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Research Article

Antifungal activity of *Ficus sycomorus* L. extracts against dermatophytes and other associated fungi isolated from Camels ringworm lesions

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Abstract

Ficus sycomorus antifungal efficacy against some dermatophytes and other associated fungi isolated from Camels ringworm lesions was investigated in this study. Camel shows a high importance part of the national livestock population in Egypt including transportation in the desert and as a source of milk and meat. In this study, a total of 26 fungal species appertaining to 13 genera were recovered from camel hair and skin lesion samples. The most dominant fungal genera were *Aspergillus* followed by *Trichophyton* and *Microsporum*. The medicinal plant, *Ficus sycomorus* methanol fractions 20% and 40% of root, stem-bark and leaf showed a promised antifungal activity against isolated fungi. Whereas, all tested dermatophytic fungi (*Trichophyton mentagrophytes* var. *erinacei*, *Microsporum audouinii* and *M. gypsum*) and the cycloxamide-resistance fungi (*Fennellia nivea, Choaenophora cucurbitarum* , *Aspergillus carneus* and *A. fumigatus*) showed high sensitivity to 40% methanol fraction except *Fennellia nivea* which showed the highest sensitivity to the stem-bark (20% methanol fraction). In the HPLC analysis for flavonoids content of the methanolic fractions (20% and 40%) of the different plant part (stem-bark, leaf and fruit), twenty-one compounds were detected. *F. sycomorus* plant extracts possess a potent antifungal effect, especially with dermatophytes.

Key words: *Ficus sycomorus*, Flavonoids, Camels, Dermatophytes, wringworm, antifungal

Introduction

Dermatological problems in domestic animals are mainly caused by dermatophytes (Nweze, 2011). Some organisms are true parasites like filamentous fungi which can use keratin as a source of carbon and cause a lesion on the skin. Dermatophytosis is caused by fungi of the genera *Microsporum*, *Trichophyton*, and *Epidermophyton* (Nweze, 2010). Keratinopathogenic fungi can cause infections in horses resulting in conditions such as abnormalities of the hoof (Apprich et al., 2006). Others fungi like *Aspergillus* exploit the infected skin to control one of the body organs and called a secondary cutaneous infection (Bonduel, 2001).

The one-humped dromedary camel shows a high importance part of the national livestock population of numerous countries in arid and semi-arid regions of Asia and Africa whereas;

Bedouin use them as a mean of transportation in the desert and as a source of milk and meat (Al-Tarazi, 2001; Babeker, et al., 2011). The diseases of Camelidae are considered difficult to deal with because of having very similar and non-specific signs. They can be dealt with by both therapy and chemoprophylaxis (Volpato, et al., 2015). Fungi seem to have a certain pathogenic role in camels especially ringworm like *Trichophton mentagrophytes, T. verrucosum, T. schoenleinii, Microsporum gypseum* and *Penicillium vinaceum* (Georg, 1960; Otcenasek, 1978; Zaror, et al., 1986; Abo El Foutah, et al., 2012).

To address this problem, a lot of modern drugs have been isolated or derived from a natural source of medicinal plants, based on their use in traditional medicine (Egharevba and Kunle, 2010). The antimicrobial agent of these plants may inhibit microbes by a mechanism different from that of antibiotic. Thus, secondary plant metabolites may contribute to the treatment of resistant microbial pathogens (Srivastava, et al., 1996; Chandra, 2013).

The genus *Ficus* is considered an important member of the family Moraceae which have densely populated species in a number of all plant genera (Lansky and Paavilainen, 2011). *Ficus* plant extracts characterized by the presence of gallic tannins, saponins, reducing sugars, alkaloids and flavone aglycone, which have an inhibitory effect on both smooth and skeletal muscles contractions and contain essential constituents for pharmacological activities (Hassan, 2005; Kubmarawa, et al., 2007; Sandabe, et al., 2006). *Ficus sycomorus* is used for treating skin inflammations and warts like ringworms (Burrows and Burrows, 2003).

In this study the medicinal plant, *Ficus sycomorus* was selected to study its ability to renitency fungi isolated from ringworm lesion of camels.

Materials and Methods

Camels and Samples collection

In this study, Fifty-eight camels were studied with ages that ranged from 6 to 24 months old. Camels were selected from various farms in Aswan Governorate, (Daraw city) 23° 35′ 24″ N, 32° 49′ 12″ E. Seventy specimens of hair and skin scrapings were taken from the belly, neck and shoulders of camels with skin lesions suspected to be dermatophytes infection. All samples were collected in a sufficient amount by scrapings of the active edge of the affected skin from lesions suspected to be ringworm by sterile scalpel, after cleaning the lesions by moist gauze in 70% ethyl alcohol. Hair near the advancing border of the lesion was epilated from its root by forceps. Samples were cultured onto Sabouraud dextrose agar (SDA) containing chloramphenicol and cycloheximide. Two techniques have been done for isolation of dermatophytes; the first one (A-technique) by adding the samples immediately in the field on the surface of sterilized SDA media and the second technique (B-technique) has been done in the lab by purring warm sterilized SDA media in sterilized plated containing samples and mixing well then kept all plates to solidify. Three replicas have been made for each sample with each technique. The inoculated plates were incubated at 37ºC for 3-4 weeks. Identification of isolated fungi was made according to Frey, et al., (1979) and Moubasher, (1993). Camel's disease was identified by the help of Dr Ahmed Abdel-Kareem (Veterinary Quarantine Director in Aswan Government)

Plant material

Plant materials of *Ficus sycomorus* (Stem-bark, leaves and fruits) were collected from two sites at Aswan Governorate; The Botanical garden Island in the middle River Nile, and Gharb Sohail

Island in western bank of River Nile. The plant was identified according to Täckholm, (1974), and the Plant materials were dried separately in a dry dark place and grounded to a powder using an electric grinder.

Plant Extraction and Fractionation

Powders of dried different parts of the plant (250 g of each) were extracted separately using MeOH 80% by maceration until exhaustion to get methanol crude extract (Hamed, et al., 2011). Three grams of the crude extract of each part were dissolved separately in a small quantity of H_2O and loaded on a water preconditioned short C18 column (6x10 cm, LiChroprep_ RP-18, granules diameter 40-60 μm, Merck) and eluted with H_2O to remove sugars, 20%, 40% MeOH to obtain flavonoids (Mahalel, 2015). Flavonoids contents of 20% and 40% MeOH fractions of stem-bark, leaves and fruits were analyzed by high performance liquid chromatography (HPLC).

Antifungal activity screening

The antifungal activity of *F. sycomorus* stem-bark, leaf and fruit of 20% and 40% methanol fractions (1 mg/plate) was estimated against the isolated dermatophytic fungi on SDA agar medium and incubated at 37°C. The diameters of cultures were measured in control dishes and in the treated plates (Ismail, et al., 2016). The inhibition percentage of mycelial growth was calculated (Singh and Tripathi, 1999).

Results and Discussion

In this study, a total of 26 fungal species and one variety appertaining to 13 genera were recovered from camel hair and skin lesion samples (Fig. 1). These fungi included dermatophytes such as *Trichophyton*, *Microsporum*, and *Malassezia*, as well as true keratinophilic and cycloheximide-resistant fungal species belonging to *Aspergillus, Basidiobolus, Botryotrichum, Chrysosporium, Cladosporium, Choanephora, Fennelia, Gliocladium, pencillium,* and *Ulocladium* were also recorded.

The most dominant genus was *Aspergillus* (62 colonies, 44.9% of total counts and 88.6% of all samples) which represented by 9 species (Fig. 1 & Fig. 2).

Previous studies showed that *Aspergillus* was the second most frequent genus on the hair of goat and camel and was encountered in 53.3% and 43.3% of the samples respectively (Bagy and Abdel-Hafez, 1985). Also, *Aspergillus* spp. was the most common non-dermatophytes mould from the infected skin (Shujat, et al., 2014; Vyas, et al., 2013). In this study, *A. terreus* was associated with the dermatophytes *Trichophyton* and *Microsporum* in the severe skin infections which confirm the previous study that infected skin serves as a portal of entry for *Aspergillus* organism (Hashmi et al., 2007).

In the present study, the second dominant genus was a dermatophytic fungus *Tricophyton* emerging 22 colonies (16% and 31.4% of total counts and all samples respectively) and represented by four species (Fig.1 & Fig. 2). The most common genus in camel's lesion skin was the genus *Trichophyton* which appeared to be the main cause of ringworm camels lesions (Mahmoud, 1993; Al-Rawashdeh, et al., 2000; Abo El Foutah, et al., 2012; Almuzainia, et al., 2014; Pal, 2016) and in human which had typical dermatophytosis lesions (Muangkaew, et al., 2017). The third prevalent isolated fungus which is implicated as a pathogenic filamentous fungus in camels was the genus, *Microsporum* (16 colonies, 11.6% of total counts and 22.9 % of

all samples) (Fig.2) which represented by three species, *M. audouinii*, *M. canis* and *M. gypsum* (Fig. 1). *Microsporum gypseum* had been previously isolated from diseased camels (Kuttin and Beemer, 1975).

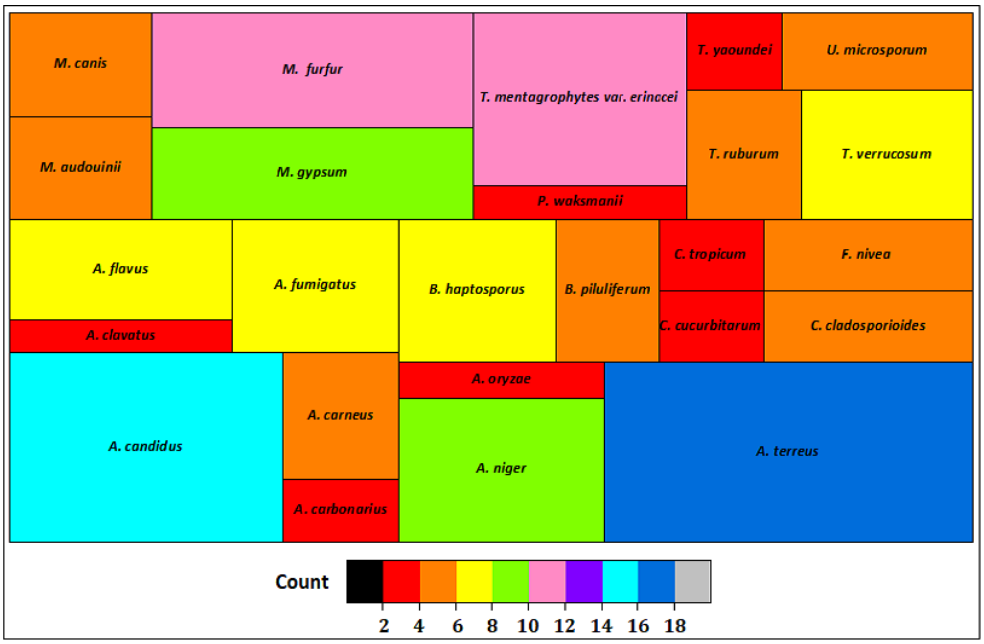

Figure 1. Treemap shows total counts of fungal species isolated from infected lesion of camels

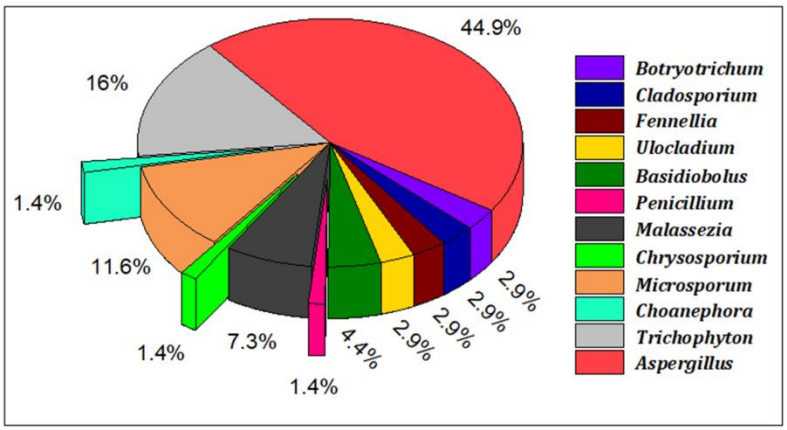

Figure 2. Percentages of fungal genera of the infected lesion of studied camels out of total counts.

A basidiomyctes fungus, *Malassezia furfur* was the next most common skin lesion fungus and emerged in 7.3% of total counts (Fig. 2) and 11.4% of all samples. *Malassezia* uses many secreted hydrolases (lipases and phopholipases C) to provide fatty acids from host lipids. In addition, this genus has historically been described as found on the skin of warm-blooded animals (White, et al., 2014). *Malassezia* was recorded in the infected skin associated with the dermatophyte *Trichyphyton* and many *Aspergillus* species that confirm the previous results as its growth is linked to vulnerability. *Basidiobolus haptosporus* recorded six colonies (4.4% of total

counts and 8.6% of all samples). *Basidiobolus* is a pathogenic zygomycetes causes a disease known as zygomycosis or subcutaneous mycoses which is generally chronic and progressive diseases. This fungus can spread slowly to contiguous tissues (Cafarchia, et al., 2013). *Choanephora cucurbitarum* is another *Mucorales* mold which is known as plant pathogen causing fruit rot but in this study, isolated from the necrotic lesion in camel. This fungus was associated with *Malassezia. Botryotrichum piluliferum, Cladosporium cladosporioides, Fenellia nivea, Gliocladium virens* and *Ulocladium microsporum* isolated as four colonies from all infected camels (3.1% of total counts (Fig. 2) and 5.7% out of all samples). *Botryotrichum piluliferum* and *Cladosporium cladosporioides* were isolated from goats hairs (Bagy and Abdel-Hafez, 1985); goat hairs and sheep wool (Awad, 2017). Both genera are known as keratinolytic fungi (Kushwaha & Agarwal, 1976; Singh, 2014). *Gliocladium virens, G. roseum G. solani, G. viride Ulocladium alternariae* and *U. chartarum* have a keratinolytic activity (Kaul & Sumbali, 1997; Ali-Shtayeh, et al., 1988 and Awad, 2017). *Chrysosporium topicum* and *Penicillium waksmanii* recorded the lowest counts (2 colonies; 1.6% of total counts and 2.9% of all samples). *Chrysosporium* have been isolated from healthy hair of camels (Shokri and Khosravi, 2011) as well as infected camels and pets (Mahmoud, 1993, Sallam and Al-Ameri, 2014). The genus *Pencillium* was isolated from dogs and cat (ringworm skin scrapings and hair) as saprophytic fungi (Aho, 1983). Mahmoud (1993) isolated *Penicillium chrysogenum, P. funiculosum, P. vadabile* as cycloheximide-resistant fungi from ringworm infected camels. Those two fungal genera could degrade keratin (Kaul and Sumbali, 1997; Ali-Shtayeh, 1988, and Singh, 2014). Interestingly, the difference in using two techniques (A and B) was clear in the incubation time whereas, B-technique incubation time was shorter (7-10 days) than A-technique (> 21 days). Also, it was interesting that *Aspergillus carbonarius, A. oryzae, Chrysosporium topicum* and *Trichophyton yaoundei* were isolated only by A-technique. In addition to late growth of *Chrysosporium topicum* was recorded (5 weeks) and by increasing the incubated temperature up to 45ºc.

Antifungal activity of *Ficus sycomorus* methanolic fractions

The antifungal activity of *F. sycomorus* stem-bark, leaf and fruit methanol extracts (1 mg/plate) of both fractions (20% and 40%) was estimated by a selected isolated dermatophytic and that cyclohexamide-resistance fungi. This activity was carried out using SDA agar medium mixed with each fraction and inoculated with each tested fungus compared with the control (without plant fraction extract).

Trichophyton is the usual causative fungus of camel ringworm (McGrane and Higgins, 1985). This dermatophytic fungus, *T. mentagrophytes* was clearly inhibited by the methanol fractions (20% and 40%). Senstivity of this fungus to 40% methanol fraction was higher than that of 20%. The highest inhibition percentage (44.7%) was recorded with 40% leaf extract followed by fruit (42.37%) and bark extract (23.73%) (Fig. 3).

In the microscopic examination, swollen in hyphae, chlamydospores and many spiral hyphae were recorded in the fungus treated with the fraction of different plant parts of *Ficus sycomorus* compared to the control. Also, ethanolic leaf extract of *F. sycomorus* inhibited the growth of *T. mentagrophytes* (Adeshina et al., 2009). The activities of the methanol extract were higher when compared with the aqueous extract (Shinkafi and Manga, 2011). The reason for this slight difference may be attributed to the solubility level of the phytoconstituents in the extracting solvents. It means that the methanol dissolved more of the active ingredients than aqueous

solvent (Shivakumar and Vidyasagar, 2015). The major content of methanolic compounds is the flavenoids whereas quercetin is the most antimicrobial active comounds (Bitencourt et al., 2014). The dermatophytic fungus, *Microsporum audouinii* was clearly inhibited by the methanol fractions (20% and 40%). The sensitivity of this fungus to 40% methanol fraction was higher than that of 20% specially in leaves extract. The highest inhibition percentage of this fungus (44.7%) was recorded with 40% leaf extract. The inhibition percentage of fruit and stem-bark extracts (20% and 40%) was almost similar (Fig. 3).

The microscopic examination of treated fungus showed modification compared to the control. Aggregation of hyphae and numerous chlamydospores were recognized with all treated fungal colonies and stained hyphae and chlamydospores were observed with 20% leaves and fruits extracts. The clinical dermatophytes *Microsporum* was significantly inhibited by different concentrations of *Ficus exasoerata* ethanol extract (Mbakwem-Aniebo, et al., 2012).

The cyclohexamide resistant fungus *Fennellia nivea* showed bizarre results. Whereas, the highest inhibition percentage (42.9% and 35%) was recorded with 20% stem-bark and 40% fruits respectively. In the microscopic examination at the third incubation day, the fungal culture was formed many hull cells by the fungus treated with 20% methanol extract of stem-bark comparing with that of control and other treated fungi. Hull cells in the untreated and treated culture other than 20% stem-bark methanol fraction was recognized at 7-10 days. Interestingly, 20% and 40% methanol extract from fruits caused a condenstaion of the vegetative mycellium with less number of conidial heads and conidia. Methanol extract (40%) of all plant parts caused excess staining of conidial head which was already in fewer counts comparing with untreated culture. In addition to stem-bark methanol extraction (40%) enhanced the fungus on the third day of the incubation to form a limited number of asci which was not recgonized in the untreated culture. Thus, *F. nivea* showed sensitivity to *F. sycomorus* methanol fraction compounds especially those of stem-bark.
Choanephora cucurbitarum is known as plant pathogen but it was isolated in this study from camel's infected skin. This fungus was associated with the dermatophyte *Malassizia furfur* and the purification of these two fungal species was a very hard task. Airborne spores or spores in the surrounded tree may fall into the specimen or onto the culture medium during sampling. It could also come from camels' food products which explain their presence in clinical specimens.

Treating the fungal culture with *F. sycomorus* stem-bark and fruits methanol fractions (40%) inhibited the fungus growth by 42.5% and 72.5%.

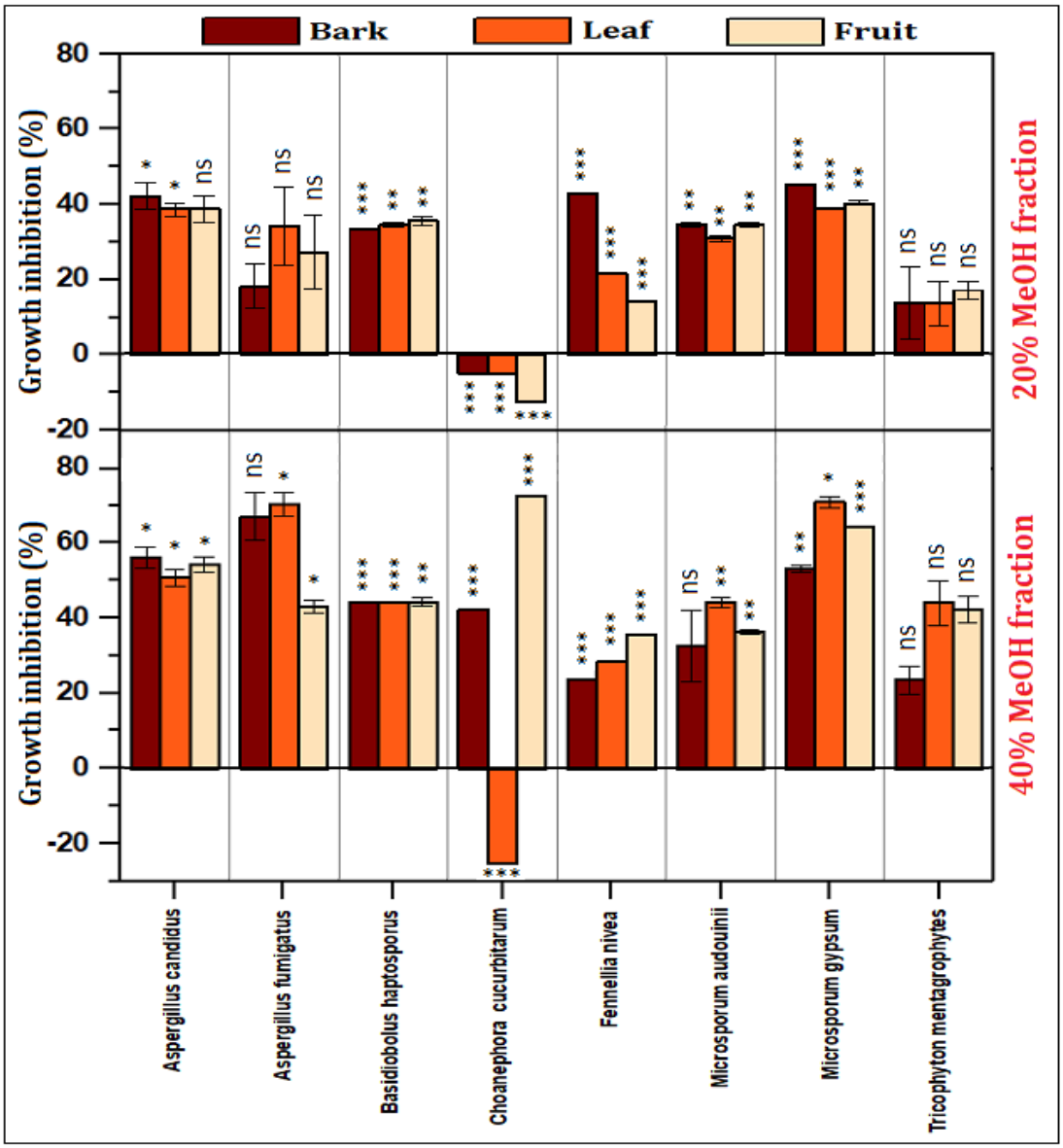

Figure 3. The Antifungal activity of *Ficus sycomorus* stem-bark, leaf and fruits methanol fractions (20% and 40%) on selected dermatophytes and non-dermatophytes isolated fungi

Another cyclohexamide-resistant fungus known as *Aspergillus candidus* has been studied. This fungus showed sensitivity to all plant extract with relative ratios. The inhibition percentage of 20% methanol extracted from stem-bark, leaves and fruit was 42.1%, 38.6% and 38.6% respectively (Fig. 3). While 40% methanol extract of different plant parts showed little high inhibition percentage (56.1%, 50.9% and 54.4%) extracted from stem-bark, leaves and fruits respectively. With regard to colony appearance, in control, it was grey color while in all treated cultures were white color. It seems that plant methanol fractions, 20% and 40% inhibited the fungal sporulation by fungi. The vegetative mycelium was condensed comparing with control in the examined slide, especially in the stem-bark methanol fraction. In addition to swollen hyphae with a brown stain which recommended being melanin. Several *Aspergillus* species are considered as dermatophytic including: *A. flavus, A. fumigatus, A. niger, A. versicolor, A. terreus*

(Torres- Rodrı´guez, et al., 1998; Tosti & Piraccini, 1998; Gupta, et al., 2003). *Aspergillus candidus* is a rare cause agent of onychomycosis. Recently *A. candidus* has been demonstrated as an agent of toenail onychomycosis (Ahmadi, et al., 2012).

The last cyclohexamide-resistant fungus, *Aspergillus fumigatus* exploits the presence of wounds in camel's skin to his way to the respiratory tract (Bonduel, 2001). Stem-bark, leaves and fruits compound extracted in 40% methanol showed effective growth inhibition of this pathogen (67.1%, 70.5%, 43.2%) respectively (Fig. 3). The other test of methanol fraction (20%) showed less potent result than 40% methanol fraction whereas stem-bark, leaves and fruits recorded 18.2%, 34.1% and 27.3% inhibition percentage for *A. fumigatus* respectively. Nicely, the distinctive blue green color of *A. fumigatus* colony was vanished in all treated cultures. The Microscopic examination showed swollen of hyphae and few germinating conidia in the base of hyphae with stem-bark methanol fraction (40%) and enlarged hyphae in 20% methanol fraction. In addition to condensation of mycelium and sparse sporulation were recorded in all treated samples. In a previous study, micafungin has an effect on the morphology of growing hyphae of *A. fumigatus*, the hyphal growth of cultures was inhibited and aberrant morphology of hyphae induced when incubated with micafungin. Characteristic morphological changes of hyphae were frequent formation of branches on hyphal lateral walls, disruption of the tips of both hyphae and branches, and collapse or crushing of the whole hyphae (Nishiyama, et al., 2005). As in many other fungi, *A. fumigatus* contains 1,3-b-D-glucan in the cell wall as the major skeletal component (Bernard and Latge, 2001). Therefore, it is the rational speculation that inhibition by micafungin of fungal 1,3-b-D-glucan synthesis can induce abortive cell-wall formation in susceptible fungi (Nishiyama, et al., 2005). Similar morphological changes were reported also by Chiou, et al., (2001).

The hierarchical cluster analysis dendrogram of the sensitivity of dermatophytes and non-dermatophytes showed that *A. fumigatus*, *A. candidus* and *M. gypsum* had similar sensitivity to *Ficus sycomorus* methanol fractions while the remaining fungal species except *Choanephora cucurbitarum* recorded similarity in sensitivity to this plant fraction (Fig. 4).

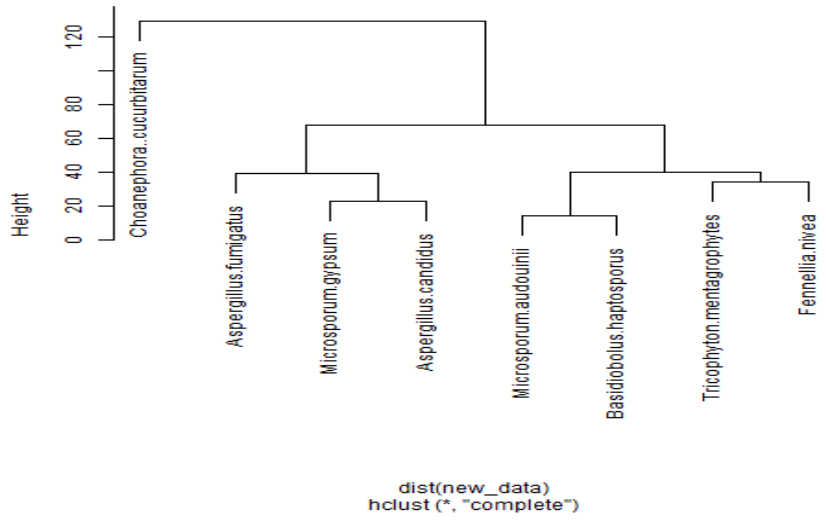

Figure 4. Dendrogram of the hierarchical cluster analysis for antifungal activity against some dermatopytes and non-dermatophytes isolated fungi to *Ficus sycomorus* methanol fractions

HPLC analysis of flavonoids contents of 20% and 40% methanol fractions of *Ficus sycomorus* Plant parts (stem-bark, leaf and fruits)

One of the main aims of this study was to evaluate the antifungal efficacy of flavonoidal compounds that present in methanolic fractions (20% and 40%) of *Ficus sycomorus* tree as leaves, bark and fruits. Flavonoids content of 20% and 40% fractions of *Ficus sycomorus* was analyzed using HPLC analysis. Results represented in Figure 5, showed that twenty-one compounds were detected namely; Hesperidin, Hespertin, Narengin, Naringenin, Acacetin, luteo.6-arbinose 8 glucose, A pig. 7-glucose, Quercetin-3-O-Glucoside, Quercetrin, Quercetin, Apigenin, A pig.6 - rhamnose 8 glocose, A pig.6- glucose 8-rhamnose, A pig. 7-O-neohespiroside, luteo. 7-glucose, luteo.6-glucose 8 arbinose, Rutin, Rhamnetin, RosmarinicKaempferol, and Kaemp.3, 7-dirhamoside. The major compounds were Hesperidin, Narengin, Acacetin, luteo.6-arbinose 8 glucose and A pig 7-glucose.

Flavonoids are considered one of the most common polyphenols, and they exhibit interesting and beneficial medicinal effects on human health (Harborne and Williams, 2000). Genus *Ficus* characterized by the presence of gallic tannins, saponins, reducing sugars, alkaloids and flavone aglycones (Hassan, 2005; Kubmarawa, et al., 2007). Flavonoids have an antifungal activity that inhibits spore germination of plant pathogens. They have been proposed to be used against fungal pathogens of human. However, the antimicrobial efficacy of flavonoids varies depending on their chemical structure and the strain of microorganism (Iranshahi, et al., 2015).

The data of the current investigation exhibited a remarkable effect of flavonoids compounds recorded in 20 % and 40% fractions of the plant under investigation against the isolated fungi from diseased camels. Hesperidin recorded the highest concentration in both 20% and 40% fractions (Fig. 5 and Fig. 6). Furthermore, fruits fraction (20%) contain the highest concentration (3898.6 µg/gm followed by bark 1066.33 µg/gm and leaves 1449 µg/g. In contrast, 40% MeOH fraction of bark recorded the highest concentration of hesperidin (818.14 µg/gm) followed by leaves and fruits (604.26 µg/gm and 552.8 µg/gm) respectively. Hesperidin (Hsd) molecule is composed of an aglycone unit, namely hesperetin (Hst), and a disaccharide, rutinose (Garg, et al., 2001). Both, Hesperidin and its aglycon, hesperetin have a lot of biological properties like, antioxidant, anti-inflammatory, anticancer, antimicrobial, and so it has protective effect against toxicity (Sun, et al., 2013; Roohbakhsh, et al., 2014). In this study, the second highest content of the *Ficus* plant methanol fractions was Narengin (Fig. 5 & Fig. 6). Accordingly, 20% methanol extracts of fruits, stem-bark and leaves contain 224.98 µg/gm, 194.13 µg/gm and 175.05 µg/gm respectively. Comparing with 20% methanol fraction of different plant parts, narengin content of 40% MeOH extract was low whereas, fruits contain only 127.09 µg/gm followed by leaves (86.86 µg/gm) and then stem-bark as the lowest concentration of Narengin (72.13 µg/gm). According to Tsui, et al., (2008), naringin possesses significant antimicrobial properties on periodontal pathogens *in vitro*.

Acacetin is an O-methylated flavone found in *Robinia pseudoacacia*, *Turnera diffusa*, *Betula pendula* (Adcock, 2002). In this study, 20% MeOH fraction of fruits contain the highest concentration of acacetin (241.72 µg/gm) among all parts of *Ficus sycomorus* followed by leaves (165.82 µg/gm) and stem-bark (127.79 µg/gm). In contrast of naringin, 40% MeOH fractions contain a higher amount of acacetin than 20% methanol fractions whereas, the leaves extract contain 794.52 µg/gm acacetin followed by fruits and bark 157.09 µg/gm and 142.51 µg/gm respectively (Fig. 5 & Fig. 6).

Luteo 6-arbinose 8 glucose content in 20% MeOH plant extract was higher than that in 40% MeOH fraction (Fig. 5 & Fig. 6). In 20% MeOH extract, the highest content of luteo –arbinose 8 glucose was recorded in fruits (1960.88 µg/gm and 969.43 µg/gm) in 20% and 40% respectively. Luteo 6-arbinose 8 glucose is a derivative of Luteolin where this compound is a naturally occurring flavonoid and abundant in our daily dietary intake. It exhibits a wide spectrum of pharmacological properties (Brown, 1980).

No significant difference of A pig.6- glucose 8-rhamnose content in 20% methanol fraction and 40% methanol fraction of *Ficus* plant parts (Fig.5 and Fig. 6). Apigenin, known also as 4', 5, 7-Trihydroxyflavone is a natural phytochemical product of flavones species which is present in fruits and vegetables and is common in apple, parsley, celery, rosemary, oregano, thyme, basil, coriander, chamomile, cloves, lemon balm, artichokes, and spinach and present a chemo-preventive potential against skin cancer (Gupta, et al., 2001).

Quercetin and its glyconerutin (quercetin-3- O-glucoside) were detected in both methanol fractions. The glucoside form was higher than quercetin

Quercetin and Rutin that detected in both 20 % and 40% methanol fractions of tee plant are polyphenolic flavonoids; stand out among the natural products, through many studies.

Most of the antimicrobial studies carried out on it have reported that quercetin has activity on *Clostridium botulinum* and *Staphylococcus aureus*, and in vitro antibacterial activity against the periodontal pathogens, *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* (Geoghegan, et al., 2010). Rutin was shown to be more effective against arthritis caused by *Candida albicans* (Han, 2009). Recently, according to Oliveira, et al., (2016), quercetin and rutin were recorded as potential antifungal agents against *Cryptococcus spp*.

The remaining compounds (kaempferol, rhamnetin, agipenin, rosmarinic and kaemp-3, 7-dirhamoside) were present in low concentrations in all plant parts of both methanol fractions 20% and 40% (Fig. 5 & Fig. 6).

The result obtained in the current study attributed the antifungal effect to the major flavonoidal compounds present in different fractions of the plant according to the HPLC analysis as well as the compounds that characterized by its antifungal activity according to previous studies. Furthermore, studies are needed to detect the antifungal efficacy of these flavonoidal compounds separately from each other. That most probably antifungal property of these fractions is resulted from synergy of all these compounds.

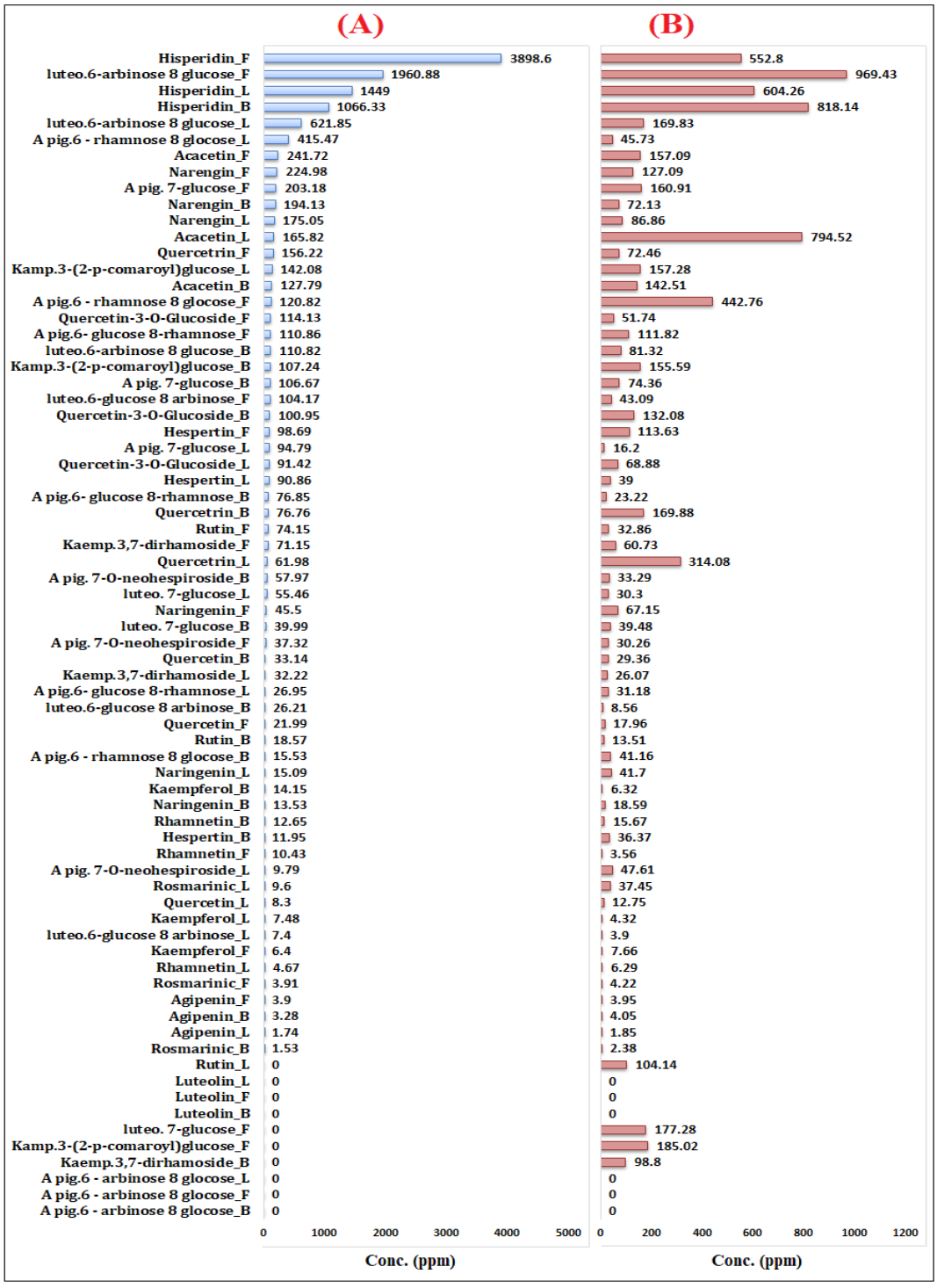

Figure 5. HPLC analysis of flavonoid concentrations (ppm) in 20% (A) and 40% (B) aqueous methanolic fractions of *Ficus sycomorus* stem-bark (B), fruits (F) and leaves (L).

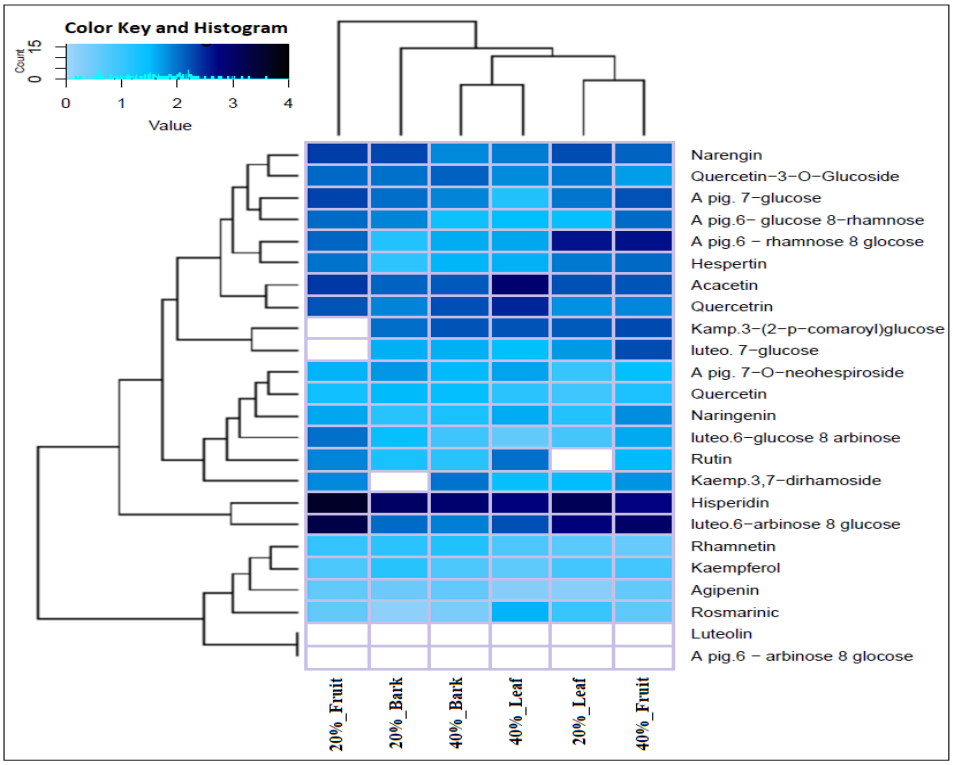

Figure 6. Heatmap clustring of total flavonids of *Ficus sycomorus* plant parts (stem-bark; B, leaf;L, and flower; F) in both methanol fractions (20% and 40%).

Conclusion

Interestingly, these results clarified that all test dermatophytic fungi (*Trichophyton mentagrophytes* var. *erinacei*, *Microsporum audouinii* and *M. gypsum*) showed high sensitivity to 40% methanol fraction of leaves followed by fruits. While the cycloxamide-resistant fungi (*Fennellia nivea*, *Choaenophora cucurbitarum* , *Aspergillus carneus* and *A. fumigatus*) had a variable reaction to both plant methanol fractions (20% and 40%) and different parts of *F. sycomorus* plant (stem-bark, leaves and fruits) but generally, 40% methanol fraction had a greater potent effect than 20% methanol fraction except *F. nivea* which showed the highest sensitivity to the stem-bark (20% methanol fraction). Thus, *F. sycomorus* plant posses a potent antimicrobial effect especially on dermatophytes. The synergy between flavonoides content of the plant methanol fraction is very important to control dermatophytic fungi, so far.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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