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Antifungal Activities of Some Plant Extracts against Pathogenic Fungi

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ABSTRACT

Soil samples were collected from different localities in Cairo, and were assayed for keratinophilic fungi. Five species of fungi classified in two genera were isolated from Giza zoo (animal cages and parks), hospital, public park, local market, primary school, club, and garbage dumping site. Five plants were chosen to investigate their antifungal activity against five isolated dermatophytes: *Microsporum gypseum, Microsporum boullardii, Trichophyton mentagrophytes, Trichophyton terrsetre,* and *Trichophyton verrucosum*. The tested plants were *Punica granatum* (Pomegranate), *Aloe vera*, *Foeniculum vulgare* (Fennel), *Allium ampeloprasum* var. *Kurrat* (kurrat), and *Ricinus communis* (*Castor bean*). Plant extracts were prepared by three different solvents, hexane, ethyl acetate, and (80%) ethanol. The study shows that ethanolic extract of *Punica granatum* (Pomegranate), hexane, and ethanolic extract of *Allium ampeloprasum* var. *Kurrat* (kurrat) were effective against most of the tested organisms. Ethanolic extract of pomegranate and hexane extract of kurrat were chromatographed by column chromatography. Fractions from column chromatography were tested for antifungal activity. Ethyl acetate: ethanol (9:1) fraction of pomegranate (*Punica granatum*) and hexane: ethyl acetate (1:9) fraction of kurrat (*Allium ampeloprasum var. Kurrat*) showed antifungal activity against the fungal strains. These fractions were analyzed by Gas Chromatography- Mass Spectrum (GC-MS).

INTRODUCTION

Throughout human history, natural products from plants have many uses of social and economic importance as medicines, fragrances, food additives, and pesticides. The last decade has seen the large demand for plant material for drugs.

Most of bioactive compounds of plants are produced as secondary metabolites which are often produced in a phase of subsequent to growth, and have no function in growth. Thus, a definition of bioactive compounds in plants is: secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals (Bernhoft, 2010). Bioactive compounds of plants belong to several chemical classes such as phenolics, alkaloids, terpenoids, saponins, flavonoids, quinones, and coumarins (Aqil *et al.*, 2010).

Antimicrobial activity of extracts of plants depends on some parameters like plant material used, extraction technique, extraction solvent, and technique employed.

Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds i.e., any part of the plant may contain active components. Both fresh and dried plant materials can be used as a source for the extraction of secondary plant components (Azwanida, 2015).

Bioactive compounds from plant materials can be extracted by conventional and non-conventional extraction techniques. The conventional techniques are Soxhlet extraction or hot continuous extraction, maceration, decoction, infusion, and percolation. The non-conventional techniques are such as ultrasound assisted extraction (UAE) or sonication extraction, microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and Pressurized liquid extraction (PLE) (Azmir *et al.*, 2013; Azwanida, 2015). Variation in extraction methods usually depends upon length of the extraction period, solvent used, polarity, temperature, particle size of the plant tissues, and the solvent-to-sample ratio (Das *et al.*, 2010).

Properties of a good solvent in plant extractions include, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, and inability to cause the extract to complex or dissociate. Examples of the various solvents that are used in the extraction procedures are water, acetone, alcohol, ether, chloroform, ethyl acetate, dichloromethanol, benzene, and toluene (Tiwari *et al.*, 2011).

There are several methods available for antifungal activity testing, which can be classified into three main groups, they are diffusion such as agar disk-diffusion and agar well diffusion, dilution such as broth dilution and agar dilution, and Thin-layer chromatography (TLC)–bioautography methods (Balouiri *et al.*, 2016).

The aim of this study was to determine the antifungal effect of some plant extracts on the tested pathogenic fungi. Finding out the best solvent for extraction of the active antifungal compounds and identifying these compounds which can be used as drugs.

MATERIALES AND METHODS

Plant Material:

Five plants were chosen to investigate their possible antifungal activity. Samples of these plants were purchased from local market and plantation (Table 1).

Table 1: Different plant parts used for screening the antifungal activity

Scientific Name of the Plant	Common Name	Plant Part Used
Punica granatum (Lythraceae)	Pomegranate	Peel of fruit
Aloe vera (Asphodelaceae)	*Aloe vera*	Leaves
Foeniculum vulgare (Apiaceae)	Fennel	Fruit
Allium ampeloprasum var. *Kurrat* (Amaryllidaceae)	Egyptian leek or salad leek (kurrat)	Bulb
Ricinus communis (Euphorbiaceae)	Castor bean or castor-oil-plant	Leaves

Preparation of Plant Extracts:

The selected parts of chosen plants were air dried in shade in room temperature (25°C). Dried plant samples were ground using grinding machine. One hundred grams of ground plant samples were macerated with 800 ml of organic solvent (1:8 ratio) at room temperature for 48 hours with frequent agitation. Plant samples were then subjected to sequential extraction using hexane (H), followed by ethyl acetate (EA) and finally with ethanol (E) 80%. Then the liquid extracts were filtrated through Whatman filter paper no. (1). The extracts were collected and the plant materials were subsequently extracted twice in fresh solvent. The filtrates were concentrated by removing the solvents under reduced

pressure at 40°C using a rotary evaporator. The dried crude extracts were cold stored till use (Othman *et al.*, 2011).

Identification of the Fungal Isolates:

Dermatophytes were isolated from different soils by hair baiting technique (Vanbreuseghem, 1952). They were identified by recognition of cultural and microscopic characteristics. In general, they were recognized by the presence of microconidia, macroconidia, chlamydospores, and other special mycelial structures. Identification of fungi was carried out using the manual of (Frey *et al.*, 1979).

Fungal Inoculum Preparation:

The fungi were cultured on Sabouraud dextrose agar (SDA) in 9 cm plate and incubated at 28ºC for 7 days. When fungal mycelium covered 80% of plate surface, the fungal spores were harvested aseptically using 5 ml of sterile water. The spore suspensions were adjusted to a concentration of 1-2 × 10^6 spores/ml in sterile water, corresponding to absorbance of 0.6 at 450 nm wavelengths (Yazdani *et al.*, 2009).

Antifungal Activity of Crude Extract by Well Diffusion Method:

One hundred µl of the standardized fungal spore suspension was spread on Sabouraud dextrose agar (SDA) using a glass spreader. Sterile cork borer with diameter 15 mm was used to bore well into the SDA. 100 µl of 10 mg of plant extract was introduced into the well and allowed to stand one hour for proper diffusion of the extract into the media. The plates were incubated at 28°C for 7 days and observed for zones of inhibition (Pathan *et al.*, 2012).

Determination of the Active Components of the Potent Extracts:

According to results obtained from the antifungal activity assay, the most potent two extracts were subjected for further chemical analysis to identify their possible effective compounds which could be responsible for their antifungal activity as follows:

Extraction of the Most Potent Plant Material:

One kilogram of peel of pomegranate fruit (*Punica granatum*) was extracted with ethanol 80% and 10 Kg of bulb of kurrat (*Allium ampeloprasum var kurrat*) was extracted with hexane according to Othman *et al.*, (2011) method.

Column Chromatography:

Glass column was packed with a solution of silica gel (60-120 mesh) with hexane using the wet slurry method. In this method, a ball of glass wool was pushed into the column. Then a solution of silica gel with hexane in a beaker was stirred and quickly added to the column before the gel settles. A substantial amount of hexane was poured continuously into the column and allowed to drain. This method was used to prevent the trapping of air bubbles. Crude extract of plant was chromatographed on the column eluted using a hexane, gradient of hexane: ethyl acetate (9:1; 8:2; 7:3; 6:4; 1:1; 4:6; 3:7; 2:8; 1:9), ethyl acetate, gradient of ethyl acetate: ethanol (9:1; 8:2; 7:3; 6:4; 1:1; 4:6; 3:7; 2:8; 1:9) ethanol, and gradient of ethanol: water (9:1; 8:2; 7:3; 6:4; 1:1; 4:6; 3:7; 2:8; 1:9) affording fractions. The collected fractions were analyzed by TLC on Silica Gel plates F254 (Merck®, 0.25mm thick) (Patra *et al.,* 2012 and Santos *et al.,* 2015).

Antifungal Activity of Fractions by Well Diffusion Method:

The collected fractions were tested for their antifungal activity according to Pathan *et al.*, (2012) method.

Gas Chromatography-Mass Spectrum Analysis (GC-MS):

GC-MS technique was used to identify the phytocomponents presence in the active fractions. The active fractions of *Punica granatum* (ethyl acetate: ethanol (9:1)) and *Allium ampeloprasum* (hexane: ethyl acetate (1:9)) were chromatographed by GC-MS technique. The GC/MS analysis was performed using a thermo scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30 m, 0.250 mm, and 0.25 µm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as the carrier gas at constant flow rate of 1mL/min. the

injector and MS transfer line temperature was set at 280˚C.

The quantification of all the identified compounds was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

RESULTS

Identification of the Fungal Isolates:

The obtained dermatophytes isolates which were isolated from soil of Giza zoo (animal cages and parks), hospital, public Park, local market, primary school, club, and garbage dumping site, were morphollogically examined and identified by macroscopic and microscopic features.

Five species belonging to two genera were identified: *Microsporum gypseum, Microsporum boullardii, Trichophyton mentagrophytes* (downy type (1) and granular type (2)), *Trichophyton terrsetre, Trichophyton verrucosum.*

Antifungal Activity of Plant Extract by Well Diffusion Method:

Hexane, ethylacetate, and ethanol 80% extracts of *Foeniculum vulgare* (Fennel), *Aloe vera*, and *Ricinus communis* (Castor bean) give negative result with all tested fungi.

Hexane and ethyl acetate extract of *Punica granatum* (Pomegranate) did not affect all fungi while 80% ethanolic extract significantly affected most tested fungi (Table 2 and Figs. 1 and 3). The inhibition effect of 80% ethanolic extract of pomegranate was determined as the following sequence:

Table 2: Antifungal activity of crude extract of *punica granatum* (pomegranate)

Isolated Organisms	Inhibition Zone Diameter (cm)			
	Hexane	Ethyl acetate	Ethanol 80%	Control
Microsporum gypseum	0	0	0^{d}	2.5^{b}
Microsporum boullardii	0	0	1.6^{c}	2.8^{a}
***Trichophyton mentagrophytes 1* (downy)**	0	0	2.7^{a}	2.9^{a}
Trichophyton mentagrophytes* 2*(granular)	0	0	2.4^{b}	2.5^{b}
Trichophyton terrsetre	0	0	2.5^{ab}	2.7^{ab}
Trichophyton verrucosum	0	0	2.6^{ab}	2.9^{a}
Significance level	NS	NS	*	*
LSD (0.05)	0	0	0.18	0.1

a, b, ab, c, d : each letter differ significantly in the activity of inhibition from each other. Values followed by the same letter within column do not differ significantly (P>0.05); * = Significant (P≤0.05); NS= non-significant

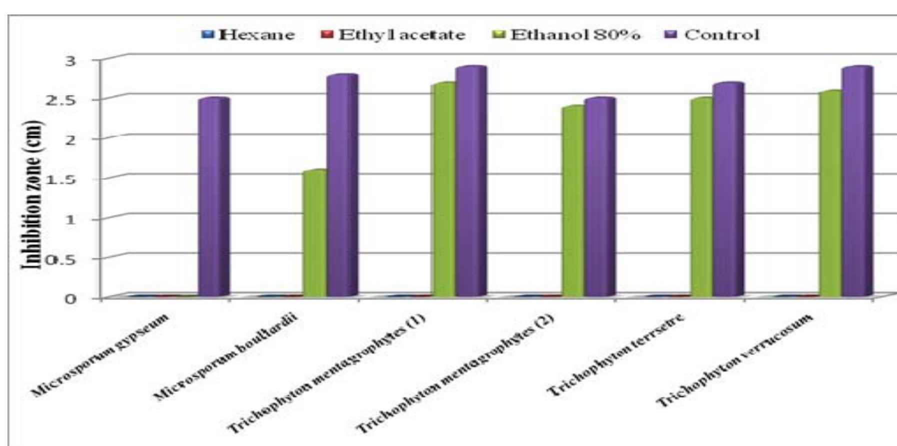

Fig. 1: Antifungal activity of crude extract of punica granatum (pomegranate).

Trichophyton mentagrophyte (1) > *Trichophyton verrecusom* and *Trichophyton terrestre* (as the antifungal activity did not differ significantly) > *Trichophyton mentagrophytes* (2) > *Microsporum boullardii.* no activity was recorded on *Microsporum gypseum.*

Ethyl acetate extract of *Allium ampeloprasum* var. *Kurrat* (kurrat) gave negative result while hexane (Figure 4) and ethanolic extract (Figure 5) gave positive result with most tested fungi (Table 3 and Fig. 2).

The antifungal activity of hexane extract of kurrat on the tested fungi was determined as the following sequence: *Trichophyton verrecusom* > *Trichophyton mentagrophytes* (2) and *Trichophyton terrestre* (as the antifungal activity did not differ significantly) > *Trichophyton mentagrophyte* (1) > *Microsporum gypseum* and *Microsporum boullardii.*

For 80% ethanolic extract of kurrat, high antifungal activity was recorded on *Microsporum boullardii* followed by *Microsporum gypseum*, *Trichophyton terrestre*, *Trichophyton verrecusom* and no activity was recorded on *Trichophyton mentagrophyte* (1) and *Trichophyton mentagrophytes* (2).

Control drug itraconazole showed positive results with all the isolated fungi (Figure 6).

Table 3: Antifungal activity of crude extract of *Allium ampeloparsum var kurrat* (kurrat).

Isolated Organisms	Inhibition Zone Diameter (cm)			
	Hexane	Ethyl acetate	Ethanol 80%	Control
Microsporum gypseum	2.2^{d}	0	2^{ab}	2.5^{b}
Microsporum boullardii	1.9^{d}	0	2.2^{a}	2.8^{a}
***Trichophyton mentagrophytes 1* (downy)**	2.8^{c}	0	0^{c}	2.9^{a}
Trichophyton mentagrophytes* 2 *(granular)	3.5^{b}	0	0^{c}	2.5^{b}
Trichophyton terrsetre	3.5^{b}	0	1.9^{ab}	2.7^{ab}
Trichophyton verrucosum	4^{a}	0	1.7^{b}	2.9^{a}
Significance level	*	NS	*	*
LSD (0.05)	0.31	0	0.26	0.1

a, b, ab, c, d : each letter differ significantly in the activity of inhibition from each other. Values followed by the same letter within column do not differ significantly (P>0.05); * = Significant (P≤0.05); NS= non-significant

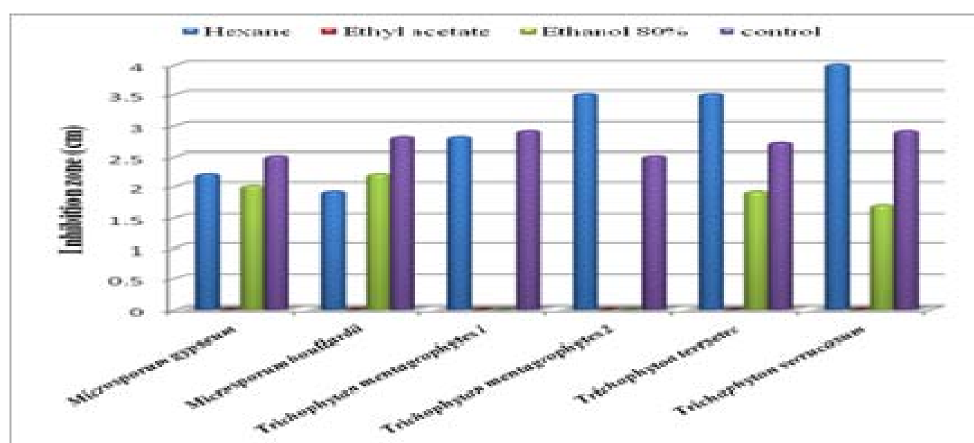

Fig. 2: Antifungal activity of crude extract of *Allium ampeloparsum var kurrat* (kurrat).

Determination of the Active Components of the Potent Extracts:

The active crude ethanolic extract of pomegranate (*Punica granatum*) and active crude hexane extract of a kurrat (*Allium ampeloprasum var. Kurrat*) were chromatographed by column chromatography. Fractions from column chromatography were tested for their antifungal activity.

Ethyl acetate: ethanol (9:1) fraction of pomegranate (*Punica granatum*) and hexane: ethyl acetate (1:9) fraction of kurrat (*Allium ampeloprasum var. Kurrat*) showed antifungal activity against fungal strains.

These fractions were analyzed by Gas Chromatography-Mass Spectrum (GC-MS). On analysis by GC-MS, several compounds were identified and tabulated (Tables 4 and 5). The chromatograms are shown in (Figs. 7 and 8).

Table 4: Phytochemical compounds identified in hexane: ethyl acetate (1:9) fraction of kurrat (*Allium ampeloprasum var. Kurrat*).

Serial Number	Retension Time (min)	Phytocmical Compound	Area %	Molecular Weight	Molecular Formula
1	9.17	Diethylmethylborane	1.03	83.969	C5H13B
2	9.39	PENITREM A or Tremortin A	0.95	633	C37H44ClNO6
3	9.96	(4-Bromophenyl)bis(2,4-dibromophenyl)amine	1.23	635	C18H10Br5N
4	10.28	Disulfide, methyl 1-propenyl or 1-(Methyldisulfanyl)-1-propene	1.94	120	C4H8S2
5	14.25	2,2',7,7'-Tetrabromo-9,9'-spirobifluorenone	2.31	628	C25H12Br4
6	14.61	3-Hydroxy-1-(4-{13-[4-(3-hydroxy-3-phenylacryloyl)phenyl]tridecyl}-phenyl)-3-phenylprop-2-en-1-one	0.87	628	C43H48O4
7	15.35	2,2'-Dibromo-5,5'-di(4-methoxyphenyl)-4,4'-di-tert-butylbiphenyl	1.48	634	C34H36Br2O2
8	15.74	Methy-2-benzothiazolinthion	7.94	181	C8H7NS2
9	16.01	trans-propenyl propyl disulfide	14.27	148	C6H12S2
10	16.14	2,5-Dimethylthiazole	1.33	113	C5H7NS
11	16.31	Allyl trisulfide	1.22	178	C6H10S3
12	16.69	10,11-Dioxatricyclo[6.2.2.0(1,6)]dodecane-7,7,8-tricarbonitrile, 9-imino-12-thiophen-2-yl-	4.38	338	C17H14N4O2S
13	17.16	4-Nitrophenyl tert-butyl sulfide	1.28	211	C10H13NO2S
14	17.61	CIS-METHYL PROPENYL SULPHIDE	1.76	88	C4H8S
15	18.14	2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene	4.93	146	C6H10S2
16	19.67	2-Methoxy-2,3-dihydro-3-furancarbaldehyde	0.89	128	C6H8O3
17	22.17	Propyl trisulfide	1.33	182	C6H14S3
18	22.58	3,5-Diethyl-1,2,4-trithiolane	4.22	180	C6H12S3
19	22.75	3,5-Diethyl-1,2,4-trithiolane	1.21	180	C6H12S3
20	23.92	Propyl sulfide	2.14	118	C6H14S
21	24.14	2-cyclohexylidene-1,3-dithiolane	1.21	186	C9H14S2
22	28.39	5-Chlorobenzo[h]-(1,6)-naphthyridine	1.69	214	C12H7ClN2
23	28.75	(2-Decyl)benzene	0.73	218	C16H26
24	29.35	6-PHENYLUNDECANE	1.75	232	C17H28
25	29.45	Valeroylpentamethylbenzene	3.37	232	C16H24O
26	29.7	1H-1,2,4-Triazole, 3-(2,4,6-trimethylbenzylthio)-	2.65	233	C12H15N3S
27	30.22	Undecane, 3-phenyl	2.59	232	C17H28
28	30.43	1H,3H,5H,6H,7H-7-Methoxy-3-oxopyrido[3,2,1-ij][3,1]benzoxazine	1.11	219	C12H13NO3
29	30.86	ZYGADENINE 3-MONOACETATE or ZYGAZINE	0.82	535	C29H45NO8
30	31.11	Undecane, 3-phenyl	3.56	232	C17H28
31	31.58	7-(Isopropoxy)-2,2,5-trimethylchromene	4.57	232	C15H20O2

Table 4: Continued:

Serial Number	Retension Time (min)	Phytocmical Compound	Area %	Molecular Weight	Molecular Formula
32	31.7	4-Diethylaminomethyl-7-methoxycoumarin	3.76	261	C15H19NO3
33	31.97	Dodecane, 4-phenyl-	2.75	246	C18H30
34	32.47	Dodecane, 3-phenyl-	2.11	246	C18H30
35	33.37	3(S)-Methyl-5-oxo-1-(1'-phenylethyl)-3-pyrrolidinecarboxamide	3.97	246	C14H18N2O2
36	33.72	Clovene	2.59	204	C15H24
37	33.85	Tridecane, 5-phenyl-	1.56	260	C19H32
38	34.13	Tridecane, 4•phenyl	0.89	260	C19H32
39	34.63	9a,11,12,12a-Tetrahydro-10H-cyclopenta[b]phenanthro[9,10-d]furan	0.76	260	C19H16O
40	35.46	2-Methylbenzylamine, N,N-dinonyl-	0.84	373	C26H47N

Table 5: Phytochemical compounds identified in Ethyl acetate: ethanol (9:1) fraction of pomegranate (*Punica granatum*).

Serial Number	Retension Time (min)	Phytocmical Compound	Area %	Molecular Weight	Molecular Formula
1	16.21	2,2'-Dibromo-5,5'di-(4-methoxyphenyl) 4,4'di-tert-butylbiphenyl	2.55	634	C34H36Br2O2
2	17.37	6-(4-Chlorophenyl)-3-cyano-4-[N-[bis (4 fluorophenyl)methyl]piperazino]-2H-pyran-2-one	2.50	517	C29H22ClF2N3O2
3	19.79	16-Deomethoxy-15,16-dehydroveratroylpseudaconine	2.62	615	C33H45NO10
4	20.07	3,4-Pyridinediamine, 6,6'-(1,3-phenylene)bis[2,5-diphenyl	2.57	596	C40H32N6
5	20.35	{12,12,17,18,22,23-Hexamethyl-2,7-anthraquinono[26,27-b]phthalocyanine}zinc	2.51	638	C38H30N4O2Zn
6	20.58	Dodecachloro-3,4-benzophenanthrene	2.62	636	C18Cl12
7	20.85	4,4',4'',4'''-Tetrabromotetraphenylmethane	2.67	632	C25H16Br4
8	21.19	[4,4'-bidibenzofuran]-2,2',8,8'-tetratetrakis[trimethyl]silane	2.50	622	C36H46O2Si4
9	22.27	N,N'-Dicyclohexyl-1-cyano-7-pyrrolidinylperylene-3,4:9,10-tetracarboxylic acid Bisimide	2.52	648	C41H36N4O4
10	22.43	3,3-DIDEUTERIO-ENDO-6-HYDROXY-9-OXABICYCLO (3.3.1)NONAN-2-ONE	2.56	584	C30H34N6
11	22.53	Methylsulfinato[2,3,7,8,12,13,17,1-octaethylporphyrinato]indium	2.61	726	C37H47InN4O2S
12	24.44	5,10-bis(3-aminophenyl)-15,20-diphenylporphyrin	2.70	644	C44H32N6
13	25.19	26,28-Dihydroxy-25,27-dioxaocta-4-ene-2,6-diynyl-p-tert-butylcalix[4]arene	2.51	748	C52H60O4
14	26.58	5,5•Dimethyl-2-[2'-(trimethylsilyl)ethynyl]cyclohex-2-enone	2.71	220	C13H20OSi
15	27.4	5,5''-Dibromo-3,3'',4,4''-tetrabutyl-2,2':5',2''-terthiophene	2.54	628	C28H38Br2S3

Table 5: continued:

Serial Number	Retension Time(min)	Phytocmical Compound	Area %	Molecular Weight	Molecular Formula
16	27.52	tetra-tert-butyl 2,6-di(3-propenyl)-3,7-dimethoxybicyclo[3.3.0]octa-3,7-diene-2,4,6,8-dicarboxylate	2.49	646	C36H54O10
17	27.82	2,9-Bis(5-tert-butyl-2-hydroxy-3-pyridylphenyl)-1,10-phenanthroline	2.61	630	C42H38N4O2
18	29.19	(4-Bromophenyl)bis(2,4-dibromophenyl)amine	2.87	635	C18H10Br5N
19	30.68	5,10-bis(3-aminophenyl)-15,20-diphenylporphyrin	2.50	644	C44H32N6
20	33.27	(R,S)-{5-[4(e)•(2•(1,4, 5,8,9,10-Hexahydro•1, 4,5,8-tetraoxo-9,10-(o•b enzeno)anthracenyl)cy clohex-(e)-yl]-10,15,20 -tri-p-tolylporphyrinato}zinc (II)	2.50	1036	C67H48N4O4Zn
21	33.51	N,N'-Bis[3-methoxy-4-hydroxy-5-bromobenzylidene(cyano)acetyl]-1,4-butanediamine	2.55	646	C26H24Br2N4O6
22	33.99	à,á-dichloro-6,7-bis[2•(methoxycarbonyl)ethyl]-1,3,5,8-tetramethylporphyrin	2.50	606	C32H32Cl2N4O4
23	34.16	N,N'-Dicyclohexyl-1-cyano-7-pyrrolidinylperylene-3,4:9,10-tetracarboxylic acid Bisimide	2.50	648	C41H36N4O4
24	34.35	N-propyl-3-aza-5à-cholestane	2.60	415	C29H53N
25	34.59	2,7,12,17-tetrabrom-(al l-às)cyclotetrathiophen (2,7,12,17-tetrabromcyc loocta[1,2-b:4,3-b':5,6 -b'':8,7-b''']tetrathiophen	2.49	640	C16H4Br4S4
26	36.37	5,10-bis(3-aminophenyl)-15,20-diphenylporphyrin	2.49	644	C44H32N6
27	38.13	2-ethoxycarbonylmethy l-9(2,3,5-tri-O-(2•methy lprop-2-yl)dimethylsily loxy-á-D-ribofuranosyl) purine	2.56	680	C32H60N4O6Si3
28	38.59	11,23-Di-tert-butyl-5,17-diethoxycarbonyl-25,26,27,28-tetrahydroxycalix[4]arene	2.63	680	C42H48O8
29	39.19	Milbemycin B, 5-demethoxy-5-one-6,28-anhydro-25-ethyl-4-methyl-13-chloro-oxime	2.49	589	C32H44ClNO7
30	39.5	Anodendroside A	2.52	572	C30H36O11
31	39.86	2,6-Bis[5-cyano-6-(4-bromophenyl)-1,2,4-triazin-3-yl]pyridine	2.56	595	C25H11Br2N9
32	40.09	(4-Bromophenyl)bis(2,4-dibromophenyl)amine	2.54	635	C18H10Br5N
33	42.28	4',5,7,8-Tetramethoxyflavone	2.62	342	C19H18O6
34	42.78	(22E)-Ergosta-14,22-dien-3-yl acetate	2.51	440	C30H48O2
35	43.27	1,2,3,4-Tetrahydro-1,1,4,4,5-pentamethyl-6-(p-tolyl)anthracene	2.54	342	C26H30
36	47.11	26,28-Dihydroxy-25,27-dioxaocta-4-ene-2,6-diynyl-p-tert-butylcalix[4]arene	2.52	748	C52H60O4
37	47.31	Methylsulfinato[2,3,7,8,12,13,17,18-octaethylporphyrinato]indium	2.66	726	C37H47InN4O2S
38	47.68	2,2'-Dibromo-5,5'-di(4-methoxyphenyl)-4,4'-di-tert-butylbiphenyl	2.55	634	C34H36Br2O2
39	49.22	Bis(methoxymethyl) Ether of 2,3-Dihydro-5,7-dihydroxy-2-[1-(2-phenylthio-3-pentenyl)]benzofuran	2.49	416	C23H28O5S

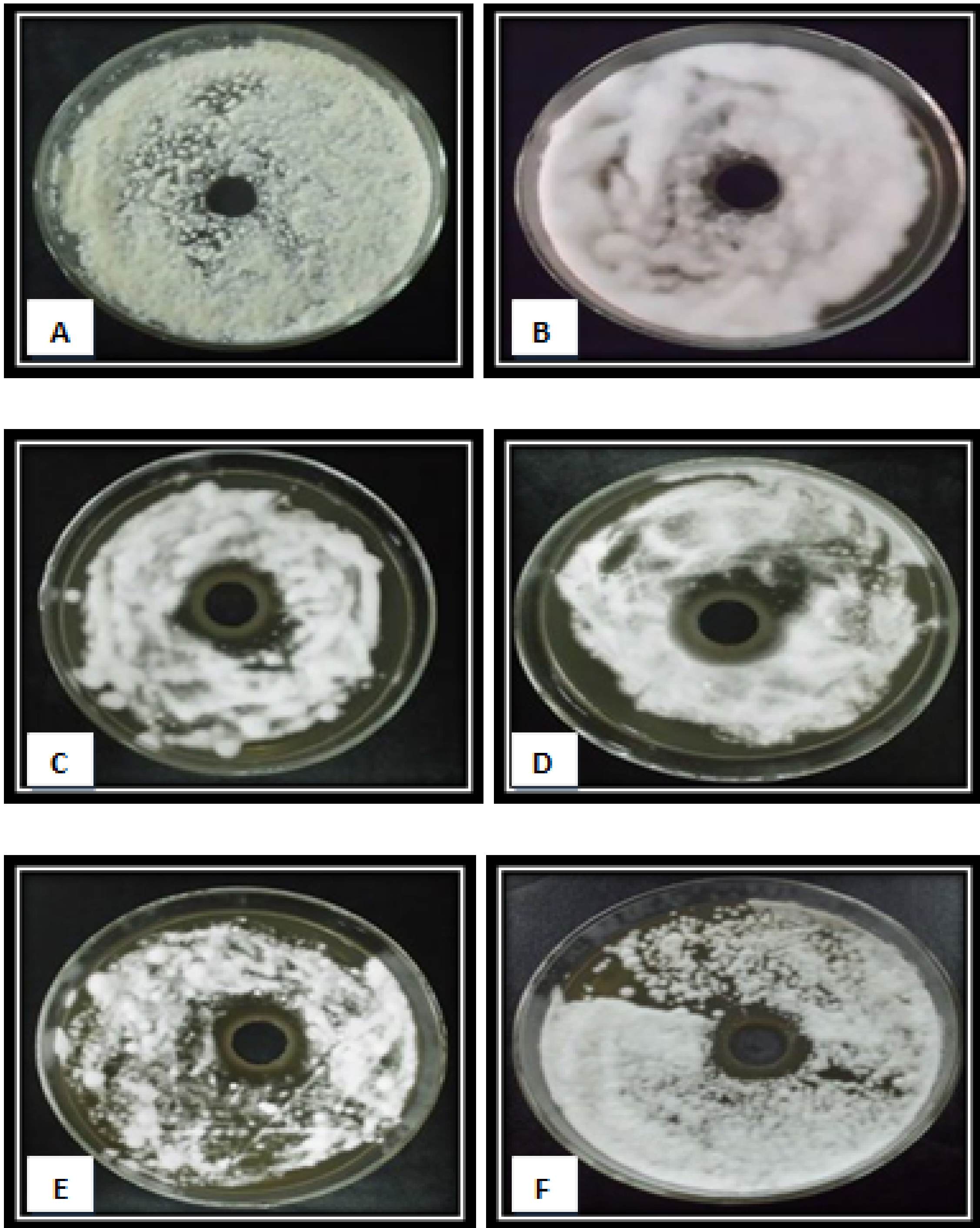

Fig. 3: Antifungal activity of ethanolic crude extract of *punica granatum* (pomegranate) against fungi (A) *Microsporum gypseum* (B) *Microsporum boullardii* (C) *Trichophyton mentagrophytes* (1) (D) *Trichophyton mentagrophytes* (2) (E) *Trichophyton terrsetre* (F) *Trichophyton verrucosum.*

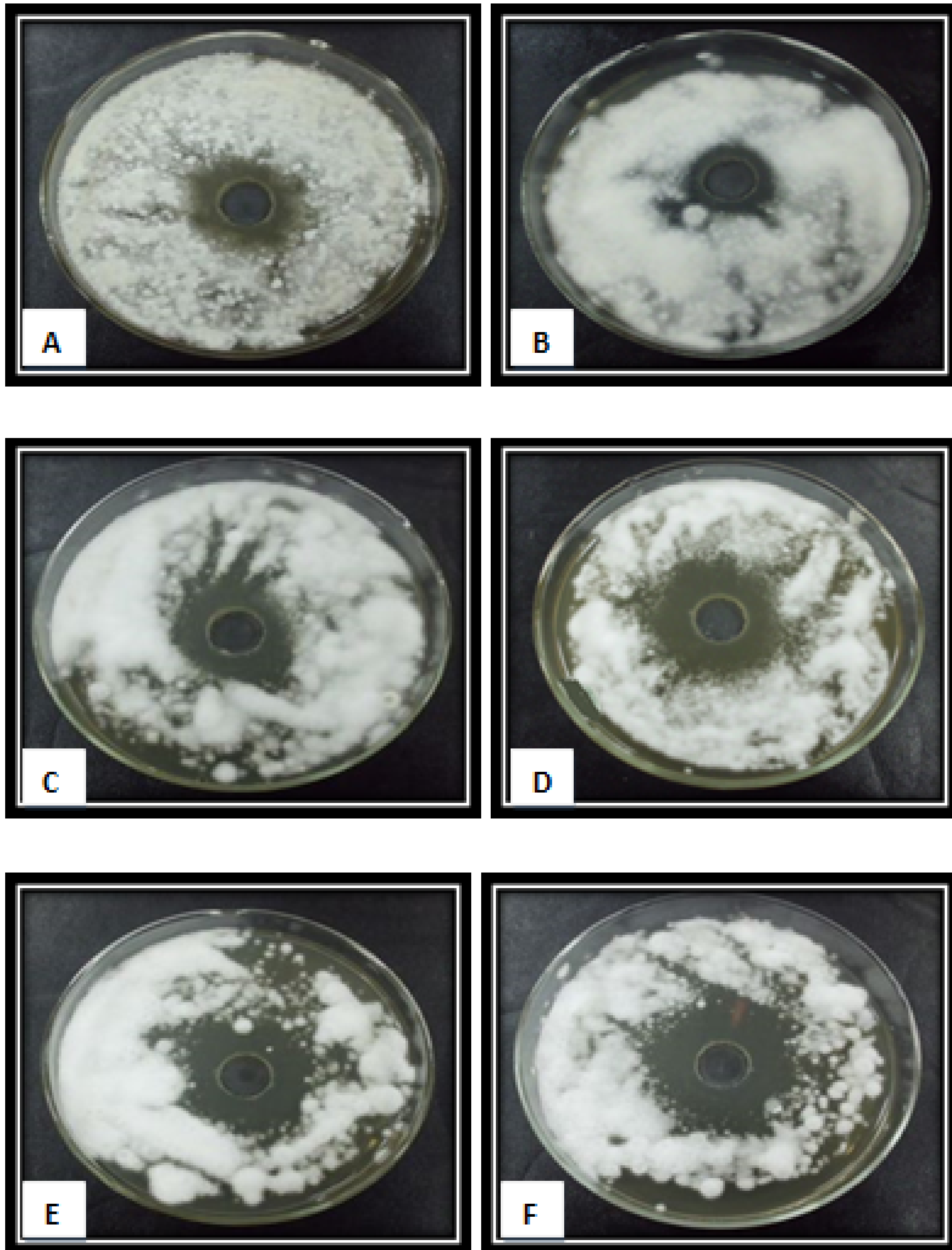

Fig. 4: Antifungal activity of hexane crude extract of *Allium ampeloparsum var kurrat* (kurrat) against fungi (A) *Microsporum gypseum* (B) *Microsporum boullardii* (C) *Trichophyton mentagrophytes* (1) (D) *Trichophyton mentagrophytes* (2) (E) *Trichophyton terrsetre* (F) *Trichophyton verrucosum.*

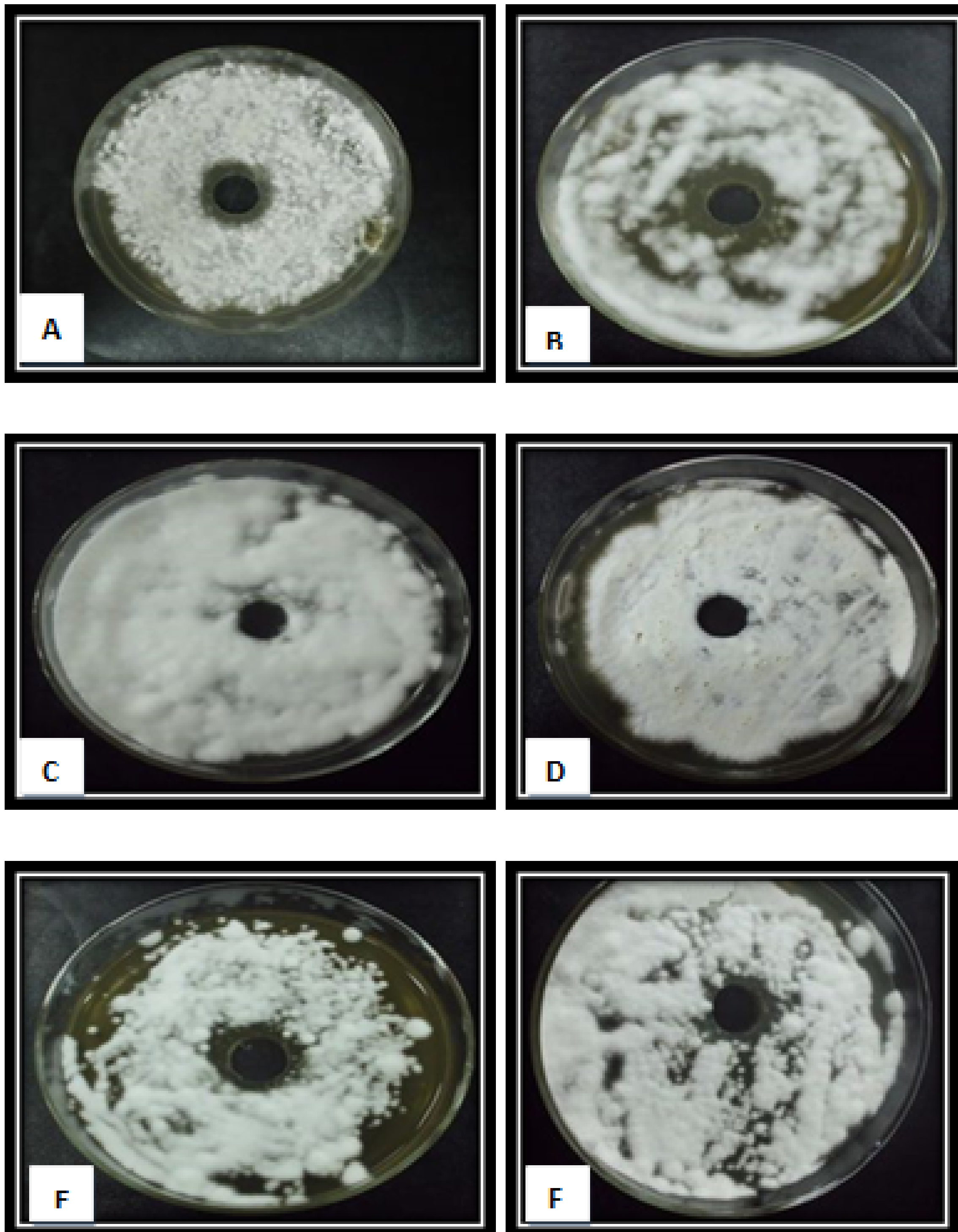

Fig. 5: Antifungal activity of ethanolic crude extract of *Allium ampeloparsum var kurrat* (kurrat) against fungi (A) *Microsporum gypseum* (B) *Microsporum boullardii* (C) *Trichophyton mentagrophytes* (1) (D) *Trichophyton mentagrophytes* (2) (E) *Trichophyton terrsetre* (F) *Trichophyton verrucosum.*

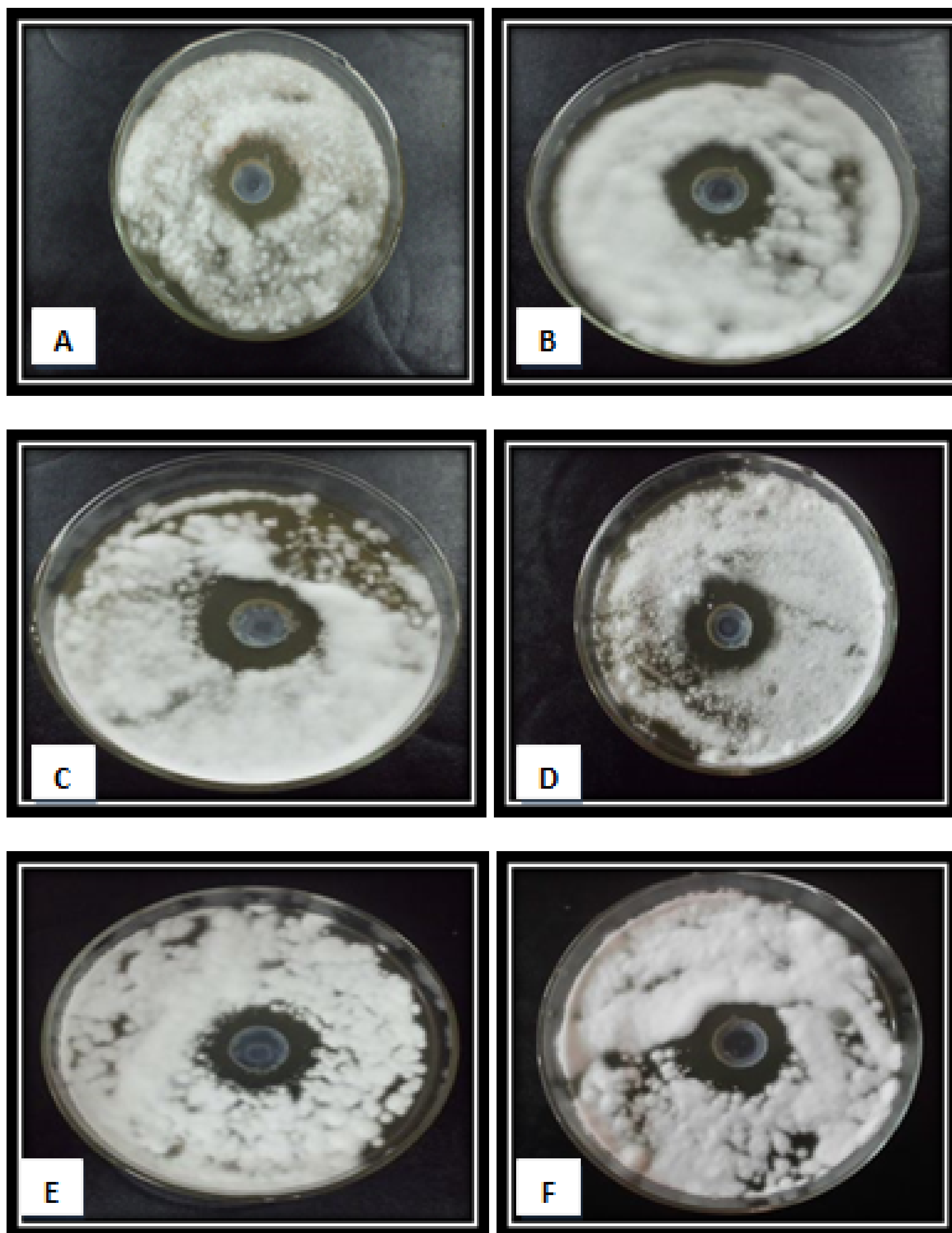

Fig. 6: Antifungal activity of control drug itraconazoles against fungi (A) *Microsporum gypseum* (B) *Microsporum boullardii* (C) *Trichophyton mentagrophytes* (1) (D) *Trichophyton mentagrophytes* (2) (E) *Trichophyton terrsetre* (F) *Trichophyton verrucosum.*

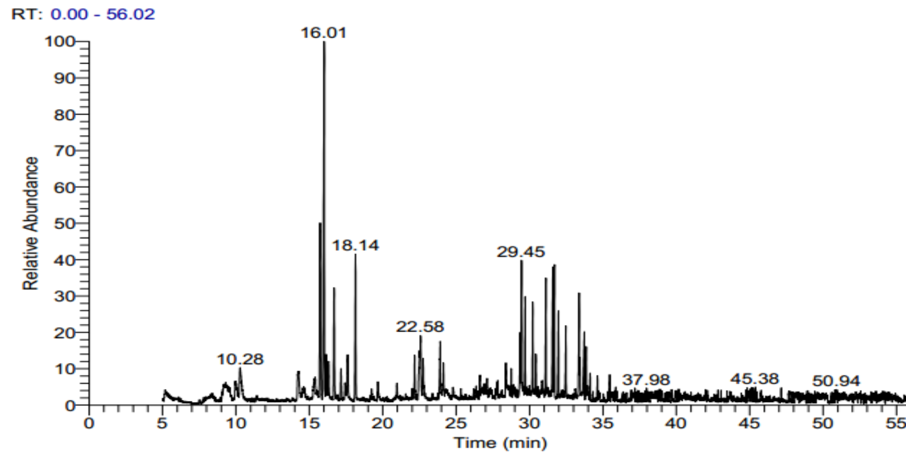

Fig. 7: GC-MS chromatogram of hexane: ethyl acetate (1:9) fraction of kurrat (*Allium ampeloprasum var. Kurrat*).

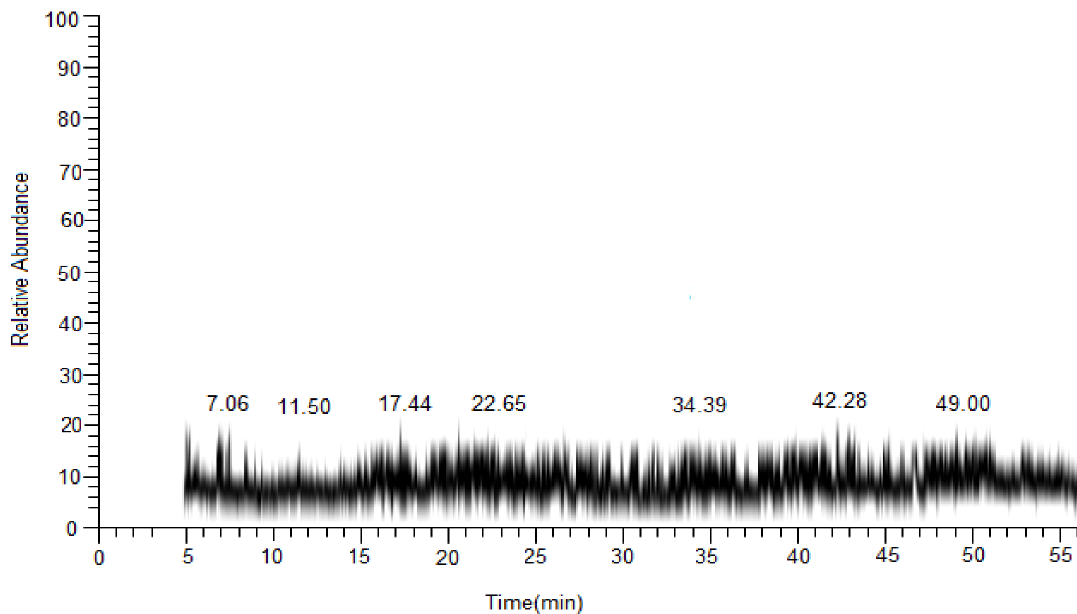

Fig. 8: GC-MS chromatogram of Ethyl acetate: ethanol (9:1) fraction of pomegranate (*Punica granatum*).

DISCUSSION

In the present investigation, dermatophytic fungi were isolated from soil collected from different localities in Cairo. Areas of the collected soil samples were Giza zoo (animal cages and parks), hospital, public Park, local market, primary school, club, and garbage dumping site.

The antifungal activities of five plant extracts were tested against the identified dermatophytes *Microsporum gypseum, Microsporum boullardii, Trichophyton mentagrophytes, Trichophyton terrsetre*, and *Trichophyton verrucosum.*

The five plants were pomegranate, fennel, kurrat, *Aloe vera*, and castor bean.

In our study, ethanol 80% extract of pomegranate fruit peels showed antifungal activity against isolated dermatophytes; *Trichophyton mentagrophyte* 1, *Trichophyton verrecusom*, *Trichophyton terrestre, Trichophyton mentagrophytes* 2 and *Microsporum boullardii* but no activity was recorded on *Microsporum gypseum.* The results were in a close agreement with that of Foss *et al.*, (2014) and Barathikannan *et al.,* (2016) who reported that hydroalcoholic and alcoholic extract of pomegranate fruit peels

showed activity against the dermatophytes *Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis*, and *Microsporum gypseum*.

Mahajan *et al.*, (2014); Khaleel *et al.*, (2016); Mohammed *et al.,* (2016); Akroum, (2017); Mostafa *et al.,* (2017); Rosas-Burgos *et al.*, (2017) clarified that extracts of pomegranate fruit peels showed activity against bacterial and fungal strains other than dermatophytes such *Staphylococcus aureus, Salmonella typhi, Vibrio cholera, Klebsiella pneumonia, Shigella flexneri, Bacillus cereus, Pseudomonas aeruginos, Escherichia coli*, *Proteus mirabilis*, *Erwinia carotovorum*, *Ralstonia solanacearum*, *Xanthomonas gardneri*, *Candida albicans, Candida krusei, Candida guilliermondii, Aspergillus niger, Aspergillus terreus, Aspergillus flavus, Aspergillus Fumigatus, Fusarium verticillioides, Alternaria alternate*, and *Botrytis cinerea*.

The present study showed that hexane, ethyl acetate, and 80% ethanol extracts of fennel fruits did not affect the tested fungi. These results were in agreement with the study of Benlafya *et al.*, (2015) and Thakur *et al.*, (2013) who demonstrated that alcoholic extract of fennel seeds was ineffective against tested bacterial and fungal strains such as *Escherichia coli, Bacillus subtilis*, *Salmonella abony*, *Candida albicans*, and *Aspergillus flavus.*

But other several studies showed that extracts of fennel fruits have antimicrobial activity.

Roby *et al.*, (2013) reported that methanolic extract of fennel fruits has effective antimicrobial activity against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus cereus*, *Candida albicans*, and *Aspergillus flavus.*

Thakur *et al.*, (2013) clarified that the aqueous and alcoholic fruit extracts of *Foeniculum vulgare* showed antifungal activity against *Alternaria alternate* and *Mucor rouxii.*

Zeng *et al.*, (2015) showed that fennel seeds essential oil has antifungal activity against dermatophytes such as *Trichophyton rubrum*، *Trichophyton tonsurans, Microsporum gypseum*, and Trichophyton mentagrophytes.

In the current work, hexane, ethyl acetate, and 80% ethanol extracts of castor bean leaves were not effective against tested dermatphytes. There are other studies which confirm this part of study.

Khan and Yadav, (2011) demonstrated that hexane, ethyl acetate, and ethanol extracts of castor bean leaves did not affect *Microsporum* sp. and also demonstrated that ethyl acetate and ethanol leaf extracts of castor bean were not effective against *Trichophyton rubrum*.

Ishnava *et al.*, (2011) reported that hexane, ethyl acetate, and methanol leaf extracts of castor bean were not effective on *Aspergillus flavus*, *Aspergillus awamori, Aspergillus parasi, Alternaria sp.,Trichoderma harzianum, Trichoderma virans,* and *Fusarium oxysporium.*

On the other hand, Naz and Bano, (2012) showed that methanol, ethanol, and aqueous extracts of *Ricinus communis* leaf have antibacterial and antifungal activity on *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Aspergillus fumigatus*, and *Aspergillus flavus*.

In the present work, hexane, ethyl acetate, and 80% ethanol extracts of *Aloe vera* leaf did not affect the tested dermatophytes. These results were compatible with results of Shrivastav *et al.*, (2013) who reported that alcoholic extract of *Aloe vera* was not effective against most tested dermatophytes such as *Trichophyton rubrum, Trichophyton equinum, Microsporum nanum*, and *Microsporum gypseum.*

In contrast to our results, Karpagam and Deveraj, (2011) and Abakar *et al.*, (2017) demonstrated that alcoholic and acetone extracts of *Aloe vera* leaf exhibited antimicrobial activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Candida albicans,* and *Aspergillus niger*.

Our study revealed that ethyl acetate extract of kurrat did not affect the tested dermatophytes . Interestingly, hexane bulb extract of kurrat showed strongest antifungal activity on *Tricophyton mentagrophytes* (*2*), *Tricophyton terrestre*, and *Tricophyton verrecusom* as compared to reference antifungal agent (itraconazole). Also ethanolic extract of kurrat was effective against most tested strains but not as strong as hexane extract.

Few studies are available on antimicrobial activity of kurrat. Abdou *et al*., (1972) demonstrated that the crude juice and ether extract of *Allium kurrat* were active on *Escherichia coli* and *Bacillus subtilis*. Sharaf *et al*., (1969) showed that *Allium kurrat* juice has antibacterial effect against *Pseudomonas* sp., *Staphylococcus aureus, Citrella freundii, Klebsiella aeroginosa, Carcena sp., Staphylococcus citrus, Proteus sp., Escherichia coli*, and *Streptococcus lutea.*

In the present work, the active fraction of pomegranate (*Punica granatum*) (ethyl acetate: ethanol (9:1)) and the active fraction of kurrat (*Allium ampeloprasum var. Kurrat*) (hexane: ethyl acetate (1:9)) were analyzed by Gas Chromatography-Mass Spectrum (GC-MS).

The analysis revealed that thirty-nine compounds from pomegranate and forty compounds from kurrat were identified. Several of these compounds were responsible for the antifungal activity found in the extracts against the fungal strains.

The major phytoconstituents present in the kurrat extract were trans-propenyl propyl disulfide (14.27%) and Methy-2-benzothiazolinthion (7.94%). Also many of compounds of kurrat extract identified as organosulfer compounds (Table 4). These results were in accordance with those of Gibbons (2004); Chung *et al.,* (2008) ; Kyung (2012); Dey and Khaled, 2013; Mnayer *et al.,* (2014) and Ramirez *et al.,* (2016) whom indicated that Alliaceae Family is rich with sulfer-containing compounds such as linear sulfur compounds (diallyl disulfide, diallyl trisulfide, allyl methyl trisulfide, diallyl sulfide, diallyl tetrasulfide, allyl methyl disulfide, dipropyl disulfide, dipropyl trisulfide, 1-propenyl propyl disulfide, methyl propyl trisulfide, dimethyl disulfide , methyl propenyl disulfide , propyl propenyl disulfide , dimethyl trisulfide , methyl propyl trisulfide, and methyl propenyl trisulfide) and heterocyclic sulfur compounds (4-methyl-1,2,3-trithiolane, 5-methyl-1,2,3,4-tetrathiane, and 6-methyl-1,2,3,4,5-pentathiepane) which are responsible for antimicrobial activity against bacterial and fungal strains.

The GC/MS analysis of the fraction ethyl acetate: ethanol (9:1) of pomegranate ethanolic extract revealed many compounds of diverse chemical structure; viz. pyridine, anthraquinones, phenanthrene, benzofuran, nonan-2-one, thiophenes, phenanthrolines, cholestane, flavone and anthracene (Table 5). Most of these compounds were found to have antimicrobial activity. Our results agree with the work done by many authors (Bnina *et al*., 2010; Alarif *et al.,* 2011; Kumar *et al*., 2011; Juhan *et al*., 2013; Yadav *et al*., 2013; Azeredo and Villar, 2014; Bagla *et al*., 2014; Mamtora *et al.,* 2015; Rodik, 2015; Sidjui *et al.,* 2015; EL Hamdani *et al.,* 2016; Kamlesh *et al*., 2016 and Mabkhot *et al.,* 2016). They found that these compounds have antimicrobial activity on *Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus pyogenes, Streptococcus pneumonia, Streptococcus faecalis, Serratia entomophilia, Serratia marsescens, Escherichia coli, Pseudomonas aeruginosa*, *Shigella flexneri, Entrecoccus coaccae, Salmonella typhi, Klebsiella pneumoniae, Clostridium perfringens, Cryptococcus neoformans, Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis, Candida tropicalis, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium monilliformae, Fusarium oxysporum*, *Alternaria solani, Rhizoctonia betaticola, Trichophyton viridae, Colletotrichum dematium, Syncephalastrum racemosum and Rhizoctonia solani.*

From our results we can conclude that hexane extract of kurrat bulb and ethanolic extract of pomegranate peel could be used as

antifungal agent that could be used for treating some tinea diseases.

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