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INDIGENOUS MEDICINAL PLANTS POTENTIAL AS ANTIMYCOTIC DRUG TO CURE FOOT FUNGAL DISEASES AND PRODUCTION OF ITS COMMERCIALABLE HERBAL PRODUCT TO FACILITATE LOCAL COMMUNITIES OF DISTRICT BHIMBER, AZAD KASHMIR

Maria Maroof[1*], Muhammad Fezan Arshad[1], Amin Ullah Shah[2], Khizar Hayat Bhatti[3]

[1]*Department of Botany, Mirpur University of Science and Technology (MUST), Mirpur-10250 (AJK) Pakistan*

[3]*Department of Botany, University of Sargodha, Pakistan*

[4]*Department of Botany, University of Gujrat, Hafiz Hayat Campus, Gujrat-50700, Pakistan*

***Corresponding Author's Email:** mariamaroof72@gmail.com

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ABSTRACT

In present research project a comprehensive investigation was conducted to explore the contaminants in herbal drugs. In this research, indigenous medicinal plants of District Bhimber which has been used by local communities since long past; were selected and tested for their medicinal potential and plethora of contaminations by unconscious under/or deliberate adulteration by similar types of phenotypic plants. Mycopathogen *Candida albicans* and others (*Microsporum canis, Trichophyton mannum*) were tested by four plants (*Azadirachta indica, Eucalyptus citridora, Allium sativum,* and *Mentha piperita*) In this analysis, ethnobotanical approach consisting of survey was used for exploring indigenous medicinal knowledge and herbarium and fresh specimens were used to study morphological description to study plants for identification purpose. The values were measured and data was statistically analyzed throughout research of different medicinal plant's properties against the infection *Candida albicans.* Based on these results, it is existed that the MIC diverges amongst extracts which differs significantly from the control. Overall, the presence of terpenoids in *Azadirachta indica* which inhibits fungal hyphae growth in *Candida albicans* illness. *Azadirachta indica* showed best antifungal activity that devastates progression of fungus after comparing the antifungal activity of extracts from four plants against foot fungal disease caused by pathogens. In comparison to the other three therapeutic plants, Eucalyptus contains a molecule called 1,8-cineole, which inhibits fungus development. However, because Neem oil includes Quercetin and sitosterol, it was assessed that it performed

better against *Candida albicans* in all four extracts to cure Athlete's foot illness. In this study, it is concluded that ethanol solvent gets better results and minimum inhibitory concentration (MIC) value differ in different plant extract.

Key Words: Herbal drugs antifungal activity, *Candida albicans, Eucalyptus citridora,* Athlete's foot, Minimal Inhibitory Concentration (MIC)

INTRODUCTION

Herbal medicines are the products that are obtained naturally either from aerial portions of plants or from underground parts of plants, or from a combination of both. Juices, gums, fatty oils, and resins, among other plant products, might be used (Rauf *et al.*, 2013). Traditional herbal medicines have gained worldwide popularity in recent decades as a source of alternative and complementary therapies. Herbal remedies are used by the majority of people to cure a variety of diseases and illnesses. These herbal medications, in contrast to conventional drugs, are thought to be the best because of their natural origins. Traditional herbal medicines have been utilized in the treatment of human illnesses for many years in numerous nations, including China, India, Italy, Iran, and Pakistan (Ghosh *et al.*, 2009).

Fungal illnesses affect people all around the world. Fungi can cause everything from a minor infection like athlete's foot to a life-threatening bloodstream infection. The Mycotic infections division of centers for disease control is including with three types of infections: Community acquired infections, Opportunistic infections and hospital acquired infections (Ahmed *et al.*, 2009).

The fungi are nucleated, achlorophyllous individuals with filaments that may or may not have septa in their body arrangement called hyphae, which in most cases contain an inaccurate physique, the mycelium. Cell walls made of chitin or cellulose, or both, demarcate the hyphae. They imitate sexual and asexual reproduction through the formation of spores (Raza *et al*., 2009).

Community-acquired infections are the second type of mycotic infection. These are typically caused by soil-borne fungal spores. People inhale spores when soil is disturbed while resultant in infections such as Histoplasmosis, Blastomycosis and Coccidiomycosis. The blastomycosis is an infection caused by the spores that comes from *Blastomyces dermatitides.* In most circumstances, spores persist dormant inside the host. The histoplasmosis is an alternative

illness initiated by *Histoplasma capsulatum* spores that distresses the lungs by posing a serious threat to immunocompromised people such as AIDS patients. Coccidiomycosis, affected by the Coccidiosis species which causes Pneumonia. Antifungal medications now on the market can treat diseases of this type (Ahmed *et al*., 2009).

Diverse fungi cause various diseases in various parts of the human body. Dermatophytes can enter the host body through scars, wounds, injured skin, and burns. The pathogens have capability to use Keratin as food supply, thus they enter the nonliving, keratinized stratum corneum layer of the skin and create the enzyme keratinase. They cause an inflammatory reaction at the infection site, and redness, swelling, heat or burning are all signs of an inflammatory reaction. (Raza *et al.*, 2009).

Trichophyton is a genus belonging to family Arthrodermataceae, order Onygenales of class Eurotiomycetes belonging to division Ascomycota. The genus Trichophyton consisting up of large varieties of parasitic dermatophytes which may become the reason of many dermatological illnesses such as Tinea, Athlete's foot, Ringworm, Jock itch and other skin, scalp, beard and hair infections are caused by it. It is a mold that may have smooth walled conidia that may be micro or macro according to size. Macro conidia are of about 8um to 50um in size and mostly grow laterally on hyphae's may be thick or thin walled and these are very rare or totally absent in many species (Mirza *et al*., 2017). *Candida albicans* is a specie of genus Candida of family Saccharomycetaceae belongs to order Saccharomycetales of class Saccharomycetes of division Ascomycota (Fang *et al.*, 2009).

The optimal temperature used for the growth and sporulation of Trichophyton (Candida) is almost 25 to 30^0C. Optimum pH value for compatible growth is less than 7. Macro conidia are most abundant at pH 8. Light exposure to light had no effect on rate of growth, rate of sporulation or production of pigments (Sabir *et al*., 2003). Mannose, glucose (carbohydrates), mannitol and maltose are the carbon compounds that enhance the growth. However, urea, ammonium sulphate asparagin show very poor growth. While nitrates were not added. *Trichophyton persicolor* can prepare vitamins so can easily grow on medium free of vitamins. However, zinc, iron, manganese and copper enhance the growth and spore production of *Trichophyton persicolor*. Usually dermatophytes cultivate between 0^0C to 30^0C. Variation in temperature and humidity causes the fungus to move towards margin, thus creating a concentric ring. Dermatophytes produce enzymes

such as chitinase or proteinase to derive nutrition from surrounding epidermal structures (Bokhari *et al.*, 2001).

Different types of Trichophyton possess different habitat according to its need and requirement. Some species are geophilic that live in soil and some are anthrophilic that prefer to infect the humans and some species of Trichophyton are zoophilic that infect different kinds of animal (Hussain *et al*., 2002). *Trichophyton ajelloi, Trichophyton flavescens, Trichophyton gloriae, Trichophyton terrestre, Trichophyton vanbreuseghemii, Trichophyton onychocola* and *Trichophyton phaseoliforme* are geophilic. According to the survey done by of World Health Organization abbreviated as WHO on the prevalence of infection of dermatophytic fungi, there is about 20 percent people have cutaneous infections on whole world. *Tinea corporis* is the most common fungal disease (about 70 percent) as compared to the *T. cruris, T.pedis* and Onychomycoses. Nor any race neither person of any age free from dermatophytic infections (Ali *et al.*, 2009).

AIMS AND OBJECTIVES

- Conduct an ethnobotanical survey in the Samahni area of District Bhimber, AJK to identify the medicinal potential of indigenous medicinal plants.
- To develop a low cost, ecofriendly product from indigenous herbs by using different indigenous herbs to prevent fungal skin infection.
- Testify an antifungal ability of selected plant species from the study area against dermatophyte.
- Examine a fungal illness such as athlete's foot and its phytotherapist action.

MATERIAL AND METHODS

Data Collection

Normally, a myco-pathogenic experimental strategy yields better results. Numerous field survey plans were designed and casual interviews with the residents were conducted in order to extract as much information as possible from the people or farmers. The field survey was conducted in the months of March, April, May, and June of 2020 &2021. Several visits to the

District were undertaken on a seasonal basis in Bhimber. All necessary material was meticulously studied before travelling into the field. The information was gathered utilizing the questionnaire method throughout the field trips. People reported their own recipes and other precautions they employed to protect themselves from fungal infections during the research. (Abbasi *et al.*, 2010).

Before heading to the field, we acquired as much information as possible about the location so that we might take preventive precautions. Photographs of the flora were also taken during the field trip.

Survey

An interview is the most effective technique to gather relevant information on the subject. Conducting interviews allows you to gather precise and up-to-date data and information about the subject. The survey was done in four villages of tehsil Samahni, namely Hillah, Sandar, Poona, and Potha. Each village was given ten informants. There were 45 girls and 15 males among the forty informants. Their ages ranged from 20 to 85 years old.

During the survey, it was discovered that females are more interested in supplying information than guys. The information gathered by the informants was accurate and trustworthy. According to the informants:

i) Wear used garments only after washing them in hot soapy water and drying them in the dryer.

ii) Combs, brushes, caps, hair clips, and barrettes should not be shared with others.

iii) Except for a bath, try to keep your skin dry. The rash is worse by wet skin.

iv) Use Tara mira oil for hairs to prevent dandruff.

iv) Ringworms and athletes foot can be treated with neem leaf oil.

v) Cotton socks are preferable to synthetic socks.

vi) Allow sweaty shoes to air dry before re-wearing.

vii) Wet suits or wet divers should be changed as soon as possible since moisture might exacerbate a fungal septicity.

viii) Garlic oil scorching is likewise recommended as a therapy for fungal skin infections.

ix) Dry Powder of lemon pericarp and Clove is mainly useful for fungal diseases.

x) Ringworm can similarly be spread by cats and dogs. So, take your dogs to veterinarian for a checkup and to seek advice on how to care for them.

xi) To get rid of dandruff from your hair, mix yoghurt with eggs.

xii) Eucalyptus oil is an excellent treatment for fungal infections.

xiii) Cloves are also quite helpful when included in one's everyday diet.

Selection

The flora of Tehsil Bhimber is stunning. Antifungal properties can be found in a wide range of plants, including mint (*Mentha piperata*), eucalyptus (*Eucalyptus citridora*), garlic (*Allium sativum*), neem (*Azadirachta indica*). However, eucalyptus, mint, garlic, and neem were used in the current study. Garlic's botanical name is *Allium sativum*, and it belongs to the Lilliaceae family. *Corymbia citridora* Hook is the botanical name for eucalyptus, and it belongs to the Myrtaceae family. *Azadirachta indica* is the scientific name for neem, and it belongs to the Meliaceae family. Mint's botanical name is *Mentha piperata*, and it belongs to the Lamiaceae family. These plants were chosen for their therapeutic capabilities as well as their availability in the chosen area.

Extraction Methodology

Maceration was used to carry out the extraction process. 50 grams of eucalyptus powder were steeped in 250 milli liters of ethanol and stored in an airtight container. I left it for seven days, shaking it every now and then. Whatmann filter paper, size 10, was used to filter the solvent. The extract was then dried at 600°C using a rotary vacuum evaporator. It was possible to obtain a dark green extract. The extract was saved for later use. To achieve around 3g of extract, the same procedure was performed three times. The same technique was used for finely ground mint, garlic, and neem powders. Plant extracts in methanol, ether, water, and chloroform were obtained using the same technique (Ahsan *et al.*, 2010).

Statistical Analysis

All of the values were taken in triplicates by repeating the method to confirm the mic value and analyze it using statistics, and the data was then described as mean value standard error. Microsoft Excel was used to calculate the mean value and standard deviation. The data was also analyzed using Minitab and SPSS software (Al-Abed *et al.*, 2003).

ANTIFUNGAL ACTIVITY PROTOCOL

Collection, Growth and Identification of Fungal Species

I visited various dermatologists, including the combined military hospital in Jhelum (Dr. Mahnoor) for one day and the district headquarters Mirpur AJK (Dr. Ahmed) for three days, in order to check out various common fungal diseases. I also spent nearly 15 days at the district headquarters in Bhimber, observing and photographing patients infected with fungal skin spores, as well as taking spores as a sample.

Collection of Specimens

The samples were collected from various parts of the body of Bhimber district headquarters patients. By using new clinical blades for each patient, this procedure was eliminated. The most common method for collecting specimens was scraping the affected area onto soft, clean paper, which was then transferred into sterile bottles, which were then labelled with the individual's information (Fang *et al*., 2009).

Fungus Recognition

The colony characteristics and conidial morph were used to identify inaccessible fungi. A colony counter was used in the laboratory of Department of Botany (MUST Bhimber Campus) in order to account the colony of fungi grown-up on petri dishes. Then the slides were created via using an immunizing needle to cut a thin piece of a fungal collection from the developing boundary and recognizing it under a microscope at 40X and 100X magnification. Digital camera was used to take the photos. Every measurement and observation was carried out 3 intervals. These photographs were then transmitted to Punjab University's microbiology department and the micro lab of University of Veterinary and Animal Sciences in Lahore for documentation (Pankaj *et al.*, 2011)

Antifungal Assay

Potato dextrose agar was prepared and poured into flask again after three days. In an autoclave with agar, 25 petri dishes were washed and sterilized. All petri dishes were arranged in five rows and five columns in laminar airflow. The same specimen and different solvents were labelled on each row of Petri dishes. Then, all 25 petri dishes were filled with Potato dextrose agar and each specimen was placed in a petri dish in the same row. The fungal specimens were spread using a spreader, and 5 wells were created in each petri dish using the corn borer. Trichophyton were spread across the first row of five petri dishes, with each dish labelled as Trichophyton in ethanol, Trichophyton in water, Trichophyton in methanol, Trichophyton in ether, and Trichophyton in chloroform. In addition, Trichophyton in ethanol plants solvent (Terbinafine 125mg) was placed into the fifth well of each petri dish. The Petri plates were transported from the air flow to the incubator to be incubated, and each well was labelled. Each row's petri dishes were prepared in the same way (Salazar *et al.*, 2015).

ZONES OF GROWTH INHIBITION CALCULATION

4 tables were drawn on each specimen's paper and the growth inhibition zone were restrained in cm using a scale after 5 days and recorded in each table. After that, each unit was multiplied by ten to convert the measurements to millimeters (mm) (Sen *et al.*, 2011).

Two-Way Analysis of Variance

When each observation is classified according to two criteria (or variable) of classification concurrently, we utilize the two-way analysis of variable methodology. The classified data are stored in a table, in which the column indicate one criterion (or variable) of classification and the row indicate the other criterion. If there are c-columns and the r-row in the table, then there will be entirely all cells. Each cell may hold a single observation or serval observation. There are two basis versions of two-way analysis of variance, depending upon whether the two variables of classification are independent or whether they interact. Two variables (or criteria) of are said to interact when they together have an extra effect that they do not have alone. Consider how we might categorize salespeople based on their age and educational attainment in order to see if age and education have an impact on sales volume (Keta *et al.*, 2019).

Let's say we find that while the two variables do not have a significant effect on their own, they do have a significant effect when combined with certain age groups and educational attainment. We can say that there is an interaction between age and educational attainment. The variables, on the other hand, are independent if a specific combination of age and educational attainment has no significant effect. When factors of categorization are independent, one observation per cell is collected. Several observations are made for each cell in the event of interaction (Khanal *et al.*, 2019).

Least Significant Difference (LSD)

The least significant difference was calculated using the formula in the statistics section below.

$$LSD = \frac{t\alpha}{2v\sqrt{2MSE}} \times r$$

Fidelity Level (F.L)

The following formula was used to determine the level of fidelity:

$$F.L = \frac{Np}{N \times 100}$$

RESULTS

The study used four medicinal plants: *Corymbia citridora* Hook (commonly known as Lemon scented gum and Sfaida in Bhimber), *Azadirachta indica* Linn. (Belongs to the genus Allium, family Amaryllidaceae, order Aspergales, division Tracheophyta of kingdom plantae), *Allium sativum* Linn. (Belongs to the genus Allium, family Amaryllidaceae, order Aspergales, division Tracheophyta of kingdom plantae, division Tracheophyta of kingdom plantae) (commonly known as neem, belongs to family Lamiaceae) (Table 1).

Table 1: Shows a plant's description as well as the sections that are being studied.

Name of Plant	Part taken	Vernacular name	Family	Part used
Allium sativum	Bulb	Garlic	Amaryllidaceae	Bulb

Azadirachta indica A. Juss	Leaves	Neem	Meliaceae	Leaves
Mentha piperita L.	Leaves	Podina	Lamiaceae	Leaves
Eucalyptus citridora Hook	Leaves	Sfaida	Myrtaceae	Leaves

A survey was conducted in four villages in the Tehsil Samahni District Bhimber before the investigation began (Parat, Kahala, Potha, and Kotla). Ten informants were chosen at random from each hamlet. There were ten males and thirty females in total. The number of males and females affected by skin fungal illnesses in each village is provided in tabular form (Table 2).

Table 2: Number of Informants of survey

Villages	**Number of informants**	**Number of male informants**	**Number f male informants**
Parat	10	2	8
Kahala	10	3	7
Potha	10	2	8
Kotla	10	6	4
Total	40	10	30

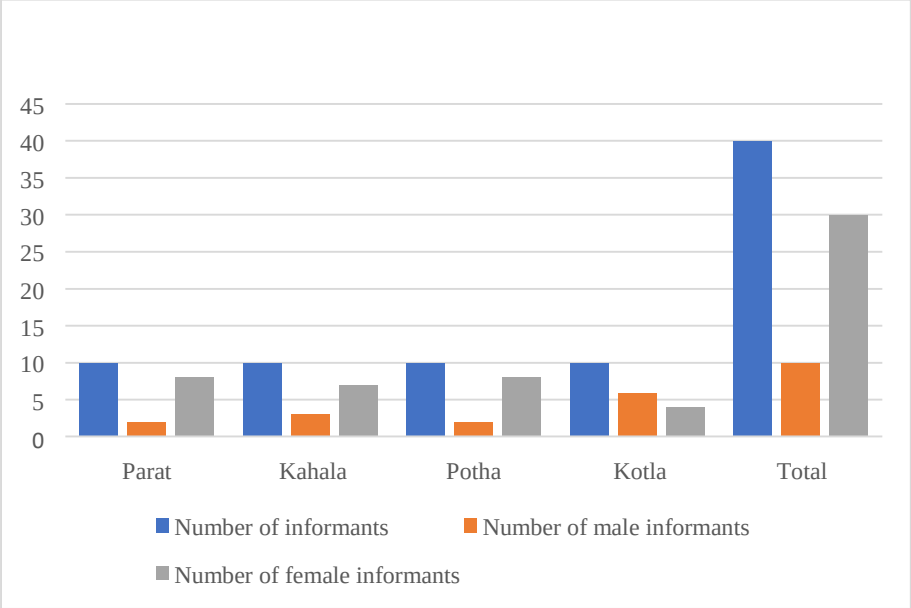

Figure 1 Number of informants in the Tehsil Bhimber study areas during the survey.

FIDELITY LEVEL

Residents were asked to describe how medicinal plants were used in their daily lives, as well as the remedies and precautions they used to combat these diseases. An unstructured interview was conducted for this purpose. During the interview, any plants were discussed. Thirty-two interviewers discussed the use of specific plants, while eight interviewers had little or no knowledge of plants. Although most people use synthetic antifungals, they did have some knowledge of herbal remedies. The following is a list of plants and their fidelity levels (Table4.3).

Table 3: List of anti-fungal plants mentioned by informants used in Traditional ethnomedicine from District Bhimber AJK.

Plants	Total	No. of informants	Fidelity level
Azadirachta indica A. Juss	40	6	15
Allium sativum L.	40	4	10
Corymbia citridora Hook.	40	7	17.5
Mentha piperita L.	40	3	7.5

The value was measured and data was statistically analyzed in table 4.5(a+b), during research on the effects of several medicinal herbs on Athlete's foot, a dermatophytic

pathogen caused by Candida albicans. Based on these findings, it was found that the minimum inhibitory concentration (MIC) changes significantly between extracts and the control. The Azadirachta indica plant is the first to be investigated (Neem). This plant had the highest minimum inhibitory concentration (MIC) against ethanol extract, which was 18mm, and the lowest MIC against methanol extract, which was 5mm (9 mm). *Allium sativum* (garlic) was the second plant picked.

Allium sativum (Garlic) was next plant that revealed utmost minimum inhibitory concentration contrary to ethanol (13mm) and lowermost minimum inhibitory concentration against the chloroform (7mm). Eucalyptus plant extracts were also used to check out antidermophytic plant effect. The extracts of this plant showed the highest minimum inhibitory concentration against ethanol that is 15mm unit. While lowest minimum inhibitory concentration was observed against water extract which is 9mm. The fourth and last plant is *Mentha piperita* (Mint) that showed that ethanol has a high minimum inhibitory concentration (12mm) and lowest minimum inhibitory concentration in contradiction of chloroform that is 6mm.

Overall, *Azadirachta indica* was calculated to be the plant extract (Neem) showed highest minimum inhibitory concentrations due to presence of terpenoids that inhibits fungal hyphae growth. When I looked back through the literature and research papers, I noticed that this discovery could be linked to Mondali's work in 2009, when he used *Azadirachta indica*. For *Aspergillus niger*, the Juss (Neem) plant was used, and it was discovered that neem extract is the cheapest and most beneficial concentration against chloroform, which is 6mm. Overall, *Azadirachta indica* was calculated to be. Because of the presence of terpenoids that limit fungal hyphae growth, the Juss (Neem) plant extract demonstrated the highest minimum inhibitory doses. When I looked back through the literature and research papers, I noticed that this discovery could be linked to Mondali's work in 2009, when he used *Azadirachta indica*. Juss (Neem) plant for *Aspergillus niger* and initiate that neem extract is a very affordable and effective way to suppress fungal growth. (Table 5). Statistically data was analyzed and showed in next table 4.5a+4.5b.

Table 5: The effect of extracts from four medicinal plants on the Candida albicans fungal pathogen, as well as the MIC value, were determined.

Plant extracts→ Solvents	Terbinafine 125mg (mm)	*Mentha piperita* L. extract (mm)	*Corymbia citridora* L. extract (mm)	*Azadirachta indica*. Juss extract (mm)	*Allium sativum* L. extract (mm)
Methanol	20±0.33	10±0.874	13±0.972	8±1.02	8±0.882
Ethanol	20±0.41	12±0.675	15±0.334	11±0.908	13±0.914
Chloroform	23±0.33	06±0.962	11±0.642	9±0.879	7±0.981
Ether	21±0.56	7±0.892	11±0.723	6±1.92	12±0.923
Water	22±0.53	9±0.573	9±0.569	10±1.45	10±0.971
Mean± standard error					

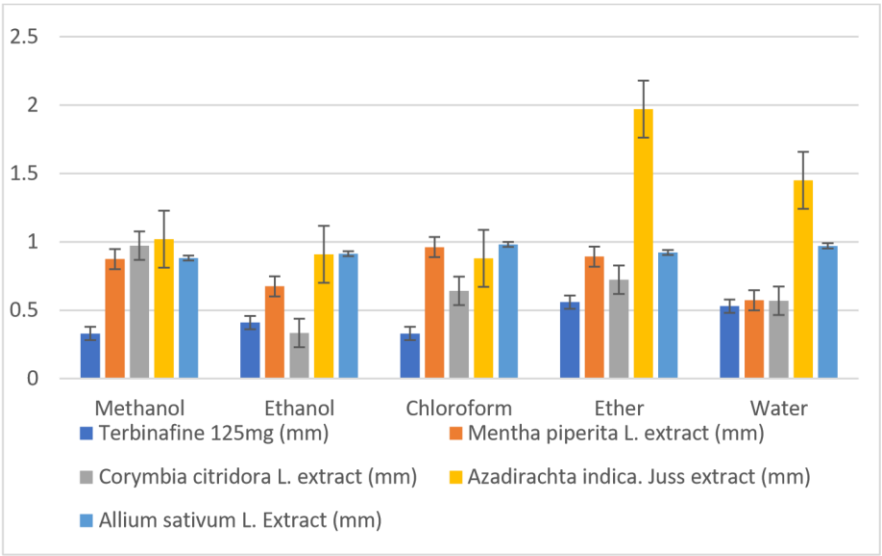

Figure 2 The effect of extracts from four medicinal plants on *Candida albicans*, as well as the minimum inhibitory concentration value.

Table 6: Statistical examination of *Candida albicans* fungal pathogen MIC values of four medicinal plants

Source of variation	DF	Sum of squares	Mean square	F
Solvents	4	48.16	12.4	2.56
Plants extracts	4	477.76	119.44	24.67
Error	16	77.44	4.84	
Total	24	603.36		

The effect of extracts from four plants on Athlete's foot infection, which is initiated by the fungal dermatophytic pathogen *Candida albicans* that was computed and tabularized in table 7. The minimal inhibitory values of several plants in various extracts are shown in this table. These values differed considerably from those of the control group. Plant extracts from *Azadirachta indica* Juss (Neem) displayed the extreme minimum inhibitory concentration (20mm) in contradiction of ethanol and the lowermost minimum inhibitory concentration contrary to chloroform extract (11mm) (Dhakal *et al.*, 2016)

Garlic was the second plant tested for antifungal properties. This had the utmost minimum inhibitory concentration (11mm) contrary to the ethanol and has lowermost MIC value (6mm) beside Ether. Eucalyptus was also tested for its antifungal properties. This plant's extract has the highest MIC value (17mm) compared to ethanol and the lowermost MIC value (17mm) contrary to ether (12mm). Mint plant had the greatest MIC value of 15mm against ethanol and the lowest in water (9mm). The best antifungal activity is found in the Juss (Neem) plant, which inhibits fungal development. In a 2015 study, Ospina Salazar *et al*. discovered that neem has therapeutic potential in reducing the growth of dermophytic infections such as *Epidermophyton floccosum* (*Candida albicans*), *Microsporum canis*, and several Trichophyton species (Tables 8) (Khanal *et al.*, 2019).

Table 7: Shows the effect of extracts from four medicinal plants on the *Candida albicans* spp. fungal pathogen, as well as the MIC value.

Plant extracts→ solvents↓	**Terbinafine 125mg (mm)**	***Allium sativum* L. Extract (mm)**	***Azadrachta indica.* Juss extract (mm)**	***Mentha piperita* L. extract (mm)**	***Corymbia citridora* L. extract (mm)**
Methanol	19±0.812	8±1.02	12±1.231	12±1.98	13±0.882
Ethanol	29±0.242	11±0.908	20±1.934	15±1.09	17±0.972
Chloroform	25±0.821	9±0.879	11±1.028	13±0.976	15±0.987
Ether	20±0.231	6±1.92	18±1.987	11±0.753	12±1.243
Water	20±0.324	10±1.45	12±0.672	9±1.023	13±1.98
Mean ±standard error					

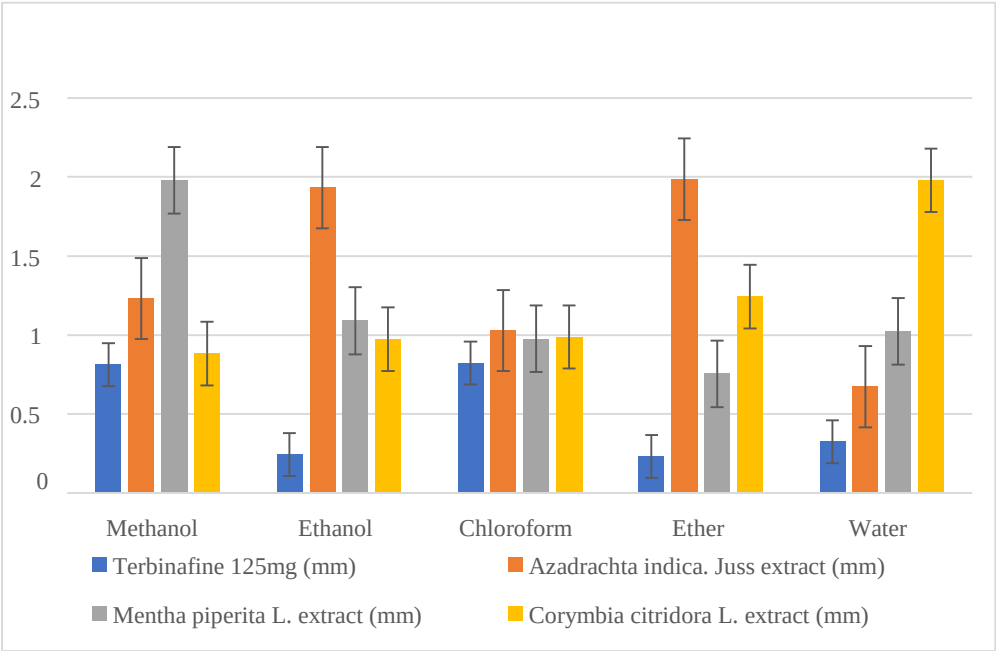

Figure 3. The effect of extracts from four medicinal plants on *Candida albicans*, as well as the minimum inhibitory concentration value.

Table 8: Statistical examination of the MIC values of four medicinal plants against *Candida albicans*, a fungal pathogen.

Source of variation	DF	Sum of squares	Mean square	F
Solvents	4	110.8	27.70	5.50
Plant extracts	4	522.80	130.70	25.98
Error	16	80.40	5.03	
Total	24	714.00		

The influence of extracts from four homoeopathic plants on the disease Ringworm triggered by the dermatophytic fungal pathogen *Trichophyton mannum* was measured and described in table 9. In compared to the control, all of the acquired Minimum Inhibitory Concentration values indicated an important change. Neem extract has the highest MIC (23mm) against ethanol and the lowest MIC (8mm) against water. The second plant garlic showed maximum Mic (15mm) after the use of ethanol as solvent and minimum MIC (6mm) after the use of chloroform solvent.

Similarly, eucalyptus plant extract indicated maximum MIC value 925mm) against ethanol extract and minimum MIC (9mm) was obtained against water solvent (Khan *et al.*, 2015).

After using ethanol as a solvent, the second plant garlic showed a maximum MIC (15mm) and a minimum MIC (6mm) after using chloroform as a solvent. In the same way, eucalyptus plant extract had a maximum MIC of 925mm against ethanol extract and a minimum MIC of 9mm against water. Plant four (Mint), on the other hand, had the highest MIC value (13mm) in the ethanol solvent and the lowest (5mm) in the water solvent. Overall, it was felt that eucalyptus extract outperformed the other three therapeutic plants. Eucalyptus includes the chemical 1,8-cineile, which inhibits the growth of fungi. These findings were compared to Baptisa *et al*., 2015's earlier findings. It was explained that the plant eucalyptus has a 62.5ug/mL^{-1} antifungal activity against Trichophyton species and had been shown as the best antifungal activity. Table 10 showed the statistical analysis and documentation of the data (Bhowmik *et al.*, 2010).

Table 9: Minimum inhibitory concentrations of extracts from four medicinal plants against the fungal pathogen *Trichophyton mannum* (MIC)

Plant extracts→ solvents↓	**Terbinafine 125mg (mm)**	***Allium sativum* L. Extract (mm)**	***Azadrachta indica*.Juss Extract (mm)**	***Mentha Piperita* L.Extract (mm)**	***Corymbia citridora* L. Extract (mm)**
Methanol	32± 0.764	9±1.442	1±1.959	18±1.875	11±2.143
Ethanol	38± 0.836	15±1.433	23±1.584	13±0.982	25±1.133
Chloroform	35± 0.683	6±2.343	10±0.894	10±2.988	16±2.134
Ether	34± 0.995	11±1.224	15±2.123	9±3.098	17±1.224
Water	23± 0.573	9±0.979	8±1.442	5±0.882	9±0.979
Mean ± Standard error					

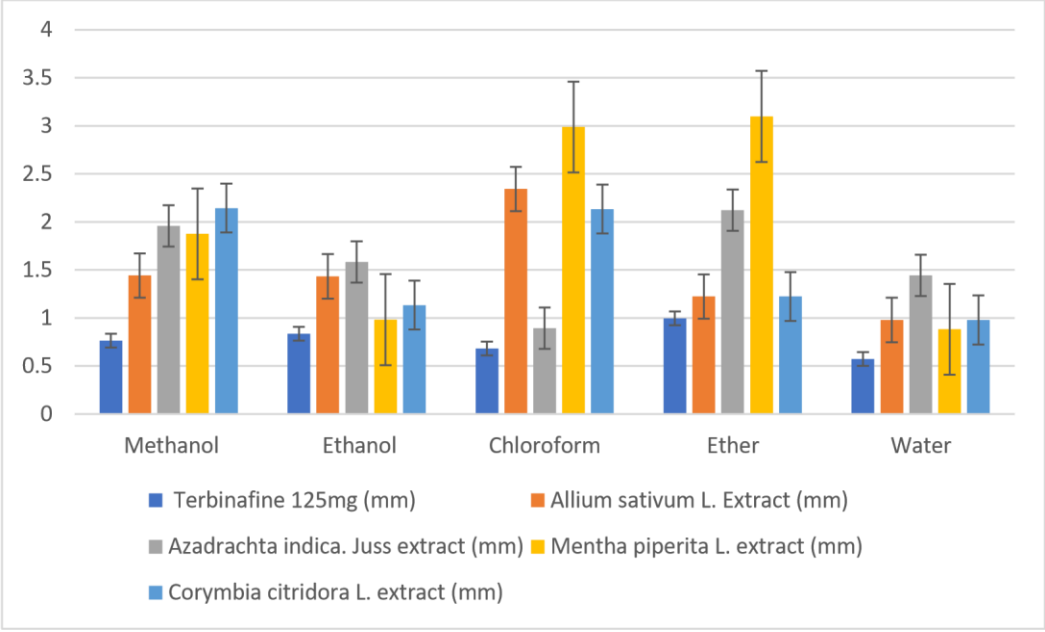

Figure 4 Shows the minimal inhibitory concentration and effect of four medicinal plants on the fungal pathogen *Trichophyton mannum*.

Table 10: Statistical investigation of the MIC values of four medicinal plants against the fungal pathogen *Trichophyton mannum*

Source of variation	DF	Sum of squares	Mean square	F
Solvents	4	373.6	93.4	7.65
Plant extracts	4	1643.4	410.8	33.67
Error	16	195.4	12.2	
Total	24	2212.6		

The *Allium sativum* L. (garlic) plant obligated the utmost MIC value (18mm) against chloroform and the lowest MIC value (18mm) against methanol (8mm). The addition of a third plant extract *Corymbia citridora* is a species of Corymbia. The extract in chloroform and ethanol (21mm) showed the highest minimum inhibitory concentration (MIC), while water had the lowest MIC value (8mm). *Mentha piperita* L. was the fourth plant (Mint). Mint plant extract had the greatest MIC value (23mm) in the ethanol solvent and the lowest in the ether solvent (1mm). Garlic, on the other hand, was found to have better results in all four extracts when compared to the other three therapeutic plants. This is due to allicin, an antifungal substance found in garlic. It was discovered that an extract of *Allium sativum* inhibits hypha growth and can be used as a fungicide as the most effective natural treatments for dermatophytes. The statistical analysis was reported in table 4.8(a+b) (Salazar *et al.*, 2015).

CONCLUSION

From all the methodologies used in present research, it is concluded that the adulterated source mixed in the genuine source of herbal medicines could be separated by using taxonomic study of herbarium, fresh collection of plant specimens, survey, analysis of phytochemicals and presence of phytochemicals indicates that all the four selected plants retain antifungal properties. These selected plants show best results in ethanolic solvent comparatively so ethanol serves good solvent for extraction of phytochemicals. Qualitative tests demonstrated that the difference in antifungal properties is due to difference in phytochemical constituents in different plants in ethanolic extract. Ethanolic extract of neem plant showed highest Minimum Inhibitory Concentration (MIC) value

beside *Candida albicans* (Athlete's foot disease). The MIC assay also revealed the antifungal capability of plant extracts, with the extracts demonstrating adequate efficacy against all fungal infections. Some plant extracts were shown to be particularly effective against the fungus specimen used in the study, *Candida albicans*. Furthermore, as compared to the other extracts, the extracts macerated in petroleum ether produced better results. As a result, it can be stated that all of the results are significant when the extract concentration is increased. Because of the secondary metabolites, the results are gradually becoming more evident and meaningful. Plants produce secondary metabolites in the form of various chemicals. As a result, further research is needed to isolate powerful molecules and utilize them in the development of pharmaceutical medications.

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