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Original Research Article

Mycoflora associated with Halfa-bar leaves and stems (*Cymbopogon schoenanthus* L. Sprengel), in vitro the antimicrobial activity of the plant leaves and stems secondary metabolites

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K e y w o r d s

Cymbopogon schoenanthus L.; Sprengel; mycoflora; antmicrobial activity; secondary metabolites.

A B S T R A C T

Cymbopogon schoenanthus L. Sprengel, Family Poaceae, locally known as Halfa-bar, is an aromatic densely-tufted grass growing wildly and widely in Upper Egypt. The herb is highly reputed in folkloric medicine. In the present study, we describe the mycoflora of the *Cymbopogon schoenanthus* L. Sprengel where thirty-eight species in addition to 2 varieties belonging to 16 genera were isolated from the plant leaves on glucose and cellulose-Czapek□s agar at 28°C by using dilution-plate method. The most frequently encountered fungal species on the two types of media were: *Aspergillus niger*, *A. terreus* var. *africanus*, *A. terreus* var. *aureus*,, *Cochliobolus spicifer*, *Emericella nidulans* , *Fusarium dimerum* and *Penicillium camembertii.* Aqueous, methanol, ethyl acetate and n-butanol extracts of the plant leaves were tested at different concentrations against 17 pathogenic and non-pathogenic fungi. These extracts were also evaluated for their activity against some pathogenic bacteria. Most of the studied microbes showed high sensitivity to all tested fractions. Among the solvent extracts tested, methanol extracts gave more effective than other solvent extracts. It will be necessary to identify the active compounds or components and to evaluate their potential for use as antimicrobials.

Introduction

The genus *Cymbopogon*, a member of the family Gramineae**.** It is a perennial herb, erect, tufted 9cm. long, culms slender, erect, glabrous 3-4 noded. Leaf simple, alternate, linear 5-7 cm. long, sheathed apex spiny entire. Inflorescence spikelets highly branched. 5cm long (El tahir *et al*., 2010). It grows in Southern Egypt and Northern Parts of Sudan (Boulos, 1999). Distributed in Central and Northern Sudan (Eltahir *et al*., 2010). It is highly reputed in Egyptian folk medicine as an effective renal antispasmodic and diuretic agent. (Taeckholm 1974 & Boulos 1983 & Batanouny *et al*., 1999; El-Askary *et al*., 2003). The entire dried herb has been used

for centuries by the Bisharin and Ababda tribes of the Aswan Province in the form of a decoction to produce diuresis, to relieve colicky pains, to help the removal of small stones from the urinary tracts, and as an antipyretic in fevers. A bicyclic sesquiterpene diol, proximadiol with unique antispasmodic properties has been isolated from *C.proximus* leaves (Radwan, 1975). Proximol® possesses unique antispasmodic properties as it produces relaxation of the smooth muscle fibers without abolishing the propulsive movement of the tissue (El-Askary *et al*., 2003).

Materials and Methods

Tested Micro-organisms

The micro-organisms used in this study consisted of: Five common fungal species (*A.flavus, A.niger, C.spicifer, F.dimerum, M.circinelloides*) as well as four crop threatening pathogenic fungi, (*Alternaria alternata, Cochliobolous spicifer, Stachybotrys atra* var.*microspora*, and *Ulocladium botrytis*) isolated from *Vicia faba*. Fungal species were incubated on Potato glucose agar (PGA) (Potato, 200 g; Glucose, 20 g; Agar, 20 g and 1000 ml distilled water) for 7 days at 28C Eight dermatophytic fungi (*Candida albicans, Candida tropicalis, Candida krusei,Epidermophyton floccosum, Trichophyton rubrum, Trichophyton mentagrophytes*, *Trichophyton verrucosum* and *Microsporium canis*) collected from Assuit fungal centre.

Dermatophytic fungi employed in the screening on Sabouroud Glucose agar medium at 28C. Sabouroud glucose agar (SGA) is composed of Glucose, 40 g; Peptone; 10 g; Cyclohexamide, 0.5 g; Agar, 20 g per 1L distilled water for 15 days.

Three pathogenic bacteria (*Staphylococcus aureus* MARSA, *Escherichia coli* and *Salmonella typhi*) collected from bacteriology laboratory at Qena were grown on nutrient agar medium at 37 C. Nutrient agar composed of (peptone, 5g, beef extract, 3g; agar, 15 g).

Preparation of Plant Aqueous Extract

Powdered samples (100gm) of *Cymbopogom schoenanthus* L. Sprengel leaves and stems were macerated with 1000ml sterile distilled water in a blender for 10 min. The macerate was first filtered through double –layered muslin cloth followed by centrifugation at 4000rpm for 30 min. at room temperature. The supernatant was filtered through Whatmann No. 1 filter paper and sterilized, which served as the mother extract (Satish *et al*. 2007). For evaluation of antifungal activity of the extracts, percentage dilutions i.e. 15%, 20%, 30% and 40% of extract were obtained by adding appropriate of standard basic stock solution to stock media.

Antimicrobial Activity Assay

For screening of antimicrobial activity of powdered ingredients of *C. schoenanthus* L. Sprengel poisoned food technique was followed (Sinha *et al*. 1993). Potato Dextrose Agar (PDA) medium was prepared and sterilized. The medium was supplemented with different serial dilutions of aqueous extracts i.e. 15, 20, 30, & 40% (stock solution). About 15ml of this medium was poured into each petriplate and allowed to solidify. Ten mm disc of 7-day-old culture of each fungus were placed at the centre of the each petriplate and incubated at 28°C for 3, 4, & 5 days . After incubation, the colony diameter was measured in millimeter

(mm). For each treatment group (for a given percentage of extract) four replicates were maintained. PDA medium without the aqueous extract was taken as control but in case of dermatophytes we used Sabouroud Dextrose Agar (SDA) medium. For pathogenic bacteria we used Nutrient Agar (NA) medium. The fungitoxicity of the extracts was taken in terms of percentage inhibition of mycelial growth was calculated by using the following formula:

$$\% \text{ inhibition} = dc\text{-}dt/dc \times 100$$

Where dc= Average increase in mycelial growth in control, dt = Average increase in mycelial growth in treatment (Singh and Tripathi 1999).

Preparation of Organic Extract

Ten grams of dried plant material was successively extracted with 100 ml of methanol, 100 ml n-butanol and100 ml ethylacetate kept on a rotary shaker for 24 h at room temperature (Al-Fatimi *et al*. 2007). The solvent concentrated under vaccum, weighted and kept in frozen (-20C) for antimicrobial assays.

Antimicrobial Activity Assay

Antimicrobial activity was studied using filter paper disk diffusion method (Benson, 1990). The degree of growth inhibition was evaluated after 24hr for bacteria and 48hr for fungi and compared with the growth inhibition results obtained from the controls.

Results and Discussion

Mycoflora associated with *Cymbopogon schoenanthus* L.Sprengel

The dilution plate method showed that *C. schoenanthus* L. Sprengel leaves and stems were highly contaminated with various types of fungi where thirty-eight species in addition to 2 varieties belonging to 16 genera were isolated from the plant leaves and stems on glucose and cellulose-Czapek's agar at 28°C. The most frequently encountered fungal species on the two types of media were: *Aspergillus niger, A. terreus* var. *africanus, A. terreus* var. *aureus,, Cochliobolus spicifer, Emericella nidulans , Fusarium dimerum* and *Penicillium camembertii.* Data shown in Table (1). The results agreed with that obtained by Abou Donia, 2008. That found *Aspergillus*, *Fusarium* and *Penicillium* genera were more frequently detected than other genera of fungi on spices and medicinal plants samples. *Aspergillus* spp. was found in all examined medicinal plant samples under investigation. Bungo *et al*., 2006. reported that the predominant mycoflora on ninety-one samples of medicinal plants was distributed in 10 genera. From these 89.9% of the isolates corresponded to genera *Aspergillus and Penicillium*. Moharram *et al*. (1989) and Regina and Raman, (1993) reported that the fungi of *Aspergillus* spp., *Penicillium* spp, *Rhizopus* spp. and *Fusarium* spp. are contaminated (anise, cumin, coriander, carawy and fennel) which the most important medicinal and aromatic seeds in Egypt and in the world.

Antimicrobial Activity

The aqueous extract of *C. schoenanthus* L. Sprengel showed antimicrobial activity against the tested fungi and bacteria while *F.dimerum, U.botrytis, C.albicans, C.tropicalis, E.floccosum* and *M.canis* were tolerant to Halfa-bar aqueous extracts. Results showed in figure (1). The organic extracts (Methanol, Ethylacetate and N-butanol) were more effective than

Table.1 Average total counts , maximum values(calculated per g dry leaves and stems in all samples), number of cases of isolation (NCI, out of 20 samples) and occurrence remarks (OR) of fungal genera and species recovered from 20 samples of *Cymbopogon schoenanthus* L. Sprengel leaves and stems on glucose-and cellulose-Czapek'sagar at 28 ^{o}C

Genera and specie	Glucose		Cellulose	
	ATC±SD (MV)	NCI&OR	ATC±SD (MV)	NCI&OR
Acremonium	125 ± 1.892969 (4)	3 L	50 ±0.57735 (1)	2 R
A.fusidiodes	25000± 0.5 (1)	1 R	-	-
A.kiliense	125000± 1.258306 (3)	4 L	-	-
A.rutilum	-	-	50000 ±0.57735 (1)	2 R
A.strictum	100000± 2 (4)	2 R	125000 ±1.5 (3)	1 R
Alternaria	25000± 0.5 (1)	1 R	-	-
A.alternata	25000± 0.5 (1)	1 R	-	-
Aspergillus	14425000 ±19.20069 (160)	19 H	11650000±14.34108 (135)	18 H
A.carenus	25000± 0.5 (1)	1 R	-	-
A.flavus	150000± 1.290994 (3)	3 L	100000± 0 (1)	3 L
A.fumigatus	1425000± 7.274384 (21)	3 L	1100000± 4.320494 (15)	3 L
A.granulosus	25000± 0.5 (1)	1 R		
A.niger	3700000± 6.055301 (41)	15 H	3025000± 5.439056 (38)	14 H
A.sydowii	75000± 0.957427 (2)	2 R	50000±0.57735 (1)	2 R
A.terreus var. *africanus*	6750000± 9.848858 (80)	17 H	1725000±2.872281 (19)	7 M
A.terreus var. *aureus*	2275000± 5.619905 (29)	10 H	5650000± 9.146948 (63)	14 H
Cochliobolus	775000 ±2.5 (11)	13 H	750000 ±7.767453 (19)	14 H
C.lunatus	-	-	75000± 0.957427 (2)	2 R
C.spicifer	775000 ±2.5 (11)	13 H	675000± 7.632169 (18)	12 H
Coleophoma cylindrospora	-	-	25000± 0.5 (1)	1 R
Cladosporium	-	-	25000± 0.5 (1)	1 R
C.sphaerospermum	-	-	25000± 0.5 (1)	1 R
Curvularia	50000 ±1 (2)	2 R	75000± 0.95742 (2)	3 L
C.ovoidea	50000 ±1 (2)	2 R	50000± 0.57735 (1)	2 R
C.pallescens	-	-	25000 ±0.5 (1)	1 R

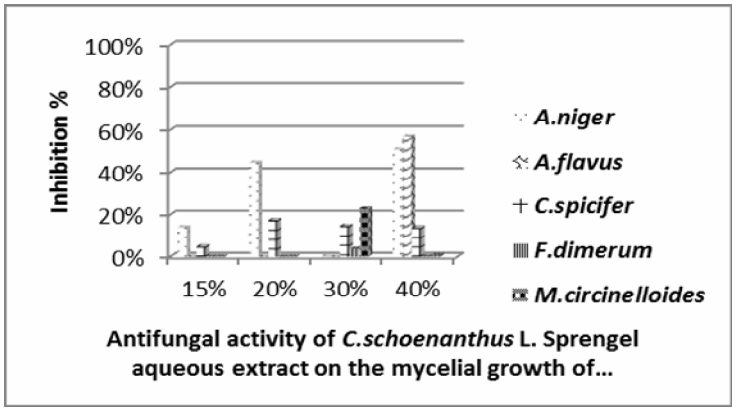
Table (1) : Cont.

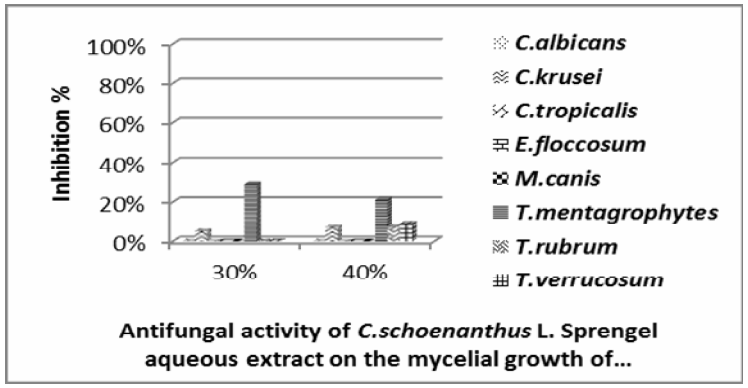
Genera and species	Glucose		Cellulose	
	ATC±SD (MV)	NCI&OR	ATC±SD (MV)	NCI&OR
Emericella	475000 ±1.5 (6)	6 M	525000± 4.787136 (12)	9 M
E.nidulans	475000 ±1.5 (6)	6 M	525000± 4.787136 (12)	9 M
Fusarium	1050000± 7.593857 (21)	12 H	1950000±6.027714 (28)	8 M
F.dimerum	1050000± 7.593857 (21)	12 H	1950000±6.027714 (28)	8 M
Gibberella	75000± 0.957427 (2)	2 R	75000± 0.5 (1)	3 L
G.fujikuroi	50000± 0.57735 (1)	1 R	50000± 0.57735 (1)	2 R
G.tricincta	25000 ±0.5 (1)	1 R	25000± 0.5 (1)	1 R
Hypocrea	25000± 0.5 (1)	1 R	-	-
H.rufa	25000± 0.5 (1)	1 R	-	-
Mucor	-	-	25000 ±0.5 (1)	1 R
M.circinelloides	-	-	25000 ±0.5 (1)	1 R
Penicillium	850000 ±3.696846 (14)	13 H	145000± 4.99166 (22)	11 H
P.camembertii	450000± 1.290994 (6)	8 M	525000± 2.886751 (8)	4 L
P.chrysogenum	25000± 0.5 (1)	1 R	-	-
P.citrinum	75000± 0.957427 (2)	1 R	-	-
P.corylophilum	25000± 0.5 (1)	1 R	125000 ±1.290994 (3)	4 L
P.dendriticum	50000± 1 (2)	1 R	-	-
P.duclauxii	-	-	325000± 0.5 (6)	4 L
P.funiclosum	75000± 1.5 (3)	2 R	25000± 0.5 (1)	1 R
P.oxalicum	100000 ±1.414214 (3)	2 R	25000± 0.5 (1)	1 R
P.puberulum	25000 ±0.5 (1)	1 R	75000± 1.5 (3)	1 R
P.viridicatum	25000± 0.5 (1)	1 R	-	-
P.waksmani	-	-	300000± 1.154701 (4)	1 R
Phoma	475000± 1.258306 (6)	8 M	375000± 2.380476 (5)	6 M
P.eupyrena			50000 ±0.57735 (1)	1 R
P.exigua	75000± 0.957427 (2)	2 R	175000±1 (2)	3 L
P.medicaginis	400000± 1.825742 (6)	8 M	150000 ±1 (2)	3 L

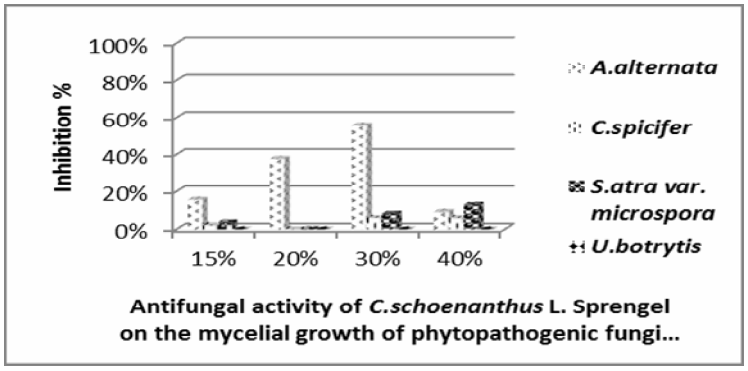
Table (1): Cont.

Scytalidium	25000± 0.5 (1)	1 R	-	-
S.lignicola	25000± 0.5 (1)	1 R	-	-
Sterile mycelia(white & dark colour)	325000± 2.061553 (5)	7 M	125000 ± 0.5 (2	5 M
Gross total count	18825000±14.77329		17225000±11.34313	
Number of genera	13		13	
Number of species	29+2var.		27+2var.	

Fig. 1 Antimicrobial activity of *Cymbopogon schoenanthus* L. Sprengel aqueous extract on the growth of tested fungi and bacteria at different concentrations**.**







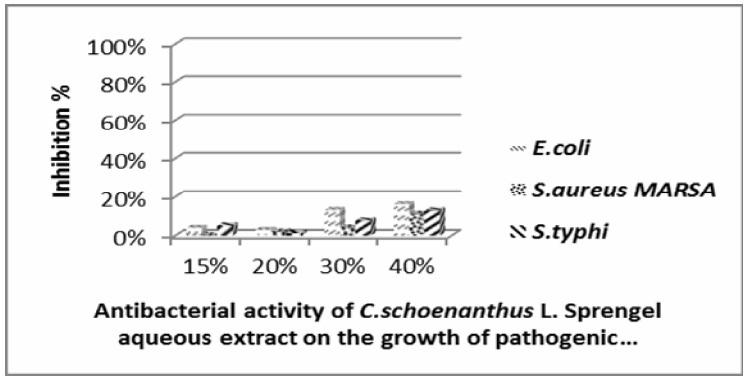
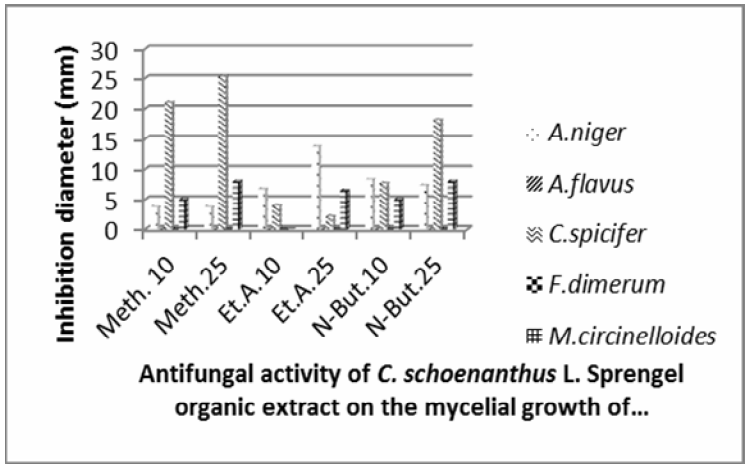
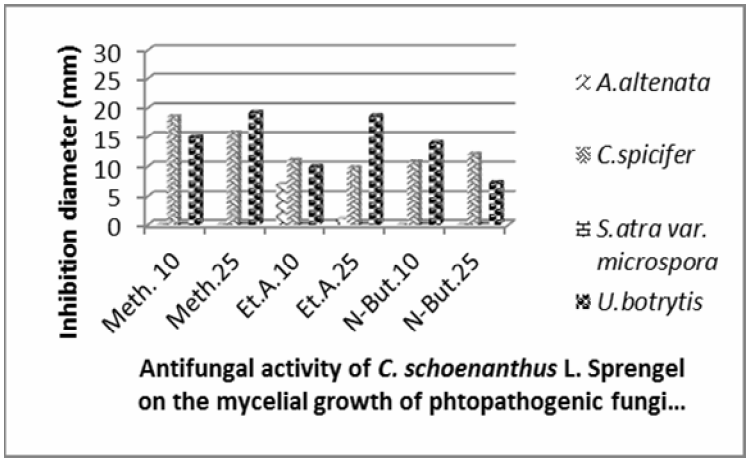
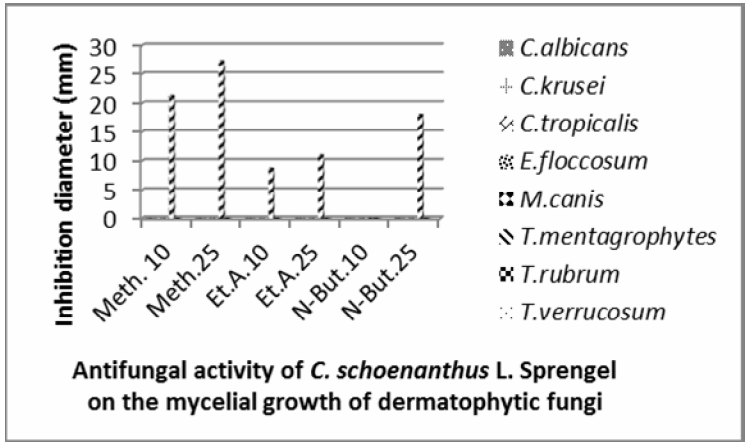
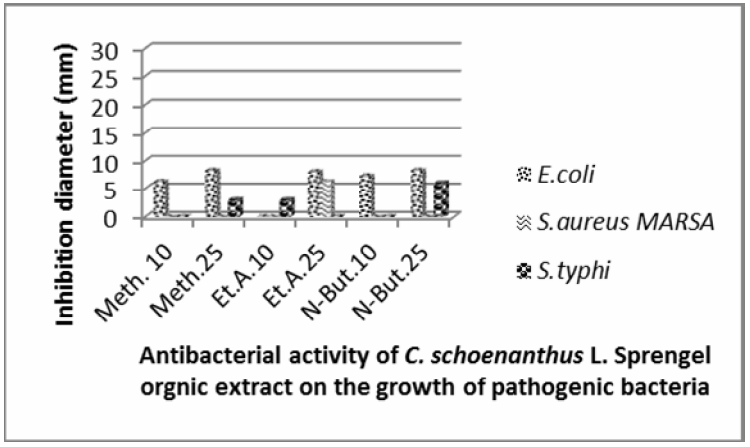


Fig.2 Antimicrobial activity of *C. schoenanthus* L. Sprengel organic extract on the growth of tested fungi and bacteria









the aqueous extract where it showed high antifungal activity against the tested fungi but *A.flavus, F.dimerum, S.atra var.microspora, C.albicans, C.tropicalis, C.krusei, E.floccosum, M.canis, T.rubrum* and *T.verrucosum* were tolerant to these fractions. Organic extraction of Halfa-bar showed high antibacterial activity against all the tested pathogenic bacteria (*S.aureus* MARSA, *S.typhi* and *E.coli*). Results showed in figures (2). The present results are inconformity with other studies, where halfa bar was shown to be the most potent among many tested plant extracts against *Fusarium verticillioides* and *Aspergillus flavus* (El-Assuity *et al.*, 2006). Proximadiol, 5α-hydroxy-β-eudesmol, 1β-hydroxy-β-eudesmol, 1β-hydroxy-α-eudesmol, 5α-hydroperoxy-β-eudesmol and 7α, 11-dihydroxycadin-10(14)-ene were isolated from the unsaponifiable fraction of extract of *Cymbopogon proximus*. These components were shown to be powerful antimicrobial agents (El-Askary *et al*., 2003). Fawzi *et al*., 2009 found that halfa bar was the most efficient among many tested plant extracts against *Alternaria alternata* and *Fusarium oxysporum* and it might be a promising material to control the studied fungi.

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