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## Phytherapeutic uses of indigenous medicinal plants of Samahni area to cure Mycodermal infirmities prevailing in district Bhimber of Azad Jammu and Kashmir, Pakistan

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Human being has been facing different types of diseases since his emergence on the planet. Microbial infection is the most prevalent and dominant for causing various infirmities in the man. Fungi are one of the major microorganisms which cause severe dermal diseases in human being. In this study, different villages of Tehsil Samahni District Bhimber of Azad Jammu and Kashmir, Pakistan were selected as model units and open-ended and close-ended interview methodology was used. The collected data was compiled and analyzed using dedicated statistical tools. The research revealed that indigenous people believe and use medicinal plants (MPs) to cure mycodermal diseases using phytherapeutics which are considered better than allopathic prescriptions. In this research work, methanolic extract of two famous MPs: *Allium sativum* (garlic) and *Azadirachta indica* (Neem) were used to check their efficacy to kill skin mycopathogens. About 56 Indigenous informants (29 male and 27 female) recommended these plants as the best herbal therapy to cure the dermal mycotic infirmities. The fidelity level of *Azadirachta indica* was higher (15) than *Allium sativum* (10). The prevalent mycotic infirmities of the area were tinea, athlete's foot, jock itch, ringworm and many other infection of skin, hairs, beard and scalp. The leaf methanolic, extract of *Azadirachta indica* depicted minimum inhibitory conc. (MIC) of  $17.5 \pm 0.80\text{mm}$  while *Allium sativum* produced MIC of  $18.5 \pm 0.50\text{mm}$  as compared with control value of  $20.0 \pm 0.52\text{mm}$ . MeOH fraction of *Azadirachta indica* leaf and *Allium sativum* clove depicted MIC values of  $16.5 \pm 1.85\text{mm}$  and  $18.7 \pm 0.90\text{mm}$ , respectively against fungus *Epidermophyton floccosum*. The extract of two plants showed good MIC against *Malassezia furfur* which causes mycosis, pityriasis (tinea) infirmities. The herbal phytherapeutics are recommended for cure of fungal skin diseases and optimization of homeopathic drug dose is required for better efficacy and treatment.

**Keywords:** Mycodermal diseases, Phytherapeutics, *Allium sativum*, *Azadirachta indica*, Bhimber

### INTRODUCTION

Human being since his emergence on planet has

been dependent on plants for life sustenance. Plants are primarily used as source of food, fodder, shelter and medicines. The man urges to have safe, secure and healthy life but the earth-environment has trillions of microbes which are both beneficial and harmful. The many microorganisms like virus, bacteria and fungi are playing positive and negative services for man. Many useful products and functions are being managed and operated by microbes. Out of these fungi is a major group with huge biodiversity from micro-to-macro level structure present. Fungi are both useful as well as harmful causing infirmities to animals, plants and particularly for human beings.

Fungal diseases are worldwide major public health issues after cancer and diabetics. The illness caused by fungi can range from superficial e.g. athletes' foot to deadly blood stream infection. The three general classes of infections that the mycotic diseases branch of the centers for disease control is concerned about are opportunistic infections, community acquired infections and hospital acquired infections (Anis, et al. 1998). Opportunistic infections refer to infections that do not generally harm healthy individuals, but can cause massive damage to immune compromised patients, including those with AIDS, individuals undergoing chemotherapy, or organ recipients. Some opportunistic infections are Cryptococci meningitis caused by *Cryptococci neoformans* and aspergilliosis that is lung infection caused by *Aspergillus* species. *Cryptococcal meningitis* is one of the primary causes of death in HIV patients and it is a serious problem on behalf of other immune compromised populations while aspergilliosis can be especially difficult in people with cystic fibrosis (Rippon et al. 1988; Ackermann, 2001; Abbas et al. 2009b).

The second class of mycotic infections is community acquired infections. These are usually caused by fungal spores that reside in soil. When soil is disrupted, people inhale the spores, resulting in diseases such as blastomycosis, histoplasmosis and coccidiomycosis. Blastomycosis is a disease that is instigated by *Blastomyces dermatitidis* spores. Usually these spores remain inactive in host.

Histoplasmosis is another disease caused by *Histoplasma capsulatum* spores that affects lungs resulting as every problem for immune compromised individuals like AIDS patients. Coccidio mycosis is also known as valley fever and is caused by Coccidiosis's species resulting disease is pneumonia. Diseases of this class are

cured by current antifungal drugs (Rippon et al. 1988; Ackermann, 2001; Abbas et al. 2009b).

The third and last one class of infections is hospital acquired infections. It is usually caused by *Candida* species in blood stream. *Candida albicans* is one of most predominant fungal pathogen causing diseases in humans and may cause many clinical and vaginitis problems (Abbas and Ghaffar, 1992). It is estimated to be about 10,000 deaths per year due to candida infections in USA (Rippon et al. 1988; Ackermann, 2001; Abbas et al. 2009b).

Various fungi cause various kinds of diseases in different areas of human being. Through scars, wounds, injured skin and burns, dermatophytes can pass into the host body (Ahmad et al. 1997; Ahmed et al., 2006). Pathogens have the ability to proceed keratin as a food source so they invade non-living, upmost and keratinized layer of skin that is stratum corneum and produce enzyme namely keratinase (Ahsan et al. 2010). At the site of infection, they cause inflammatory reaction and redness, swelling, heat or burning are the signs of inflammatory reaction which are seen at the site of infection. The pathogens stir away from the site of infection due to inflammation and produce the ringed lesion with affecting area (Ali et al. 2006).

In Pakistan including Azad Jammu and Kashmir, prevalence of fungal diseases is very common. There are many infirmities occurring in Azad Jammu & Kashmir and particularly in District Bhimber because the district has warm and humid climate. Ahmed et al. (2006) worked on dermatophytes has competed research in Karachi (Pakistan) area and he described and documented different diseases caused by fungal pathogens. However, Aman et al. worked in Quetta in the year of 2001 who described foot athletes' infections Aman et al. 2001). In Lahore the research was done by Ahsan et al. in 2010 on hair loss or damage causing diseases (Aman et al. 2001). In Karachi, skin infections are very communal due to humid environment. These diseases are more common in children as compare to adult. Fungal infections are dominated among these infections (Ansari and Siddiqui, 2005).

The purpose of current research was to explore occurrence and density of mycodermal infirmities in Samahni area of District Bhimber, AJK. The key objective was to see the efficacy of MeOH extract of two medicinal plants viz: *Azadirachta indica* and *Allium sativum* in eradication of dermal fungal diseases.

## MATERIALS AND METHODS

### Ethnomedicinal survey:

In order to extract maximum traditional knowledge (TK) about the fungal diseases occurrence and their cure mechanism; field surveys were planned to collect TK from the people or farmers employing structured and semi-structured interviews (Ishtiaq et al. 2013). The numerous field survey was conducted during the year 2016 in the months of June, July, August and September in different targeted villages. During the survey, people shared their home made recipes and the other precautions which they were using for their protection and cure therapies against fungal diseases. The informants comprises on both gender with age range 45-70 years with possessing various professions (Ishtiaq et al. 2013). The ethnomedicinal (EMs) studies provided the baseline information the MPs like *Azadirachta indica* and *Allium sativum* are predominantly used by indigenous communities for control and mitigation of mycodermal infirmities.

### Selection of Medicinal Plants

Tehsil Bhimber is eye-catching in its flora with diverse phytodiversity. A large variety of plants such as mint (*Mentha piperita*), eucalyptus (*Corymbia citriodora*), garlic (*Allium sativum*), neem (*Azadirachta indica*), lemon grass (*Cymbopogon citratus*), gandira (*Nerium indicum*), holy basil (*Ocimum basilicum*), and marigold (*Tagetes erecta*) were declared as being used as antifungal EMs. But in the current study two plants: *Allium sativum* called garlic and *Azadirachta indica* called neem have been used. The botanical name of garlic is *Allium sativum* L. and its family is Lilliaceae. Neem is scientifically known as *Azadirachta indica* and family is Meliaceae. These plants were selected due to their several medicinal properties and availability in the selected area.

### Collection of Plants Samples

*Azadirachta indica* and *Allium sativum* were collected from all four sampling sites of villages viz: Bindi, Kaschanatar, Rayal, Chowki and Samahni. Fresh leaves and clove of garlic were used for extraction of herbal medicine or fraction to testify the efficacy against fungi mitigation or retardation of its growth and proliferation. Fresh leaves of plants were cut with scissors and wrapped in polythene bags.

### Drying and Crushing of Plants

The plants were carefully washed with water for eradicating all the dirt and soil. Then these were kept in a dark room for drying. The plants were dried at normal room temperature, 25°C in darkness. Garlic took more time in drying while neem completely dried within two weeks. When plants were completely dried, then ground to powder form with the help of an electric blender. One kilogram powder of each plant was sieved with 1mm mesh sieve and deposited in air tight containers for further use.

### Extraction Procedure

The extraction procedure was carried out by maceration. 50g powder of Neem was soaked in 250 ml methanol (MeOH) and placed in an air tight container. Placed it for seven days with periodic shaking. The solvent was filtered with Whatmann filter paper of size 10. Then extract was dried by using rotary vacuum evaporator at 60°C. Dark green colored extract was obtained. Extract was stored for further use. The same process was repeated for three times to obtain about 3g of the extract. Same procedure was followed for finely ground powder of garlic. Same procedure was used to obtain plant extracts in methanol.

### Preliminary phytochemical profiling

The ethanolic extracts of plants were tested separately to find the phytochemical constituents of the plants. The presence or absence of phytochemicals such as alkaloids, tannins, glycosides, saponins, flavonoids and terpenoids were tested using Trease and Evans method (Evans, 2009). Before performing tests, 5mg of each of the extract was mixed in 20ml methanol. For qualitative, the formula of Ezeonu & Ejikeme, (2016) was employed with some modifications.

### Fungal collection, growth and identification

For collection mycopathogens for experimental analysis samples were collected from reknown dermatology labs: from DHQ lab of Bhimber and clinic of Dr. Faisal Rehman located in Kotla Arab Ali Khan of district Gujrat with due precautions and operating SOPs of biosafety lab protocols and ethics. The specimens were placid from different parts of body of different patients of district headquarter Bhimber, with assistance of concerned physician. This procedure was conceded out by using new clinical blades for separately patient. The main way to collect the specimens was to scrap the affected part into a soft and clean paper then transferred into sterile

bottle sand then these bottles were labeled with individual's data.

### Inoculation of specimens

Sabouraud dextrose agar were prepared by using standard procedure and poured into 10 Petri dishes. Specimens were transferred from sterile bottles to petri dishes and allowed for 5 minutes to solidify. The Petri dishes were labelled and placed in incubator for 3 days to incubate.

### Identification of Fungi

In accessible fungi were recognized on the basis of their colony features and conidial morph. Colony counter present in laboratory of Mirpur University of Science & Technology, Bhimber Campus, Bhimber was used to count the colony of fungi grown on Petri dishes slides were prepared by taking a tiny slice of fungal colony from the emergent margin with the help of immunizing needle and recognized by seeing under the microscope at 40x and 100X lenses. The photographs were taken with digital camera. All measurements and observations were replicated three times. These photographs were further sent in Punjab university microbiological department and micro lab of university of veterinary and animal sciences Lahore for identification (Domsch et al. 1980; Tanveer et al. 2014).

### Antifungal Assay

After three days Sabouraud dextrose agar were prepared again and poured into flask. 25 petri dishes were washed and sterilized in autoclave with agar. In laminar airflow all petri dishes were placed and grouped into 5 rows and 5 columns. Each row of Petri dish was labelled with same specimen and different solvents. Then Sabouraud dextrose agar were poured in all 25 petri dishes and each specimen were added in petri dishes of same row. The fungal specimens were spread with the help of spreader and by using corn borer 5 well were made in each petri dish. In first row of 5 Petri dishes Trichophyton were spread and each petri dish was labelled as Trichophyton in methanol. In each well of a Petri dish labelled as Trichophyton in methanol plants solvent made from garlic and neem were poured. Terbinafine 125mg were also poured in well no. 5 of each Petri dish. Each well was labelled and Petri dishes were transferred from laminar air flow to incubator for incubation. Same process was repeated for all Petri dishes of allows.

### Calculation of growth inhibition zone

After 5 days 4 tables were drawn on a paper of each specimen and growth inhibition zone were measured in centimeter (cm) with the help of scale and written in each table. These measurements were then changed into millimeter (mm) by multiplying each unit with 10.

### Statistical analysis

To check minimum inhibition conc. (MIC) value and evaluate it with the help of statistics, all the values were taken in triplicates by repeating procedure and then data were described as mean value  $\pm$  standard error. The mean values and standard error was calculated by using Microsoft excel and Minitab software was also used for analysis of data.

## RESULTS

Fungal diseases have been permanent threatening factor for human being it causes many skin diseases in man. The dermal or internal infections of fungi cause severe pain in body and skin rashes as well. Even these are painstaking as one of the core intention of loss of lives, as these accomplish shape of wide- ranging. People can only save from such diseases when they keep themselves away from fungal spores. Scientists are continuously trying to develop variety of products to stay apart from such diseases. Synthetic chemicals are mostly dedicated to avoid these skin diseases. But these chemicals cause bothersome for operators.

Now-a-days botanists are careworn to prepare cost actual and environmental friendly herbal remedies. The present research work is conducted keeping in view the above problems and necessity to vanish fungal skin diseases. During study, two medicinal plants are taken. *Allium sativum* Linn. (Belongs to genus *Allium*), *Azadirachta indica*. A. Juss (commonly known as neem belongs to family Meliaceae).

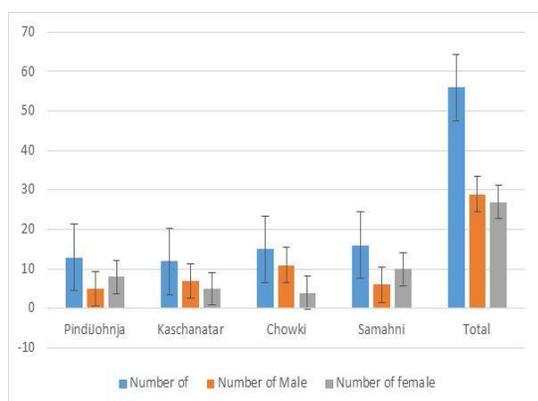
Before starting the research work, a survey was conducted in four villages (Bindi, Kaschanatar, Chowki and Samahni) of District Bhimber, AJK. From each village at least 10 informants were selected randomly with total number was 56 with 29 male and 27 female. The demographic description about the number of males and females from each village who shared their skin fungal diseases knowledge is described in tabular form (Table 2, Fig. 1).

**Table 1: Plant description with their parts under study area of District Bhimber AJK**

Plant name	Plant partTaken	Common name	Family	Part used
<i>Azadirachta indica</i>	Leaves	Neem	Meliaceae	Leaves
<i>Allium sativum</i>	Bulb	Garlic	Amaryllidaceae	Bulb

**Table 2: Number of informants of survey from study area of District Bhimber of Azad Jammu and Kashmir**

Villages	Number of informants	Number of Male informants	Number of female Informants
PindiJahnja	13	5	8
Kaschanatar	12	7	5
Chowki	15	11	4
Samahni	16	6	10
Total	56	29	27



**Figure 1: Number of informants in study areas of tehsil Bhimber during survey**

The residents were asked to tell about the usage of medicinal plants in their daily matters, remedies and precautions they applied against these diseases. For this purpose, structured and unstructured interview procedure was conducted. Many plants came under discussion during interview. Fifty two interviewees told about the usage of certain plants while four interviewees have no significant knowledge about the used plants (called here MPs). Mostly new generation people use synthetic antifungal drugs and tubes or lotion but still their ancestors and people of old ages have very good and handsome traditional knowledge about the herbal remedies because indigenous people think that herbal phytotherapeutics are cost effective, accessible

and with least or nonth side effects or toxicity. The list of plants used as MPs in cure of mycodermal diseases in form fidelity level (FL) is presented in Table (3).

**Table 3: List of anti-fungal plants mentioned by informants from District Bhimber of AJK**

Plants	Total informants	Number of informants	Fidelity Level
<i>Azadirachta Indica</i>	56	42	75.0
<i>Allium sativum</i>	56	48	85.7

From statistical verification, it was proved that plants are used as alternative to commercial medicines. To realize the anti-fungal potential of the plants, ethanolic extracts of the plants are qualitatively verified. It was found out that plants have this potential because of their phytochemical constituents. The difference in the ability of the plants to treat as antifungal depends on the presence or absence of variety of chemical constituents including alkaloids, phenolic and terpenoids. The methanolic extract of *Allium sativum* L. was tested, it was noticed that in this extract alkaloids, saponins, flavonoids, terpenoids and glycosides were present while tannin was absent. The methanolic extract of *Azadirachta indica* was qualitatively tested, it was found out that it contained only tannins, flavonoids and saponins while terpenoids, glycosides and alkaloids were absent (Table 4).

During the study of effect of different medicinal plants against disease *tinea corporis* caused by dermatophytic pathogen named as *Microsporum canis*, the values was measured and data was analyzed statistically (Table 5). From these values it was judged that minimal inhibitory concentration (MIC) varies in different extracts and have great difference as compared to control. The first under observation plant is *Azadirachta indica*. Juss (Neem). This plants exhibited highest minimum inhibitory concentration (MIC)  $17.5 \pm 0.80\text{mm}$  while *Allium sativum* produced MIC of  $18.5 \pm 0.50\text{mm}$  against methanol extract with

minimum inhibitory concentration (MIC) against fungal species. The second plant taken was (garlic) which showed highest minimum inhibitory concentration against ethanol ( $18.5 \pm 0.50\text{mm}$ ). It was reviewed previous literature and research papers, it was proved that the current results are correlated to Mondali who worked in 2009 who used *Azadirachta indica*. Juss (Neem) plant for

*Aspergillus niger* and concluded that neem extract is very cheapest and useful source for controlling growth of fungal species (Table 5, Fig 2).

The effect to methanolic extract of plants against diseases athlete's foot that is caused by fungal dermatophytic pathogen *Epidermophyton floccosum* was calculated and tabulated (Table 6).

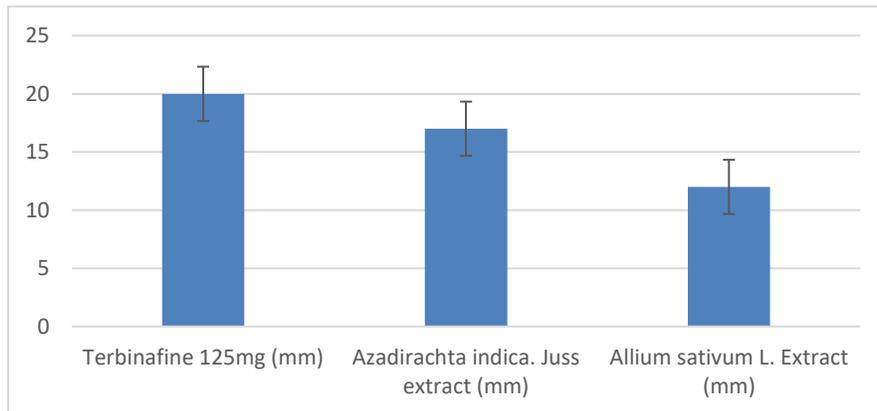
**Table 4: Phytochemical constituents of *Allium sativum* and *Azadirachta indica* from Bhimber AJK**

Plant names	Solvent	A	Sa	Ta	F	Te	G
<i>Allium sativum</i> L.	Methanol	++	++	--	++	++	++
<i>Azadirachta indica</i> A. Juss.		--	++	++	++	--	--

A=Alkaloid, Sa=Saponin, Ta=Tannin, F=Flavonoid, Te=Terpenoids, G=Glycoside, += Presence of phytochemical constituent, - = Absence of phytochemical constituent.

**Table 5: Effect of extracts of two medicinal plants against *Microsporum canis* fungal pathogen and measured minimum inhibitory concentration (MIC) value**

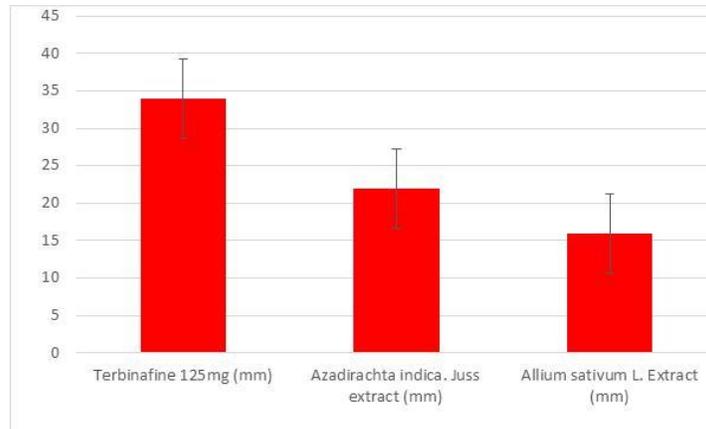
Plant extracts → Solvents ↓	Terbinafine 125mg (mm)	<i>Azadirachta indica</i> . Juss extract (mm)	<i>Allium sativum</i> L. Extract (mm)
<b>Methanol</b>	$20 \pm 0.55$	$17 \pm 0.80$	$12 \pm 0.50$



**Figure 2: Effect of extracts of two medicinal plants against *Microsporum canis* fungal pathogen and minimum inhibitory concentration value**

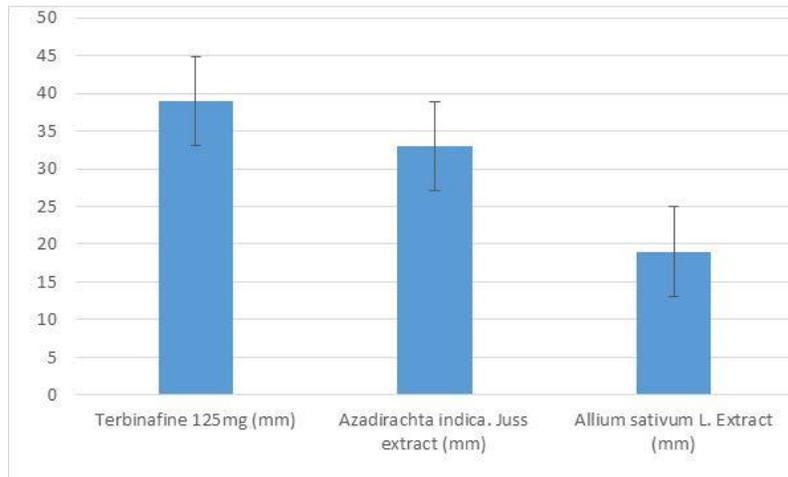
**Table 6: Effect of extracts of two medicinal plants against *Epidermophyton floccosum spp* fungal pathogen and measured minimum inhibitory concentration (MIC) value**

Plant extracts → Solvents ↓	Terbinafine 125mg (mm)	<i>Azadirachta indica</i> . Juss extract (mm)	<i>Allium sativum</i> L. Extract (mm)
<b>Methanol</b>	$34 \pm 0.20$	$22 \pm 1.85$	$12 \pm 0.90$



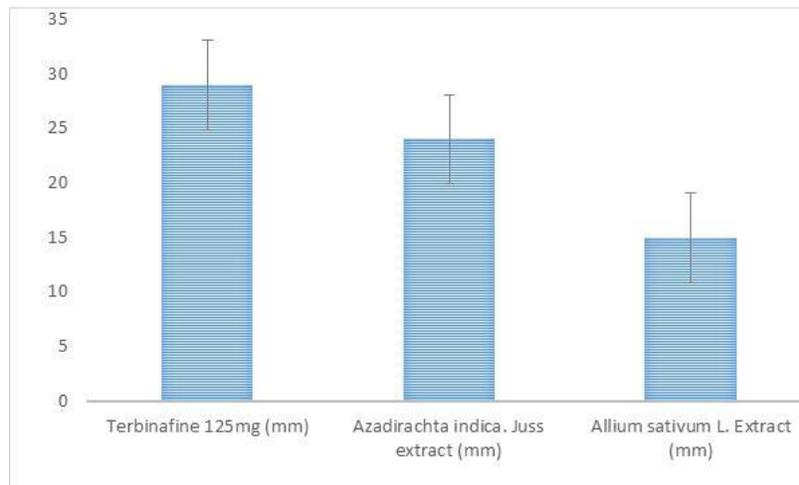
**Figure 3: Effect of extracts of four medicinal plants against *Epidermophyton floccosum* fungal pathogen and minimum inhibitory concentration value**  
**Table 7: Effect of extracts of two medicinal plants against *Trichophyton mannum* fungal pathogen and measured minimum inhibitory concentration (MIC)**

Plant extracts → Solvents ↓	Terbinafine 125mg (mm)	<i>Azadirachta indica</i> . Juss extract (mm)	<i>Allium sativum</i> L. extract (mm)
Methanol	39 ± 0.80	33 ± 1.50	19 ± 1.00



**Figure 4: Effect of extracts of two medicinal plants against *Trichophyton mannum* fungal pathogen and minimum inhibitory concentration value.**  
**Table 8: Effect of extracts of two medicinal plants against *Malassezia furfur* fungal pathogen and measured minimum inhibitory concentration (MIC)**

Plant extracts → Solvents ↓	Terbinafine 125mg (mm)	<i>Azadirachta indica</i> . Juss extract (mm)	<i>Allium sativum</i> L. extract (mm)
Methanol	29 ± 0.50	24 ± 1.50	15 ± 0.90



**Figure 5: Effect of extracts of four medicinal plants against *Malassezia furfur* fungal pathogen and minimum inhibitory concentration value.**

In this table, minimum inhibitory values of different plants were observed in MeOH extract against different mycopathogens. Comparative to control these values were different significantly. *Azadirachta indica*. Juss (Neem) plant extracts showed highest minimum inhibitory concentration against ethanol (20mm) and lowest minimum inhibitory concentration against chloroform extract (11mm). For garlic methanolic extract antifungal activity was testified against different diseases. This showed highest minimum inhibitory concentration against ethanol (11mm) and lowest MIC value against ether that is 6mm. It was observed that *Azadirachta indica*. Juss (Neem) plant has best antifungal activity that inhibits fungal growth. Ospina Salazar et al. (2008) also worked on neem in 2015 and described that the therapeutic potential of neem that inhibits the growth of dermatophytic pathogens like *Epidermophyton floccosum*, *Microsporum canis* and different species of Trichophyton and results are presented in the Table (6) and Fig 3.

The effect of extracts of two medicinal plants against the disease ringworm (that is commonly known as daddar in district Bhimber) caused by *Trichophyton mannum* that is dermatophytic fungal pathogen was measured and documented in Table (7). It was observed that all obtained minimum inhibitory concentration (MIC) values showed significant difference as comparison to control. Neem extract showed highest MIC value ( $22 \pm 1.85$  mm) against methanol solvent and Garlic showed maximum MIC ( $12 \pm 0.90$ mm) as in Table 7 and Fig 4.

To check out the effect of extract in five

different solvents of two medicinal plants against *Malassezia furfur* that is fungal pathogen which causes pityriasis versicolor (tinea versicolor) disease was calculated in Table 8. It was calculated and observed that all obtained MIC value showed significant difference as comparison to control. *Azadirachta indica* Juss. (Neem) extract showed highest MIC value ( $24 \pm 1.50$  mm) and *Allium sativum* L. (garlic) plant showed maximum MIC value ( $15 \pm 0.90$ ). This is due to presence of antifungal pigment in garlic that is allicin. Farzad et al. (2010) also worked on garlic in 2010 and concluded that extract of *Allium sativum* inhibits the growth of hypha and found that *Allium sativum* can be used as best natural remedies to treat dermatophytes as given in detail Table 8 and Fig 5.

## DISCUSSION

Fungi have been cause of many diseases in man, particularly dermatic infirmities are more teasing for people. During study, two medicinal plants are taken as trial for cure of skin diseases caused by fungi. It is known that herbal tonics are the best for cure of the skin diseases of human being. *Allium sativum* L. Belongs to genus Allium, family Amaryllidaceae, order Aspergales division Tracheophyta of kingdom Plantae. Garlic is best remedy for cardiovascular diseases by lowering blood pressure and cholesterol. Garlic lowers cholesterol level approximately by 10% and lowers blood pressure by 5-7%. Garlic is safe when eaten as food, though in some sensitive persons, it can cause gastrointestinal irritation (Kathi et al. 2000). *Azadirachta indica*. A. Juss. belongs to species indica, genus *Azadirachta*,

tribe Melieae, subfamily Melioideae, family Meliaceae, sub-order Rutinae, order Rurales. Neem plant was firstly introduced by De Jussieu in the year 1830 (De Jussieu, 1830). Neem is usually found in tropical areas and is native to Barma and east India. It grows mostly in warm areas where temperature reaches to 48°C (Leosand, 2002; Osuna, 2000). It is stated that neem is most extensively searched plant in the world (Ekanem, 1971; Udeinya et al. 2006). It displayed anti-ulcer properties (Pillai and Santhakumari, 1984), anti-tumor (Fujiwara et al. 1982), antimicrobial (Wafaa et al. 2007). Neem oils and leaf extracts show inhibitory effects against viruses, fungi and bacteria. The oil and extract of neem leaves, bark and seeds indicated effective ness against cabies and ring worm.

It is well known that biochemical constituents in plants show bio-chemical properties of plants. So for this purpose it is necessary to determine the phytochemistry of plant before the evaluation of biological properties of any plant. Flavonoids are associated with analgesic, antimicrobial and antioxidant activities of plant. Therefore, it is very important to appraise to determine their capabilities to interfere in different biological activities. The terpenoids demonstrate best activity for fast healing of wound and inflamed mucous membrane, in addition to production of lipids, blood and body fluids against free radicals. The studies of ethno- pharmacological properties of plants have pave way towards the discovery of novel biological components. The constituents play important role to improve the health standard and are cheapest source if they are obtained from local plants.(Rauf and Muhammad, 2013). Plants are natural source of producing wide number of bioactive chemical constituents in a most efficient way and with precise selectivity. Since the middle of 19<sup>th</sup> century, different class of bioactive compounds have been isolated and characterized. Many of these are used as the active ingredients of the modern medicine, or as the lead compounds for new drugs discovery. Several plant derived medicines, are rich in phenolic compounds. Mostly plants are source of non-toxic remedies but still there is a need to test toxicity of active plants to establish their administrative safety (Uddin et al. 2011).

The effectiveness of different extracts of neem plant against the growth of fungal pathogens was analyzed in laboratory and found that *Azadirachta indica* shows antifungal property due to having antifungal substances in plant tissue. This result is correlated with the results of other worker's results

(Tewariand, 1991; Al-Abed et al.1993, Qasem et al. 1996; Amadioha 1998 and Amadioha 2003). It is also concluded that ethanolic extract of *Azadirachta indica* shows better results comparatively water extract of *Azadirachta indica*. It is due to differences in constituent extraction (Shekhawrat and Prasads, 1991). This study also revealed that mature leaves shows better result and more inhibitory effect than the young leaves. It has been previously reported that the active ingredients of neem constitute mostly of triterpenoides, eg, Nimbin, Nimbidine, Azadirachtin etc (Brahmachari, 2004). Therefore, from the foregoing discussion it may be concluded that *Azadirachta indica*, a common medicinal plant could be exploited as the source of a potent biocide that have immense fungi toxic effect to several fungal pathogens (Mondali et al.2009).

Dermatophytes are a group of closely related fungi that cause dermatophytosis, which affects approximately 40% people worldwide and accounts for 30% of all cutaneous mycotic infections. The most common clinical signs are circular patches of alopecia with scales formation. May arise ring injuries with healthy central halo and follicular papules with thin crusts on the out skirts (Bouchara et al. 2008). Current treatments for dermatophytos is include synthetic antifungal drugs, which can give rise to adverse reactions and resistant strains if not administered properly (Gupta et al. 2013).Studies on the chemical composition of essential oils from various *Eucalyptus* species havefound1, 8-cineoleto be the dominant compound. However, these studies have shown large variations in the chemical composition of essential oils from different species and even in those from the same species but from different regions, because these volatile oils are affected by non-genetic factors such as leaf age and seasonal variations. *Eucalyptus* show better result for dermatophytes such as *Microsporium canis*, *Microsporium gypseum*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum* in vitro (Simmons et al. 1997).

## CONCLUSION

It is concluded that District Bhimber's inhabitants have prevalent skin diseases mainly caused by fungi. People of rural areas use wild plants and home tonics to cure these infirmities. Sometime, in case of severity of disease, people use allopathic or western medicines as well which may have side effects. It is concluded that all results are significant on the basis of increasing the 'concentration of the extract. The results are

gradually become clearer and more significant because of the secondary metabolites. The secondary metabolites present in plants in the form of different compounds. Hence, further investigation is needed to explore the potent compounds by the isolation and their use in the developing pharmaceuticals drugs.

#### Traditional knowledge based recommendations

Do not wear/use clothes unless washed in soapy hot water and then dried in drier.

Do not share combs, brushes, hats, hair clips or barrettes with other person.

Try to keep your skin dry except for a bath. Wet skin makes the rash worse.

To prevent dandruff, use the Tara mira oil for hairs.

Oil of neem leaves is very useful for ringworms and athletes foot.

Wear cotton socks instead of synthetics.

Allow sweaty shoes to dry out before wearing again.

Use antiperspirant deodorants to control excessive sweating.

Change out of wet swimmers or wet suits as soon as possible because moisture can worsen fungal infection

The oil burning with garlic is also considered best treatment for fungal skin diseases.

Dry powder of lemon pericarp and clove is very effective for fungal diseases.

Ring worm can also have caught from dogs and cats. So for check up your pets visits the veterinarian and also get tips how to take care of your pets.

Use yogurt with eggs to remove dandruff from hairs.

Use of cloves in daily diet is also very effective.

#### CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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#### AUTHOR CONTRIBUTIONS

MI designed and performed the experiments, IH revised paper, MM assisted in lab work, KHB applied statistics, MI assisted in plant collection, MA identified plants, TH supervised work, WM

assisted in paper preparation, AG revised and polished paper, RT helped in proof reading and galley proof. All authors read and approved the final version.

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