Studies on Antifungal Effects of Some Plant Extracts on Fungal Isolates of *Candida albicans* and *Trichophyton mentagrophytes* in Jimma Town, Ethiopia

1Gemechu Hailu, 2Molalegne Bitew and 3Mathewos Temesgen

1Office of livestock health, Jarso District, Western Wollega, Ethiopia
2Ethiopian Institute of Biotechnology, Addis Ababa, Ethiopia
3Department of Biology, Ambo University, Ethiopia

Abstract: *In-vitro* studies on the efficacy of crude extracts of *Croton macrostachys* and *Allium sativum* against *Candida albicans* and *Trichophyton mentagrophytes* was conducted in Jimma Town, Ethiopia. The leaves and the bulb cloves of *Croton macrostachys* and *Allium sativum*, respectively were collected and the crude plant extracts were prepared. The *in-vitro* antifungal activity was evaluated at six different concentrations (5%, 10%, 15%, 20%, 25% and 30%) by agar disc diffusion method for several replicates and the activity obtained was not concentration dependent except methanolic extract of *Croton macrostachys* on the strain of mould. The results were compared with standard antifungal drug (Ketoconazole) and distilled water. *Trichophyton mentagrophytes* was more susceptible fungal strain, while *Candida albicans* was more resistant one. The results revealed that the methanolic extract of *Allium sativum* demonstrated more growth inhibitory activity against both fungi strains which studied than methanolic and aqueous extracts of *Croton macrostachys*. There was no a significant variation in the zone of inhibition between the different concentrations (p>0.05). However, there was high significant variation between the plant species, method of extraction and the tested fungus (p<0.001). Findings from this study confirmed that plant extracts can be used as natural fungicides to control pathogenic fungi, thus reducing the dependence on the synthetic fungicides.

Key words: Antifungal activities • *Candida albicans* • Crude Plant Extracts • *Trichophyton mentagrophytes*

INTRODUCTION

Economically fungi are important in that some of them have direct public and animal health importance, some others are responsible for toxin production and others are contaminants of foods [1]. For instance, yeasts of a genus *Malassezia* inhibit the skin growth for variety of mammals and birds and are considered as opportunistic pathogens in animals and human beings [2]. *Cryptococcus neformance* is also an opportunistic fungal pathogen, which causes fatal meningoencephalitis in patients with compromised immune responses [3]. *Candida albicans* has a worldwide distribution and is common commensal of GIT and urogenital tracts of human [4]. In addition, fungal infections have been damaging the different plant species. For instance, the imperfect fungi called *Phaeoisariopsis griseola*, is one of most widely distributed and damaging disease of common bean (*Phaseolus vulgaris*), which causing a yield losses as high as 80% all over the world [5].

Fungal infections remain a significant cause of morbidity and mortality of animals despite of advances in medicine and the emergence of new antifungal agents [6]. The most responsible fungal groups causing morbidity are *Aspergillus* and *Candidia* species [7]. Of these, the *Zygomyces Absidira corrymbifera*, *Rhizopusoryze*, *Rhizommucor puzzles* and *Mortirella wolfi* are the most common infectious, which cause systemic meiotic disease. Additionally, the infections most commonly recorded in bovine mycotic abortion are *Mucor*, *Aspergillus* species, *Petrilellidiumbode*, *Candida parapoilosis* and *Mortirella wolfii* [8]. For instance, many different species of fungi have been isolated from conjunctiva sac of horses, including *Aspergillus* species and other molds,
such as Cladosporium, Mucor, Fusarium, Alternaria and Candida species [9]. Another common type of infection is a yeast infection, which caused by the fungus C. albicans [10].

Candida albicans, the agent of candidiasis, is an increasingly important disease, because it is a frequent opportunistic pathogen in its nature [11]. It is a common commensal of the gastrointestinal and urogenital tracts of human [4] and is the cause of Candidiasis in women [12]. In addition, Candida tropicalis is one of the non-albicans Candida strains currently emerging in fungal infections [13]. The treatment of systemic mycosis has been largely by amphotericin-B and nystatin, but the newer azoles compounds (Enilconazole, Fluconazole, Itraconazole and Ketoconazole) being administered orally appears to be highly effective and easy [14]. However, the cost of conventional drugs and its resistance to pathogens is the major problem, especially in rural areas where high proportion of livestock is reared and far from the modern drugs [15]. Since a strain of C. albicans with multiple antibiotic resistances is increasing, it is of great importance to find effective treatments for these pathogens. To overcome the alarming problem of microbial resistance to antibiotics, the discovery of novel active compounds against new targets is matter urgency. Therefore, researchers are increasingly turning their attention to folk medicines, looking for new leads to develop better drugs against microbial infections [16].

Natural products have been served as a major source of drugs for centuries and about half of the pharmaceuticals in use today are derived from natural products [17]. The study has indicated that about 25 to 50% of current pharmaceuticals are derived from plants [18]. Microbiologists and natural product chemists trying to discover more about phytochemicals, which could be developed for treatment of infectious diseases [19]. Recent trends favor the use of plant extracts and their essential oils show antifungal activity against a wide range of fungal effects [20]. Many of these plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicines [21]. Several authors also studied the effect of different plant extracts on the growth of fungi [22-24]. Findings from this study confirmed that plant extracts can be used as natural fungicides to control pathogenic fungi, thus reducing the dependence on the synthetic fungicides [19]. There are also some folkloric herbs still in use without any scientific evidences. Therefore, pharmacological and clinical data should be conducted for herbal medicinal products to remove the impedimentation of integration of herbal medicines in to conventional medical practices [25]. However, there is no information on antifungal effects of Croton macrostachys and Allium sativum on fungal isolates of Candida albicans and Trichophyton mentagrophytes. Therefore, this study was aimed at assessing their in-vitro antifungal activity of C. macrostachys and A. sativum on the growth of C. albicans and T. mentagrophytes in Jimma Town, Ethiopia.

MATERIALS AND METHODS

Description of the Study Area: The present study was carried out at Jimma town Southwestern part of Ethiopia, which is located on 346km away far from the capital Addis Ababa between latitude of 7013'-8056’N and longitude of 3552’-37037’E, with an elevation ranging from 880m to 3360m above sea level. The study area receives a mean annual rainfall of about 1530mm. The mean annual minimum and maximum temperature of the study area were 14.4 °C and 26.7 °C, respectively. There area is characterized by mixed farming systems of crop production and livestock rearing. The most important crops grown are; Maize, Teff, Sorghum, Horse bean and Chick pea. Majority of the animals kept in the region are; cattle, sheep and there are some goats, horses and donkeys.

Materials and Design of Study: An experimental study was used to determine the antifungal activity of selected plants that was conducted between November 2010 and May 2011 in Jimma University College of Agriculture and Veterinary Medicine. Allium sativum (Qullubbii adii in Oromic, Netch shunkurt in Amharic) and Croton macrostachys (Bakkanniisa in Oromic, Bisanna in Amharic), were collected from different places of Jimma town and identified and authenticated by a botanist in the Department of Botany, Jimma University, Ethiopia. The seeds of Croton macrostachys are used as purgatives, antihelmentics and mulluscides [26]. The two plants were extracted by methanol and distilled water at different concentrations in order to compare and contrast them with their activity against both fungi growth. The test organisms used for screening the antimicrobial activity of the extracts were fungal isolates identified as Candida albicans and Trichophytonmentagrophytes that were acquired from Ethiopian health and nutrition research institute, Addis Ababa.
Methods

Pre-Extraction Preparation of the Two Herbal Medicine:
After collection of the plants, leaves and shoots of the fresh C. macrostachys were washed using distilled water, drained and cut into very small pieces using a knife, then dried at room temperature (25 °C) for seven days in the laboratory. In addition, A. sativum purchased from the shops of Jimma town were separated into its cloves, peeled to remove its cover and cut into small pieces using a knife. After appropriate drying, the dry products of both plants were grinded into a thin powder by using a pestle and mortar, sealed properly and kept in plastic vials until used for preparation of the crude extracts [27].

Preparation of Crude Plant Extracts: To obtain different concentrations of crude plant extracts, different concentrations like; 5%, 10%, 15%, 20%, 25% and 30% by 99.5% of methanol and distilled water were used as solvents. Then, 5gm of each of the powdered plant material was macerated in 100ml of methanol (99.5%) in 500ml flasks, kept on a rotator shaker and shacked for 24hrs to prepare 5% concentration of methanolic extracts. Thereafter, it was filtered through gauze placed in a tea strainer and centrifuged at 3000rpm for 5 minutes, so that the plant material, which passed through the gauze and tea strainer was sedimented at the bottom of test tubes. The supernatant was collected and evaporated in rotary evaporator till bioactive compounds not affect. The same procedure was continued to prepare all the rest concentrations for both methanolic and aqueous extracts of both plant species by changing their weight together with change of concentrations [27].

Microbial Culture and Growth Conditions: The work surface was sterilized by 70% ethanol alcohol and 9.75gm of Sabouraud’s dextrose agar media was mixed with 150ml of distilled water and boiled on bath water and stirrer. Then, it was autoclaved at 121°C for 15 minutes. After autoclaving, chloramphenicol was added to the medium under laminar flow hood to make the medium selective for fungi. About 15ml of the medium was poured in to sterilize Petridish up to a level of approximately 4mm and kept until it was solidified. Dishes with solidified agar were incubated at 25 °C for 24hrs for sterility checkup. C. albicans and T. mentagrophytes were inoculated on the media using sterile wire loop and the inoculated dishes were incubated at 25 °C for 24hrs. Finally, the obtained colonies were identified culturally and stored in the refrigerator at 4°C.

Antifungal Assay of the Tested Plant Extracts:
To evaluate the antifungal activity of tested plant extracts, sterile agar plates were used according to the disc diffusion assay [28]. A loopful C. albicans and T. mentagrophytes from Petridishs were inoculated into test tubes containing Sabouraud’s broth media and incubated at 25°C for 24hrs. After 24hrs, the diluted broth cultures were aseptically poured and spread on sets of Sabouraud’s dextrose agar plates. Surplus suspension was decanted from the surface of the agar plates which were allowed to dry at room temperature for 5 minutes. Later sterile, Whittmann No.1 filter paper discs (5.0 mm diameter) were impregnated with different concentrations (5%, 10%, 15%, 20%, 25% and 30%) of the plant extracts and allowed to air dry in the laminar flow hood for 3 minutes [29]. Other discs impregnated in aqueous suspension of standard ketoconazole served as a positive control and discs impregnated in distilled water served as a negative control. The impregnated discs in the ketoconazole were placed firmly at the center of the culture plates, whereas discs impregnated in different concentrations of crude plant extracts and discs impregnated in distilled water were placed firmly near the periphery of the culture plates. The plates were incubated at 25°C for 72hrs. Following incubator period of 72hrs, plates were removed from the incubator and antifungal activity was evaluated by measuring zones of inhibition of fungal growth. Clear zones within which fungal growth absent were measured and recorded as the diameter (mm) of complete growth inhibition. The whole experiment was replicated five times to minimize error.

Statistical Analysis: Descriptive statistical methods was used by Microsoft office excel (2003) for data analysis and the results was present as percentages and tables for illustration. The laboratory results were analyzed using SPSS version 16.0. The significance of association between and among the considered variables was determined using Chi-square (x2).

RESULTS

On the bases of this study, all the tested concentrations (5%, 10%, 15%, 20%, 25% and 30%) of the two plant extracts (C. macrostachys and A. sativum) inhibited the two fungal strains growth with varying degree of sensitivity except aqueous extraction of C. macrostachys on the C. albicans. Out of the all 240
replicates tested with different concentrations, 158 (65.8%) of them showed inhibition zone and 82 (34.2%) of them did not show inhibition zone against the test fungus (Table 1 & 2).

The result showed that the antifungal activity of the two crude plant extracts determined by disc diffusion method. Both the aqueous and methanolic extracts of *A. sativum* exhibited antifungal activities against almost the tested organism except 15% and 25% of the plant extraction. The methanolic extract 5% of *C. macrostachys* and all its concentrations of aqueous extract were never demonstrated inhibitory activity against *C. albicans* (Table 3).

The antifungal activities of both crude plant extracts were shown according to the disc diffusion assay against mould strain. Both aqueous and methanolic extracts of *A. sativum* showed good inhibitory activity at all tested concentrations against the organism which studied. In addition, *C. macrostachys* showed antifungal activity with both extraction methods except at 25% of its aqueous extraction on *T. mentagrophyts*. This indicates that the yeast strain (*C. albicans*) is more resistant than the mould strain (*T. mentagrophyts*). According to this study, *A. sativum* showed more antifungal activity on both *C. albicans* and *T. mentagrophyts* than that of *C. macrostachys* by both methanolic and aqueous extraction methods (Table 4).

Generally, there was no a significant variation in the inhibition zone between the different concentrations which studied (*p* > 0.05). However, there was highly significant variation observed between the plant species, extraction method and the tested fungus (*p* < 0.001).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Total replicates</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. macrostachys</em></td>
<td>120</td>
<td>56 (46.7%)</td>
<td>64 (53.3%)</td>
<td>0.000</td>
</tr>
<tr>
<td><em>A. sativum</em></td>
<td>120</td>
<td>26 (21.7%)</td>
<td>94 (78.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>240</strong></td>
<td><strong>82 (34.2%)</strong></td>
<td><strong>158 (65.8%)</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Total replicates</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic</td>
<td>120</td>
<td>28 (23.33%)</td>
<td>92 (76.67%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Aqueous</td>
<td>120</td>
<td>54 (45.0%)</td>
<td>66 (55.0%)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>240</strong></td>
<td><strong>82 (34.17%)</strong></td>
<td><strong>158 (65.8%)</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Plant species</th>
<th>Methanolic extract (%)</th>
<th>Aqueous extracts (%)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td><em>C. macrostachys</em></td>
<td>5 10 15 20 25 30</td>
<td>5 10 15 20 25 30</td>
<td>18</td>
</tr>
<tr>
<td>-</td>
<td>8 6 6 6 6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>A. sativum</em></td>
<td>10 10 9 13 9 9</td>
<td>7 8</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>-</td>
<td>10 8</td>
<td>10 7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Keto. = Ketoconazole, +ve = Positive, -ve = Negative, - = Zero zone of inhibition, Dist. = distilled water, Value are mean of the five replicates

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Plant species</th>
<th>Methanolic extract (%)</th>
<th>Aqueous extracts (%)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. mentagrophyts</em></td>
<td><em>C. macrostachys</em></td>
<td>5 10 15 20 25 30</td>
<td>5 10 15 20 25 30</td>
<td>22</td>
</tr>
<tr>
<td>10 12 13 15 11 12</td>
<td>8 8 6 9</td>
<td>10 13 13 16 10 14</td>
<td>2%(Keto.+ve)</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Dist.Water(-ve)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Keto. = Ketoconazole, +ve= Positive, -ve = Negative, - = Zero zone of inhibition, Dist. = distilled water, Value are mean of the five replicates
DISCUSSIONS

The fungicidal activity of some plant extracts in controlling different plant pathogens have been reported by several researches [23, 30, 31]. The active compounds present in plants are influenced by many factors, which include the age of plant, extraction solvent, method of extraction and time of harvesting plant materials [32]. Many researchers have reported the presence of antifungal in different plant extracts, which cause inhibition of radial growth and spore germination and the reduction of in rot development by the pathogen in-vitro [22-24]. The differences observed in fungitoxic activity to the extracts were likely to be due to the solubility of the active compounds in aqueous and organic solutions or the presence of inhibitors to the fungitoxic principle [33].

The tested organisms in the present study (C. albicans and T. mentagrophytes) have been indicated in causing of dermatomysitis [34]. Our finding indicated that the presence of active antimicrobial agents in C. macrostachys and A. sativum plants thereby justifying their use for the treatment of C. albicans and T. mentagrophytes infections. A similar study of screening natural plant extracts against different fungal pathogens was well recorded in literature [35].

Of the two plants, the aqueous and methanolic extracts of A. sativum showed an induced zone of inhibition on both fungal strains. However, the effect of plant extracts was varied with the different fungal strains and extraction solvents. The results of this study corresponds with work done by Slusarenko et al.[36] who tested the effectiveness of garlic juice against range of plant pathogenic bacteria, fungi and oomycetes in-vitro.

The minimum inhibitory concentration (MIC) of A. sativum was also observed with both test organisms and extraction solvents. The effects of the antifungal compounds may be on spore germination leading to its inhibition or may be due to the effect of these compounds on the cell wall altering its permeability [37]. The inhibition zones induced by both plant extracts were observed to reduce in size and the time implying the active ingredient was losing its potency with time. Although the methanolic and aqueous extracts of C. macrostachys showed zones of inhibition on the strain of mould, but the aqueous extracts of the plant did not show any inhibition zone on the C. albicance. This may probably due to the fact that the extracts were in suboptimal doses to effect the inhibition of the organism. Moreover, the test organism used may have been a highly resistant strain [31]. The result is in agreement with that of Parveen et al. [23].

Thus, from the overall results obtained, it is evident that the two plants screened possess antimicrobial agents against some pathogenic organisms associated with skin infections. They therefore justify their popular use by local herbalists in the treatment of skin diseases.

CONCLUSIONS

In this study, the leaf of C. macrostachys and bulb cloves of A. sativum showed good antimicrobial activity against C. albicans and T. mentagrophytes, whereas the aqueous extraction of Croton macrostachys leaf has no any activity against C. albicans. The demonstration of satisfactory growth inhibition against C. albicans and T. mentagrophytes at different concentrations would see justify their potential in the synthesis of new phytoremedies and their use in treatment of microbial infections. These findings suggest that there is a potential in the discovery of novel antimicrobial agents from the two herbal medicines that are found in Ethiopia.

ACKNOWLEDGMENTS

The authors acknowledged Haramaya University for their financial support. In addition, we would like thank Jimma University, College of Agriculture and Veterinary Medicine for their laboratory and material support. Finally, all authors in this study are duly acknowledged.

REFERENCES


