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Seeds oil extract of *Mesembryanthemum forsskalii* from Aljouf, Saudi Arabia: Chemical composition, DPPH radical scavenging and antifungal activities☆

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Abstract – Vegetable oils are the subject of several studies considering their importance as biological properties. Chemical composition of plants oil depends on the plant family in which they were extracted. The study here deals with analysis of chemical composition of the extract obtained from seeds of *Mesembryanthemum forsskalii* naturally grown in the region of AlJouf located in the northern part of Saudi Arabia. Examination of anti-oxidant and anti-fungal properties of seeds oil extract was determined. Results showed that this extract contained 23 chemical elements with good amounts of phytosterols (35%). In addition, the antioxidant activity was evaluated by DPPH test which showed good activity and a value of $IC_{50} = 3.43 \pm 0.19$ mg/mL. For the determination of the antifungal activity, 11 fungal species belonging to 7 genera were isolated from children hairs. *Aspergillus carneus* and *Penicillium chrysogenium* were the most frequent fungi (32.45, 25.41%), respectively, whereas the appearance of *Penicillium chrysogenium* and *Fusarium oxysporum* were found to be (17.67 and 12.33%), respectively. Results showed that the percentage of boys infested hair by fungi was higher than that of girls with a percentage 70.85 and 55.62%, respectively. Antifungal activity of ethanolic seeds extract was carried out on the isolated non-dermatophytes keratinophilic fungi. It was found that the fungi of *Penicillium chrysogenium* and *Aspergillus. fumigatus* were inhibited by seeds oil extract with 88% followed by *Aspergillus flavus, Aspergillus carneus* with 85% of inhibition and the rest of the isolated fungi were inhibited between 60 and 75%. Based on these encouraging results, seeds oil extract of *M. forskalii* can be interesting for food, pharmaceutical or cosmetic industries.

Keywords: antifungal activity / DPPH scavenging / non-dermatophytes keratinophilic fungi / phytosterols / seeds oil extract

Résumé – Extrait d'huile de graines de *Mesembryanthemum forsskalii* de la région d'Aljouf, en Arabie Saoudite : composition chimique, piégeage des radicaux DPPH et activités antifongiques. Les huiles végétales font l'objet de plusieurs études considérant leur importance comme des propriétés biologiques. La composition chimique des huiles végétales dépend de la famille de plantes dans laquelle elles ont été extraites. L'étude porte ici sur l'analyse de la composition chimique de l'extrait obtenu à partir de graines de *Mesembryanthemum forsskalii* naturellement cultivées dans la région d'AlJouf située dans la partie nord de l'Arabie Saoudite. Les propriétés antioxydantes et antifongiques de l'extrait d'huile de graines ont été examinées. Les résultats ont montré que cet extrait contenait 23 éléments chimiques avec de bonnes quantités de phytostérols (35 %). De plus, l'activité antioxydante évaluée par le test DPPH a montré une bonne activité et une valeur de $IC_{50} = 3{,}43 \pm 0{,}19$ mg/mL. Pour la détermination de l'activité antifongique, 11 espèces fongiques appartenant à 7 genres ont été isolées à partir de cheveux d'enfants.

☆ Contribution to the Topical Issue "Minor oils from atypical plant sources / Huiles mineures de sources végétales atypiques".

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Aspergillus carneus et *Penicillium chrysogenium* ont été les champignons les plus fréquents (32,45 %, 25,41 %), respectivement, tandis que l'apparition de *Penicillium chrysogenium* et de *Fusarium oxysporum* s'est avérée être de respectivement 17,67 et 12,33 %. Les résultats ont montré que le pourcentage de garçons infestés par des champignons était plus élevé que celui des filles avec un pourcentage de 70,85 et 55,62 %, respectivement. L'activité antifongique de l'extrait de graines éthanolique a été réalisée sur les champignons kératinophiles non dermatophytes isolés. Il a été constaté que les champignons *Penicillium chrysogenium* et *Aspergillus fumigatus* étaient inhibés par l'extrait d'huile de graines à 88 %, suivis par *Aspergillus flavus*, *Aspergillus carneus* à 85 % d'inhibition et le reste des champignons isolés entre 60 et 75 %. Sur la base de ces résultats encourageants, l'extrait d'huile de graines de *M. forskalii* peut être intéressant pour les industries alimentaires, pharmaceutiques ou cosmétiques.

Mots clés : extrait d'huile de graines / activité antifongique / phytostérols / piégeage de la DPPH / champignons kératinophiles non dermatophytes

1 Introduction

Mesembryanthemum forsskalii or in Arabic "Samh" plant is one of the most popular natural plants grown in the region of Al-Jouf, localized in the Eastern North of Saudi Arabia (Showdrei, 1999). Due to the richness of plant in proteins, fats, and carbohydrates (Aljassir *et al.*, 1995), it is used in food preparations (Mustafa *et al.*, 1995). Moreover, this plant has applications in biological and medical fields (Elgasim and Alwesali, 2000; Alqahiz, 2009; Alfaris *et al.*, 2010). Fungi inhabiting human hairs are a common health problem, especially children in poor places. In this aspect, some fungi are considered risk factors because of immunity deficiency in children as well as other nutritional problems (Katona and Katona-Apte, 2008). *Tinea capitis* is a fungal pathogen that affects human hair and scalp. Kundu *et al.* (2012) recorded that out of 505 students, 52 were infected with *Tinea capitis*. Dogo *et al.* (2016) reported that 45 from 100 children were infected with ringworm, and that the dominance of ringworm infection was 51.4% among girls while the infection among boys was 41.5%. Among ringworm fungal species, it was reported that *Trichophyton rubrum* (28.8%) and *Microsporum canis* (22.7%) were predominant dermatophytes and the least common was *Trichophyton verrucosum* (4.5%) and *Trichophyton tonsurans* (4.5%). In Nigeria, 55% of school children were infected with fungal skin (Soyinka, 1978), while in India, the infection ranged from 2.9 to 13.9% (Gopinath *et al.*, 1997). Al-Mosawi *et al.* (1993) found that 5% of children without *Tinea capitis* had dermatophytes in their scalp. Common ringworm recoded species are *Microsporum* sp. and *Trichophyton* sp., but other filamentous skin fungi were isolated from keratin (a fibrous spherical protein) and infected the superficial layer of the skin that excreted hydrolyzed enzymes caused weakened and hair falling (Ali-Shtayeh *et al.*, 2001; East-Innis *et al.*, 2006; Mbata and Nwajagu, 2007; Andrews and Burns, 2008). Keratinolytic fungi are defined as those that can break down keratin, whereas they are the only one that can use naturally related substances from keratin resulting from its destruction or decomposition (Sharma and Choudhary, 2014). According to Rippon (1982), each keratin-analyzed fungus can be considered as a potential pathogen. Not only filamentous dermatophytes have the ability to breakdown keratin, but also, there are several non-dermatophytes keratinophilic fungi and many saprophytic fungi as well (Ulfig *et al.*, 2010).

In this study, seeds oil extract of *M. forsskalii* was analyzed by gas chromatography coupled with mass spectroscopy GC-MS to give more details about the chemical composition then the antioxidant activity was evaluated by DPPH scavenging. Additionally, series of non-dermatophytes keratinophilic fungi were screened and identified to evaluate the antifungal activity of the tested seeds oil extract.

2 Materials and methods

2.1 Chemicals

All chemicals used were of analytical reagent grade: ethanol, purity 99%, dimethyl sulfoxide (DMSO) extra pure. All reagents were purchased from Sigma Aldrich–Fluka.

2.2 Collection of plant material and seeds oil extraction

Seeds of *M. forsskalii* were obtained in April 2019 from desert of Sakaka-Aljouf, according to the following GPS geographical coordinates (latitude: 29.953894, longitude: 40.197044, 29° 57' 14.0184" N and 40° 11' 49.3584" E). Extract was gained from powder of *M. forsskalii* seeds by Pyrex® Soxhlet extractor apparatus. Five grams of the powder were placed in the extraction chamber of the Soxhlet extractor adapted by the condenser, and then 150 mL of ethanol were added in the distillation flask. After refluxing for 8 h, the ethanol was eliminated with a rotary evaporator at 45 °C under reduced pressure. Extraction was done in triplicate. Pure seeds oil extract was stored at 4 °C in the dark until the beginning of the analysis. Yield of the extracted oil was 0.43 g/5 g w/w (Tab. 1).

2.3 Determination of chemical composition by GC-MS

Seeds oil extract from *M. forskalii* was analyzed using gas chromatography coupled to mass spectrometry Shimadzu GC-MS-QP2010SE single quadrupole apparatus.

The gas chromatograph was equipped with SLB-5MS capillary column (characteristics: L = 30 m, d = 0.25 mm, thickness = 0.25 μm) and FID (flame ionization detection) detector. Injector temperature was fixed at 200 °C, oven temperature raised from 45 °C to 260 °C at 5 °C/min, held for 15 min then raised to 360 °C at 40 °C/min. Detector temperature was set at 365 °C. The mass spectrometer was adjusted for

Table 1. Botanic properties and extracted yield of the used plant.

Plant species	Family	Local name	Part of plant used	Extract yield (%)
Mesembryanthemum forsskalii	Aizoaceae	Samh	Seeds	8.6

an emission current of 10 μA and electron multiplier voltage at 1500 V. Trap temperature was 250 °C and mass scanning has been set from 40 to 650 amu. The total analysis time was 80 min, and components were identified based on the comparison of their retention time and mass spectra with those of standards. All determinations were performed in duplicate.

2.4 Antioxidant activity: DPPH radical scavenging activity

Antioxidant activity of the seeds oil extract from *M. forskalii* was determined *in vitro* using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, according to the method of Blois (1958). In this work, Trolox was used as an internal standard and percentage of inhibition was calculated according to the formula of:

$$\%\,\text{inhibition} = [(\text{ABS}\quad\text{blank} - \text{ABS}\quad\text{sample})/\text{ABS}\quad\text{blank}] \times 100.$$

Absorbance was measured by spectrophotometer at 517 nm after incubation in the dark within 20 minutes at room temperature (+/−27 °C). Lower absorbance indicates higher free radical scavenging activity.

ABS blank was the absorbance of the control reaction containing all reagents except the tested compound. ABS sample was the absorbance of the test compound.

2.5 Antifungal activity

2.5.1 Isolation and identification of fungi

Hair samples were collected from some primary school students in Sakaka city, Aljouf, KSA (5 boys and 5 girls) ranging in age from 5 to 7 years. Samples were transferred to the laboratory for isolation of fungi where potato dextrose agar medium (PDA) supplemented with rose-bengal was used. Examination of the purified growing colonies on the culture medium was carried out using a compound microscope. Fungi were identified according to Barnett and Hunter (1972); Pitt and Hocking (1997).

2.5.2 Percentage and frequency of the isolated fungi

Percentage and frequency of isolated species were calculated according to Krebs (1978). For statistical analysis GraphPad Prism 2.01 and comparison between averages using Least Significant Difference test (LSD) at the level of probability of 0.001 have been used (Ghoodjani, 2019).

2.5.3 Antifungal activity of ethanolic extract of *M. forsskalii*

A mL of fungal spore suspension (~10^6 spores) as placed with 1 mL of *M. forsskalii* oil seeds extract in 15 cm diameter Petri dish contained warm sterilized PDA medium and left until solidification. Five replicates of tested dishes were placed in sterile bags and incubated at 25–27 °C for 5–7 days until the appearance of fungal colonies. Control was done using all components with distilled sterilized H_2O.

3 Results and discussion

3.1 Gas chromatography-mass spectroscopy (GC/MS)

GC-MS is an efficient analytical technique for identifying and quantifying components of organic mixture. The brown extract obtained from the seeds of *M. forsskalii* plant was examined by GC-MS technique and the given analysis had shown a complex composition. Twenty-three components were identified by comparing retention time with those described in literature for the standard compounds. Composition of the extract and their percentage are presented in Tables 2 and 3.

Analysis of chemical composition of seeds oil extract had shown that 68.75% represent total amount of steroid derivatives subdivided into sterols, ketosteroids and stanols (Tabs. 2 and 3). Proportion of unsaturated aliphatic compounds was 12.94%.

Plant steroids are a diverse group of secondary metabolites that can be classified into several groups based on their structures and functions (Sultan and Raza, 2015); they play important pharmacological activities (Gunaherath and Gunatilaka, 2014). It's of importance that plant steroids were analyzed and qualifying. In this study, major steroids are beta-sitosterol (33.05%) which was a phytosterol and 3-methoxy-(3-beta,5-alpha)cholestan-6-one (22.14%) which was a ketosteroid derivative, representing 55.19% of the total composition of the extract. In addition, the extract contained two natural tri-terpenoids which were alpha and beta amyrin (isomeric mixture) (Tab. 2), that well-known by their analgesic and anti-inflammatory properties (Pinto *et al.*, 2008).

During the last decade, phytosterols became a center of interest due to their benefits values including reduction of blood cholesterol and prevention to cardiovascular diseases (Woyengo *et al.*, 2009; Othman and Moghadasian, 2011; Alemany *et al.*, 2014; Shuang *et al.*, 2016). According to literature, the three main phytosterols existing in plants extract are stigmasterol, beta-sitosterol and campesterol (Milovanović *et al.*, 2009; Yuang *et al.*, 2018). As shown in Table 2, sterols present in the seeds oil of *M. fosskalii* were beta-sitosterol (−92.20% of sterol content) and campesterol (−7.80% of sterol content) (Fig. 1).

Due to the presence of appreciable quantity of phytosterols (35.8%) in extract, seeds oil of *M. forskalii* represent a good choice for patients with high cholesterol and cardiovascular diseases.

Table 2. Chemical composition of the seeds oil extract from *Mesembryanthemum forsskalii* plant.

No.	Rt (min)[a]	KI (Ref)[b]	Components	Percentage (%)
1	34.96	437 618	Linoleic acid	0.86
2	35.05	446 541	Oleic acid	1.84
3	38.92	146 924	Tetracosane	1.04
4	40.50	153 747	Pentacosane	1.37
5	42.01	159 836	Hexacosane	1.66
6	42.86	443 833	9-phenanthrenemethylcinnamate	0.53
7	43.47	165 301	Heptacosane	1.70
8	44.88	169 719	Octacosane	1.93
9	46.38	173 139	Nonacosane	1.44
10	47.28	452 480	Cholestan-3-ol	1.62
11	48.14	454 158	Triacontane	1.10
12	49.16	174 843	3-methoxy-(3-beta,5-alpha)cholestan-6-one	22.14
13	49.66	277 722	Stigmasta-3,5-diene	4.59
14	50.06	183 961	4-iododiphenyl ether	2.08
15	52.64	171 432	Campesterol	2.80
16	54.38	287 256	Beta-sitosterol	33.05
17	54.90	176 563	Alpha-amyrin	7.32
		176 562	Beta-amyrin	
18	57.18	286 193	Cholest-7-en-3-one	4.55
19	70.29	247 373	1H-indole-2-carboxylic acid	1.26
20	70.35	415 664	2,4,6 (1H,3H,5H)-pyrimidinetrione	0.07
21	72.66	249 919	3'-hydroxy-5'-propylphenyl-2.4-dihydroxy-6-pentylbenzoate	6.54
22	72.76	247 201	2',4',dimethyloxanilic acid N'-veratrylidenehydrazide	0.31
23	72.91	166 614	Phenaleno[2,3-g] quinolin-7-one	0.19
Total			99.99%	

[a] Rt: retention time (min).
[b] KI: Kovat's Indices.

Table 3. Classification of the chemical compounds of the seeds oil extract from *Mesembryanthemum forsskalii* plant.

Class	Percentage (%)
Aliphatic compounds	12.94
Steroid derivatives	68.75
Tri-terpenoids	7.32
Alkaloids	1.45
Others	9.48
Total	99.99

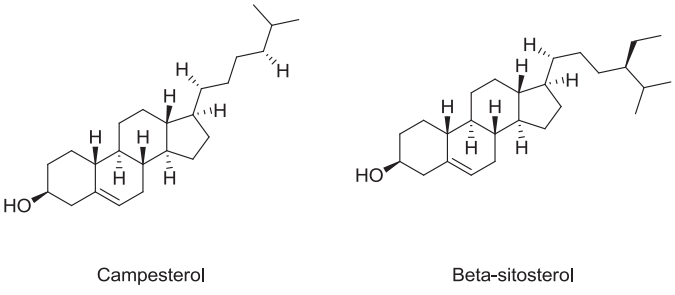

Fig. 1. Beta-sitosterol was the dominant phytosterol in the seeds oil extract of *Mesembryanthemum forsskalii*.

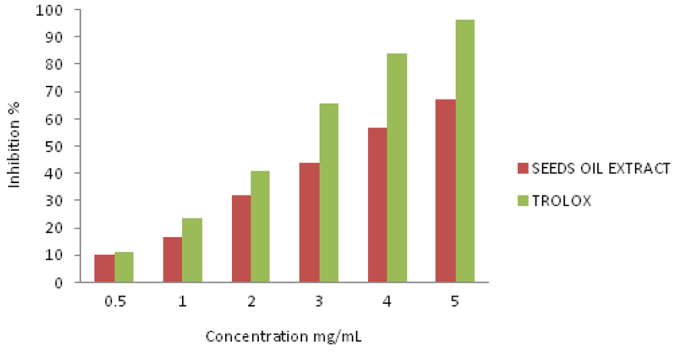

Fig. 2. DPPH radical scavenging activity of seeds oil extract from *Mesembryanthemum forsskalii*.

3.2 Antioxidant activity of the seeds oil extract

Antioxidant activity of the seeds oil extract from *M. forskalii* was evaluated by DPPH radical scavenging method using Trolox as an internal standard. The obtained results are presented in the next graph (Fig. 2).

Results showed that the extract from seeds of *M. forskalii* had good antioxidant activity; an increase in the concentration from 0.5 to 5 mg/mL raised the inhibition percentage and reached a maximum value of 69% (Fig. 2). Value of the extract

Table 4. Percentages of frequency and appearance of isolated species by childrens' sex.

Species	Percentage of appearance of isolates (%)		Percentage of frequency (%)	
	Girls	Boys	Girls	Boys
Alternaria alternata	–	8.33 ± 1.68	–	7.40 ± 0.45
Aspergillus carneus	11.50 ± 0.25	–	32.45 ± 1.13	–
Aspergillus flavus	7.60 ± 2.73	–	7.52 ± 0.54	–
Aspergillus fumigatus	–	8.93 ± 2.45	–	13.57 ± 0.28
Aspergillus niger	–	10.27 ± 1.7	–	13.44 ± 0.23
Cladosporium cladosporioides	–	6.133 ± 0.92	–	12.93 ± 0.06
Fusarium oxysporum	–	12.33 ± 0.7	–	3.87 ± 0.54
Paecilomyces lilacinus	6.66 ± 0.35	6.53 ± 0.46	22.43 ± 1.16	20.35 ± 0.19
Penicillium chrysogenium	17.67 ± 2.36	10.27 ± 1.64	23.67 ± 0.78	25.41 ± 0.23
Penicillium oxalicum	6.46 ± 3.23	8.06 ± 0.67	7.84 ± 0.69	1.24 ± 0.13
Rhizopus oryzae	5.73 ± 3.35	–	6.56 ± 0.48	–
Total (%)	55.62 ± 0.78	70.85 ± 1.55	99.87 ± 0.13	97.93 ± 1.09

concentration providing 50% inhibition IC_{50} can be calculated directly from the graph plotting inhibition percentage against extract concentration, a lower value of IC_{50} indicated a good antioxidant activity. Seeds oil extract of *M. forskalii* was an effective DPPH radical scavenging agent with an IC_{50} value of 3.43 ± 0.19 mg/mL. Anti-oxidant capacity is due to the presence of some chemical compounds responsible for this activity. According to the literature, phenolic compounds were important secondary metabolites present in plants oil (Carpa and Gonzalez, 2001) which were responsible for the stability of unsaturated fatty acids (Siger *et al.*, 2008). Abdel-Farid *et al.* (2016) declared that the plant extract of *M. forskalii* is rich in flavonols, tannins and phenolics, it has been strengthened by Lee *et al.* (2011), Sutharut and Sudarat (2012) and Abdel-Farid *et al.* (2014). Good antioxidant activity obtained may be a consequence of its total phenolic compounds present in the seeds oil extract of *M. forskalii*. Results here support the possibility of using seeds oil extract as a natural antioxidant in different pharmaceutical fields.

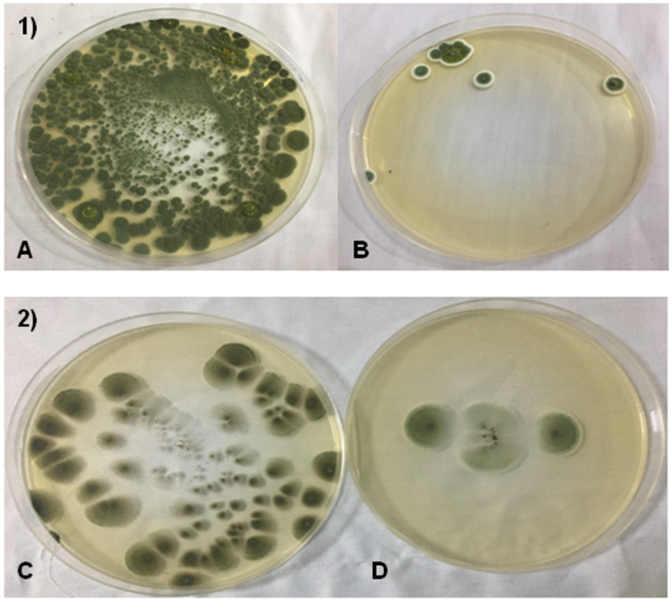

Fig. 3. 1. Inhibition of mycelial growth of *Penicillium chrysogenum*. A. Control dish containing DMSO only. B. Treated dish containing the seeds oil extract of *Mesembryanthemum forsskalii* dissolved in DMSO (1%). 2. Inhibition of mycelial growth of *Aspergillus fumigates.* C. Control dish containing DMSO only. D. Treated dish containing the seeds oil extract of *Mesembryanthemum forsskalii* dissolved in DMSO (1%).

3.3 Antifungal activity of seeds oil extract

3.3.1 Isolation and identification of fungi

All fungi isolated from the hair of boys and girls were used. It is worth noting that the presence of these fungi is a health hazard due to the ability of them to cause allergies and some skin diseases for humans and animals. Because the hair of the head is close to the respiratory tract in humans, this increases the chance that the fungi germs can enter the lungs of children, causing respiratory-like diseases (Moustafa and Abdelzaher, 2016).

Distribution of fungi from boys and girls showed that a total of 11 species belonging to 7 genera were identified (Tab. 4). Among them, 8 species were isolated from boys' hair samples and 6 species from girls' hair samples. Four species of the genus *Aspergillus* appeared, 2 species were isolated from boy's hair samples and the other two species were isolated from girl's hair samples. Additionally, two species of the genus *Penicillium* were isolated from both boy's and girl's hair samples. The predominant isolated species was *Aspergillus carneus* (32.45%) followed by *Penicillium chrysogenium* (25.41%). *Paecilomyces lilacinus* ranked the third species with a frequency of 22.43%, while, the remaining isolates showed lowest frequency ratios ranging from 1.24 to 13.57%.

According to fungal appearance, *Penicillium chrysogenium* came the first with 17.67% followed by *Fusarium oxysporum* (12.33%). *Aspergillus carneus* appeared at 11.50%, which is the third rank, the prevalence of the remaining species was distributed between 5.73 and 10.27%.

Results in Table 4 represent the distribution of species between boys and girls. *Penicillium chrysogenium* ranked first in frequency of both boys and girls samples with 25.41 and

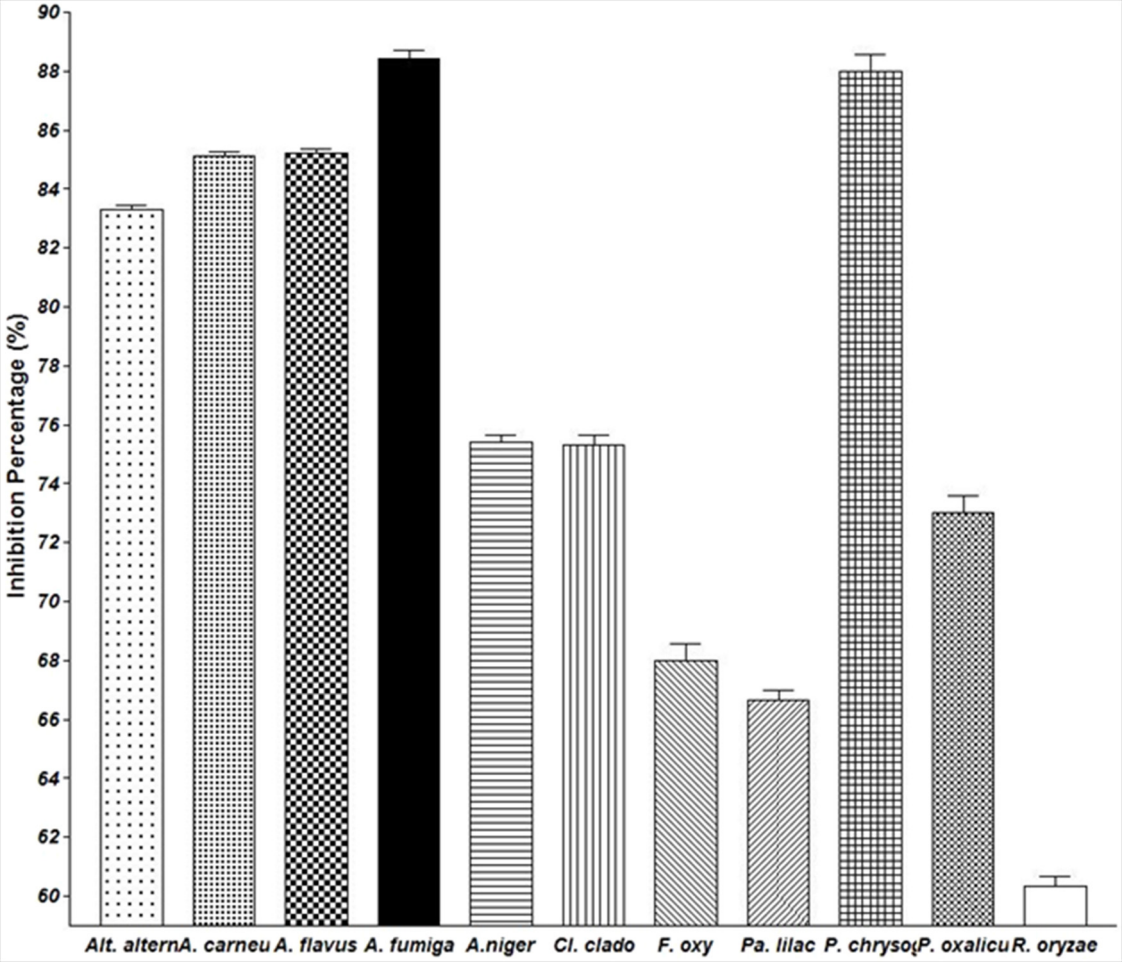

Fig. 4. Effect of seeds oil extract of *Mesembryanthemum forsskalii* on growth of isolated fungi from boys and girls hair samples in PDA media.

23.67%, respectively; it was also the most prevalent in girls with 17.67%. *Penicillium chrysogenium* was first in frequency to boy's hair samples at 25.41%. In female samples, *Aspergillus carneus* was 32.45%, represent the first rank of frequency. *Paecilomyces lilacinus* came in second in boy's hair samples with 20.35%. The current study showed that percentages of appearance were 70.85% of boy's hair samples and 55.62% of girl's hair samples gave a positive result on the PDA medium.

Findings here were consistent with several studies suggesting that males are more susceptible to hair fungi (Fathi and Al-Samarai, 2000). Uneke *et al.* (2006) pointed out that the short hair of boys compared to girls, facilitated the occurrence of scalp infection as well as the contaminated barbers sharing tools. The present investigation proved the dominance of Aspergillus species in frequency, visibility and in the number of isolated species. Penicillium species represented the second of frequency and visibility, that the dominance of these two species may be due to the nature of their spores widely spread in our surroundings.

3.3.2 Antifungal activity of the seeds oil extract

Several plant extract including ethanol extracts, resins and essential oils were reported to have an antifungal activity. These forms involved simple extraction methods with low production costs (Garcia *et al.*, 2008; Kuster *et al.*, 2009; Gahukar, 2012).

Inhibitory effect of seeds oil extract from *M. forskalii* was numerous on the isolated fungi and the results are illustrated in Figures 3 and 4.

The present study showed a great inhibitory effect of the seeds oil extract towards P. *chysogenum* and *A. fumigatus* which were inhibited by 88%, followed by *A. flavus*, *A. carneus* with 85% and the remaining isolated fungi were inhibited from 60 to 75%.

M. forsskalii seeds oil extract showed strong and significant influences on the growth of *Alternaria alternata, Aspergillus carneus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Cladosporium cladosporioides, Fusarium oxysporum, Paecilomyces lilacinus, Penicillium chrysogenium, Penicillium oxalicum,* and *Rhizopus oryzae.* Based on these results, seeds oil of *M. forsskalii* in the form of oil or lotion is recommended for treatments or prevents fungal infection of childrens' hair.

4 Conclusion

Understanding chemical composition, antioxidant and anti-fungal properties of various extracts are interesting for

food, cosmetic and pharmaceutics industries. The study of chemical and biological properties of *M. forskalii* showed that this extract had many benefits properties. Seeds oil extract of *M. forskalii* can be used as a natural food preservative to control food spoilage avoiding the use of chemical preservatives due to their good antioxidant activity. Owing to their containing of phytosterols, it can be taken by people having high amount of cholesterol. Additionally, growth of hair fungi can be treated by seeds oil extract of *M. forskalii* in the form of oil or lotion. In conclusion, our study can be considered as the first detailed document on the *in vitro* antifungal behavior of seeds oil extract of *M. forskalii* against a series of non-dermatophytes keratinophilic fungi.

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