions, and fear of amputation. A study published in 2014 based on the survey conducted in an endemic Sudanese village has shown that 96% of the villagers had substandard level of understanding about mycetoma, and only 49% of them practiced safety measures [32]. The triad of painless swelling, draining sinuses, and discharge consisting grains is a characteristic feature of eumycetoma. These grains may be yellow, black, white or pale, and yellowish-brown based on the etiological agent [11].

The infection begins with the breach of the skin through which the pathogen gains the entry and later spreads to the different regions including tissues, forming sinuses. Skin exhibits a painless and tender wooden induration. The cystic, solid mass thus formed results in tissue damage, deformation, and loss of function [33]. Direct spread to the spinal cord and vertebral bodies could result in paraplegia. Sometimes lesions in the skull can cause bone damage with subsequent neural degeneration. Bone invasion can produce cavities filled with cement-like matrix that comprises grains and fibrous tissue. The stability provided by the fibrous tissue makes it uncommon to have pathological fractures. Purely osteosclerotic lesions can be seen in case of skull infection, where the dense bone formation is observed, with dense bone formation [34]. The hyperplasia and hypertrophy of sweat glands beneath the lesions result in increased sweating. The elevated temperature is due to the inflammatory responses, which involve a series of interconnected molecular mechanisms [2,23]. Dilated arteries and veins depict the increased blood flow to the lesion. Apart from tissues, tendons and nerves are rarely affected until the extremity is reached. As the pain is absent, tracts of subcutaneous tissue get filled with mass. Metastatic lesions could occur at various lymph node regions, which might turn into suppurative. Lymph nodes are said to be recurrent and could be seen in case of surgery. Meanwhile, hematological spread is also possible. *Madurella mycetomatis* is reported to occur in intact blood vessels of the spinal mycetoma [31,35].

3.4. Mode of Transmission

Culturing of fungi including *M. mycetomatis* has been a failed attempt, though the DNA samples were found to be detected in soil and samples [36]. This indicates the presence of fungi in soil and thorns, from which the disease spreads upon interaction with the hosts. The available evidence suggests that the fungi could be dung-inhabiting, providing a new dimension to the phylogenetic analyses that investigate the natural fungal origin [37]. This was supported by the detection of thorn remnants in the lesions of mycetoma patients suggest the possible entry of fungi through trauma like cuts, and wounds [38]. Further, there is no data available on primary reservoirs that help in the transmission. Studies need to investigate the possible role of domestic animals like donkeys, cattle, sheep, chicken, and dogs that live in proximity with humans.

4. DETECTION AND DIAGNOSIS

4.1. Imaging

In scarcity of basic health facilities, diagnosis is carried out using ultrasound and fine needle aspiration. These are the minimal requirements used to confirm the diagnosis in clinical practice and are believed to diagnose most of the cases. Out of these, ultrasound is a preferred imaging technique that is commonly found in health institutions in endemic areas. It clearly distinguishes eumycetoma from other masses in subcutaneous tissue [39]. Appearance of sharp-reflective echoes which are probably caused by grain's cementing substance shows the presence of grains. Especially, cavities either with presence or absence of acoustic enhancement can be seen in case of eumycetoma. Based on grain size, type of embedding, and nature of cement, eumycetoma and actinomycetoma are differentiated. The technique is efficiently used to define the extent of lesion, which could be further utilized for surgical intervention [40].

Radiography is also an imaging technique that is used in peripheral hospitals. A survey conducted on 516 patients diagnosed with radiography revealed that only 3% had a normal radiograph of the affected limb. Among other abnormalities observed, soft tissue swelling was more common (93%), followed by (56%), and bone invasion (46%). In addition, bone cavities (32%) and osteoporosis (32%) were also observed [41]. Among computerized tomography (CT) tests, helical CT is comparatively more advanced than plain CT, as the former allows 3-dimensional reconstruction and generates precise results on the extent of organ involvement. Helical CT also aids in the visualization of the vascular involvement [42]. Nonetheless, usage of modern approaches like magnetic resonance imaging (MRI) has proved its efficacy over the others, in terms of detecting the extent of lesion and invasion. MRI is believed to possess greater sensitivity than ultrasound, radiography, and CT. It also shows the dot-in-circle sign, which is an indication of fungal grains [43].

4.2. Identification of the Pathogen

Identification of the microorganism in the patients is a key to drive the treatment further. Grains are isolated from the sinuses discharging and are further examined in microscope. Isolation of grains from the deep is preferred over the peripheral because of the lack of viability and possible contamination associated with the latter. Isolation is done with the syringe, and the grains are crushed under cover glass. The size of the septation, filaments, shape, color, and other morphological characteristics are thoroughly examined. In case of eumycetoma, fine filaments are seen with periodic acid-Schiff. This is followed by the culturing for 4 weeks. But the identification of the fungal species based on colony morphology could be difficult as they share similar morphology [23]. Apart from microscopic examination of grains, histopathology is also efficiently used by many research laboratories. Hemotoxylin and eosin stains are used to pre-identify the fungi. However, due to the similar appearance as in case of grain examination, fungal species share similar morphology, it becomes difficult to identify them at species level. For example, differentiation between *Scedosporium boydii*, *Fusarium* spp, *Acremonium* spp, and between *Medicopsis romeroi*, *Exophiala jeanselmei*, *Falciformispora tompkinsii*, *Falciformispora senegalensis*, and *Trematosphaeria grisea* is difficult. Further, a fungus like *M. mycetomatis* makes it even more difficult to differentiate as they present multiple grain types during histological examination [6].

4.3. Molecular Identification

Although chemotaxonomic methods are effective in distinguishing the etiologic agents up to their respective genera, they are referred to as tedious, time-consuming, and laborious. The results generated by these techniques are not reliable as they lack vital information on species, sub-species, and types of grains produced. These are being complemented by molecular systematic procedures that use biomolecules like DNA and proteins to determine the species of the etiologic agents [19,40]. Methods like 16S ribosomal RNA (rRNA) gene sequencing [44], polymerase chain reaction (PCR) [45], Curie-point pyrolysis mass spectroscopy, and PCR-randomly amplified polymorphic DNA fingerprinting [23]. Such techniques provide precise details on classification. Unfortunately, these procedures are yet to be deliberated in eumycetoma endemic areas [19,40]. In addition, specialized sequences known as internal transcribed spacer regions are usually amplified with the help of pan-fungal primers and are sequenced. Resultant sequences are compared with previous sequences available in databases like GenBank. This approach has led to the identification of pathogens at species level. New species like *Madurella fahalii*, *Madurella tropicana*, *Madurella pseudomycetomatis* have been identified with the help of these techniques [6,46].

Development of techniques like PCR-restriction fragment length polymorphism has even resulted in the determination of homogeneity of *M. mycetomatis* isolates from thorn and soil samples. Another novel technique known as isothermal rolling circle amplification has generated results in 6 hours of time, in which it was reported to detect different fungal species. Molecular typing techniques like random amplified polymorphic DNA (RAPD), amplification fragment length polymorphism (AFLP), and restriction endonuclease (REN) analyses have been carried out successfully [47]. Although results from RAPD are variable, AFLP and REN analyses were found to differentiate *M. mycetomatis* isolates from different origins. In comparison with the chemotaxonomic methods, molecular diagnostics appears to be expensive, and cannot be affordable by all the patients [23].

4.4. Serological Identification

Furthermore, molecular techniques based on serological examination also provide precise information. Although no reliable serological tests exist to this date, efforts have been made to utilize the serum-based antibody–antigen reactions for the pathogen identification. They include indirect hemagglutinin assays, immunoblots, immunodiffusion, counter-immunoelectrophoresis, immunoblotting, immunodiffusion, and enzyme-linked immunosorbent assay (ELISA). In case of eumycetoma, serological tests have been used for *P. boydii* and *M. mycetomatis* [6]. Indirect hemagglutinin assays, counter-immunoelectrophoresis, and immunodiffusion use crude non-standardized antigens, and do not meet up the required specificity and sensitivity. Further, an ELISA analysis based on *M. mycetomatis* pure antigens that included recombinant produced translationally controlled tumor protein (TCTP), and luminex assays based on TCTP-fructose-bisphosphate aldolase, and pyruvate kinase also showed insignificant specificity, without differentiating between patients and healthy controls. Therefore, effective diagnosis could be achieved either with reliable imaging, histological examination, molecular typing, or with advancements in serological tests to improve sensitivity and specificity [19].

5. THERAPEUTICS AND LIMITATIONS

Several chemotherapeutic agents that have been employed to treat eumycetoma were once experimented with common fungi. These antifungal drugs exhibit different mechanisms to either inhibit or to kill the fungus [48]. Much remains unknown of their mechanisms to eliminate the fungus. The chemotherapy exists for 18–24 months prior surgical intervention. The response to these drugs has been poor and has left many patients are unsatisfied. Thus, a combination of surgery and chemotherapy is preferred. Currently, a class of drugs known as azoles have been recognized as effective against eumycetoma [11,49]. Ketoconazole was used as a mainstream therapeutic agent at 400–800 mg/day for 9–12 months. However, it was restricted in 2013 for its adverse effects like drug interactions, potentially fatal liver injury, and problems with adrenal gland by the U.S. Food and Drug Administration (FDA) [50]. For the same reasons, marketing authorizations of ketoconazole were called off by European Medicines Agency. Currently, itraconazole is used to treat infections, but it is reported with incomplete cure [51]. Melanin production in the fungi could elevate the MICs of antifungal drugs like ketoconazole (32-fold) and itraconazole (64-fold), thus increasing the resistance to the next level [25]. In a similar fashion, terbinafine has been employed to treat small numbers of infections with limited efficiency [51].

Posaconazole and voriconazole have been evaluated in limited cases yet yielding promising results. Although good *in vitro* activity has been recorded, therapy involves a long duration [51]. In addition, fosravuconazole and Isavuconazole were found to possess profound *in vitro* activity. However, unlike itraconazole, MICs of fluconazole, voriconazole, amphotericin B are not affected. Pathogens are reported to exhibit susceptibility towards these drugs [25], except the fact that amphotericin B has reported with suboptimal *in vitro* activity and toxicity level. There is only minimal amount of data exists on the liposomal activity that the drug, which is essential to be deciphered, to determine the antifungal activity. In addition, it has been proved that *M. mycetomatis* is resistant to class of echinocandins as well [52–54]. Dose dependent studies regarding the MIC determination of posaconazole are yet to be conducted. In view of these figures, voriconazole could be effectively used as a monotherapeutic agent, yet considering risk factors like emergence of resistant fungal strains. Although itraconazole (200–400 mg/day) is considered for current treatment, its inability to cure the disease has been put forward [25]. Thus, a clinically proven, efficient, and safe antifungal drug with short duration of treatment is essential for eumycetoma. But considering the economic and social conditions, it would take years to develop such a drug. Frustrated with prolonged and expensive treatment, some of the Sudanese patients have been reverted to herbal treatment. But this has led to adverse effects and further delay in treatment [17]. In the coming sections, the efficacy of phytotherapy has been discussed in perspective of developing plant-based drug.

6. MEDICINAL PLANTS WITH THERAPEUTIC POTENTIAL

6.1. *Acacia nubica* Benth.

Acacia nubica Benth. is an African origin medicinal shrub belonging to the family of *Leguminosae-Mimosoideae*. It is mainly found in African countries like Sudan, Egypt, Ethiopia, Uganda, Kenya, Tanzania, Arabia, and Kenya. It measures up to 1–5 m from the base, with grayish-white to yellowish-green branchlets. The plant is inhabited to dry and rocky soil. With stipular spines and pinnately arranged leaves, the plant appears as a thorny shrub spread on the land [55,56]. A recent evaluation showed that defatted methanol extract of the plant root bark could inhibit the fungal growth at MIC_{50} value of 4 µg/ml, which ranged between 0.5 and 128 µg/ml during the experiment conducted on *M. mycetomatis* [18]. However, there was no significant activity reported by this study. With no individual component with antifungal potential reported, *A. nubica* requires further attention of researchers to obtain antifungal constitutes. With the chemical extraction followed by the application of standard drug discovery procedure, phytochemcials of the plant could be efficiently used for treating eumycetoma.

6.2. *Nigella sativa* Linn.

Nigella sativa is an annual flower plant commonly known as black cumin or black caraway. It belongs to the family of *Ranunculaceae*, and can be found in Middle Eastern Mediterranean region, Northern Africa, Southern Europe, Indian subcontinent including India, Pakistan, Saudi Arabia, Turkey, and Syria. The plant usually grows up to 12 m in length with finely divided leaves. Flowers are blue to white colored and delicate. Fruits are large and comprise follicles, which in turn contain numerous seeds that could be used as spice, a replacement to cumin seeds [57]. The plant is reported to possess a feeble anti-fungal activity against *M. mycetomatis* with MIC_{50} value of 4 µg/ml, which ranged between 0.25 and 128 µg/ml. The gas chromatography–mass spectrometry analysis of the plant is yet to be conducted to reveal the phytochemical diversity of the plant, as there were no components reported during the study [18]. Alongside *A. nubica*, *N. sativa* could also be applied with drug discovery procedures to develop plant-based drugs with antifungal potential.

6.3. *Boswellia papyrifera* (Del.) Hochst

Boswellia papyrifera (Del.) Hochst, also known as Sudanese frankincense, is a flowering plant of African origin and native to the countries Eritrea, Ethiopia, and Sudan. Being a member of the family *Burseraceae*, the plant could grow up to 12 m of length with a straight regular bole and rounded crown. Leaves are large and compound. It usually grows on river gorges, grasslands, and rough soil. The mature tree is protected by bark which comprises resin, which is said to have diverse nature of chemical constituents that could be exploited for the medicinal purposes [58,59]. In a

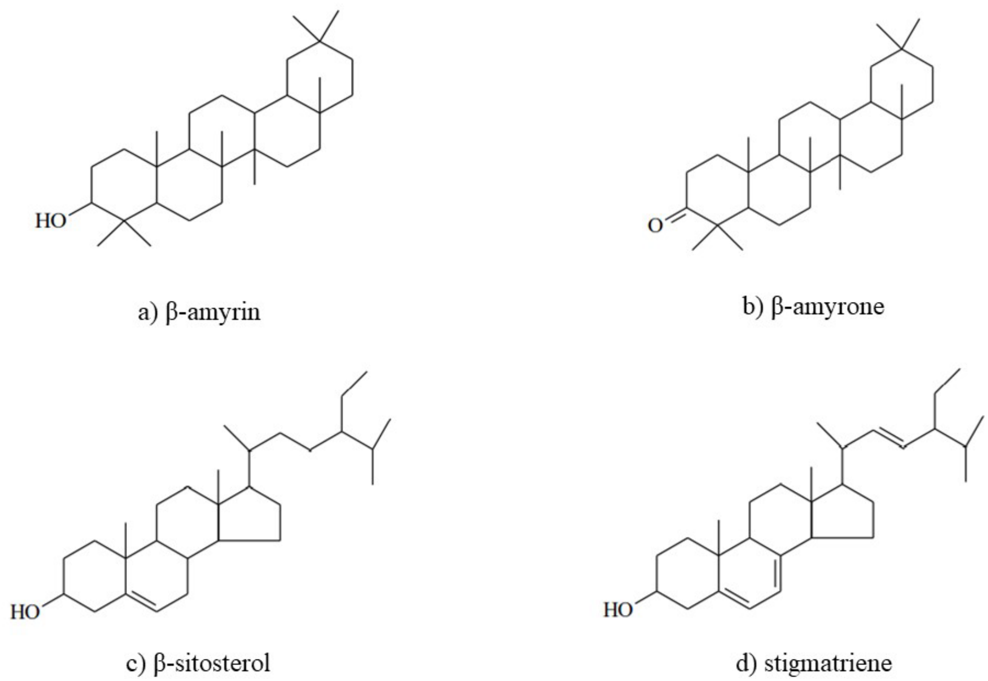

Figure 3: Triterpenoids from *B. papyrifera* with antifungal activity.

recent evaluation, gum resin of the plant using different extracts examined for antifungal activity against *M. mycetomatis*. Defatted methanol extract of *B. papyrifera* gum resin showed an MIC_{50} value of 1 µg/ml, which was ranged between 0.5 and 128 µg/ml. This was low and efficient in comparison with other plants reported (*A. nubica* and *N. sativa*). It has also been reported that the crude methanol extract comprises triterpenes like β-amyrone, β-amyrin, and stigmatriene, whereas soluble ethyl acetate extract contains β-sitosterol in addition to the three components listed above (Fig. 3). Furthermore, the *in vitro* antifungal activity of these individual compounds revealed that, MIC_{50} values of β-amyrin (0.5–>256 µg/ml), β-amyrone (0.25–>128 µg/ml), β-sitosterol (0.125–>128 µg/ml), and stigmatriene (0.125–>128 µg/ml) were significantly lower than that of crude extracts [18]. These findings show that plants like *B. papyrifera* should be evaluated for the individual compounds that possess antifungal activity.

6.4. *Melaleuca alternifolia*

Melaleuca alternifolia, popularly known as tea tree is a tall shrub used worldwide for the commercial production of tea tree oil. Being a member of the family *Myrtaceae*, it is endemic to the Australia and grows on swampy areas and alongside streams. The mature tree grows up to 7 m, surrounded by thin and whitish bark with a bushy crown. Leaves are whorled and contain numerous oil glands. Flowers are small, whitish cream colored and give a fluffy appearance in the spring season. Tree bears small woody, cup-shaped fruit that are present all over the branches [60,61]. A study conducted using tea tree oil on *M.* mycetomatis depicted the MIC of the range 0.008%–0.25% (*v/v*). The study revealed that a mere concentration of 0.06% (*v/v*) was required to reduce 50% of the fungal isolates and 0.25% (*v/v*) required to reduce 90% of the same. The study also predicts the presence of 40% terpinen-4-ol could be able to penetrate through the fungal cell wall and could be used as topical agent against the infection [16]. Advanced studies in this regard are essential to decipher the complete phytochemical as well as antifungal profile to develop plant-based drugs against eumycetoma.

6.5. Other medicinal plants

M. oleifera is a fast-growing, drought-resistant tree belonging to the family *Moringaceae,* and is commonly found in Indian subcontinent [62]. Though the plant is believed to possess significant antifungal activity, it has been neglected since many years since the incident of Sudanese patients declining the herbal treatment. But further examinations, including dose-dependent *in vivo* studies could reveal the actual true potential of the plant [17]. *Zingiber officinalis* or ginger is a flowering plant originating from Southeast Asia of *Zingiberaceae* family, whose rhizome is extensively utilized as a spice as well as a folk medicine [63]. Even though the rhizome part is believed to possess anti-fungal activity, plant failed to deliver the accurate results at expected rate [18]. Advanced assessments could further be carried out in a dose dependent manner to completely depict the antifungal potential of the plant. In addition, *Piper nigrum* is a flowering vine of Indian origin belonging to the family *Piperaceae* commonly known as black pepper or pepper. Seeds of the plant are extensively used as spice and folk medicine [64]. Fruit extract of the plant needs more attention like that of *Z. officinalis* [18]. Further, *Eugenia caryophillus* or clove is an evergreen aromatic plant bears flowering buds that are used as spice for their unique aroma. It belongs to the family of *Myrtaceae* and is native to the Southeast Asia [65]. *Cinnamomum verum* also known as true cinnamon tree, is a member of the family *Lauraceae*, and is commonly found in Southeast Asian countries [18]. Like all the other plants described

Table 2: A summary of phytotherapy against Eumycetoma.

Categories	Particulars			
Plant species	*A. nubica*	*N. sativa*	*B. papyrifera*	*M. alternifolia*
Fungi species	*M. mycetomatis*	*M. mycetomatis*	*M. mycetomatis*	*M. mycetomatis*
Type of study	*in vitro*	*in vitro*	*in vitro*	*in vitro*
Control	Inoculum and solvent	Inoculum and solvent	Inoculum and solvent	Artemisinin, itraconazole
Result	MIC_{50} value of 4 µg/ml, which ranged between 0.5 and 128 µg/ml	MIC_{50} value of 4 µg/ml, which ranged between 0.25 and 128 µg/ml	MIC_{50} value of 1 µg/ml, which was ranged between 0.5 and 128 µg/ml	MIC values of the range 0.008%–0.25% (*v/v*).
Inference	Confirmed antifungal activity	Confirmed antifungal activity	Significant antifungal activity with confirmed chemical compounds with MIC_{50}: β-amyrin (0.5–>256 µg/ml), β-amyrone (0.25–>128 µg/ml), β-sitosterol (0.125–>128 µg/ml), and stigmatriene (0.125–>128 µg/ml)	Concentration of 0.06% (*v/v*) required to reduce 50% of the fungal isolates and 0.25% (*v/v*) required to reduce 90% of the same. Possible fungal cell wall penetration by available 40% of terpinen-4-ol
Antifungal mechanism	Not defined	Not defined	Possible anti-inflammatory activity against mycetoma wounds. Confirmed antifungal activity of triterpenoids β-amyrin, β-amyrone, β-sitosterol, and stigmatriene	Possible antifungal activity of terpinen-4-ol, an essential oil component that inhibits the growth of the fungi

Adapted from van de Sande *et al*. [16] and Elfadil *et al*. [18].

above, it is used as a spice [66]. Along with the *Z. officinalis* and *P. nigrum*, the plant needs to be assessed further for potential antifungal activities. A summary of phytotherapy has been depicted in Table 2.

7. PERSPECTIVES AND PROJECTIONS

Eumycetoma has been an ominous infectious disease that has been categorized under NTDs. The extensive lethality is equally attributed to the economical setbacks of the developing countries, people with substandard level of education and awareness about health. The chemotherapeutic agents have been used so far have not given good results except itraconazole; but the risk of adverse effects and possible drug resistance prevail the efforts of public health agencies and researchers [19,51]. In the vista, phytochemcials from various plants irrespective of their total medicinal potential could be useful in treating eumycetoma. It could be a noteworthy effort to induce health awareness programs in endemic regions to practice safety measures while working, as the fungal invasions occur through open wounds. In addition, people must be advised to go for timely diagnosis, which could substantially reduce the burden of the disease. This could be further modified with contributions from different sectors of the society, including scientists, academics, non-governmental organizations, pharmaceutical companies, and health officials to practice "One-Health Approach" [67]. Designing efficient and accurate diagnostic tools and techniques like DNA sequencing, 16S rRNA, PCR, PCR-restriction fragment length polymorphisms, PCR-RAPD, and AFLP has been done [6]. However, advanced methods like RNA interference technology, and proteomic analysis for possible target search need to be developed.

In the treatment sector, development of chemotherapeutic models for the drug analysis, targeting the virulent proteins and enzymes for the inhibition of the associated pathogenic mechanisms could be practiced. Even in this regard, there have been some gaps to be taken care of. Most of the people cannot afford these expensive diagnostic and therapeutic tools, as they are economically backward. Considering these options, treatment of the infected becomes difficult despite colossal amount of research and funding [19].

As many of the endemic countries belong to the substandard levels in the global economy, it becomes essential to utilize available natural resources as a source of medicine. Thus, usage of cost-effective measures like phytotherapy becomes a feasible approach. A limited amount of research has been conducted in herbal treatment [16,18]. Though it takes a considerable amount of time to develop plant-based drugs, one must notice the resistance of the fungi to the available chemical therapeutic agents, that in turn could reverse the entire landscape of treating eumycetoma. The reported phytochemicals can be easily extracted from highly efficient plants like *B. papyrifera*. These phytochemical compounds need to be characterized, evaluated for their cytotoxic effects and possibilities of their synthesis *in vitro* conditions need to be studied. In addition, qualitative and quantitative assays using individual compounds in a dose dependent and combination approaches could reduce the burden of multifactorial results. These isolated compounds could be further modified according to the requirement based on cytotoxic evaluations done in primary screening. These compounds could be analyzed *in silico* for effective binding and inhibitory effect on specific component or any part of the identified target. Further, the bioavailability of the compounds could be dealt to modify the compound to match the physiological conditions of the target organ. Usage of *in silico* methods like molecular modeling and docking could save time and money spent for pre-clinical trials. Following these protocols would surely help the treatment of eumycetoma through phytotherapy.

8. CONCLUSION

Eumycetoma has been an extensively pathogenic and drug-resistant fungal ailment spread all over the world, especially in Mycetoma belt. The invasive infection of this disease is attributed to both the causative agents that resist the therapeutic treatment. It is equally supported by the humans practicing no safety measures and deficient medical treatment, due to poor economic background. The review focuses on the various factors of the disease like infection, transmission, epidemiology, pathogenesis, diagnosis, available therapeutic agents, and phytotherapy. The aim of the review is to highlight the role of medicinal plants and their constituents, which may aid the phytochemical based approach to cure the disease. No major research breakthrough has been completed in this aspect, leaving behind much of the facts unknown about the reported phytochemcials from the plant extracts. This review has highlighted the work done so far, in the phytotherapeutic approach and it further dwells on the requisite in research and development. It can thus be concluded that with the following of projections discussed, it could be easier to find a plant-based cure for eumycetoma.

9. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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