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Evaluation of Efficiency of Some Medicinal Plant Extracts on Dermatophytes Isolated in Saudi Arabia

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In *vitro* susceptibility testing of forty five isolated dermatophytes against four crude extracts of henna (*Lawsonia inermis*), handal (*Citrullus colocynthis*), indian fig (*Opuntia ficus-indica*) and pomegranate (*Punica granatum*) were evaluated. A minimum Inhibitory Concentration of the crude plant extracts required to inhibit the growth of 50 % (MIC_{50}) and of 90 % (MIC_{90}) of dermatophytes were estimated by a broth macrodilution method [National Committee for Clinical Laboratory Standards for filamentous fungi NCCLS, 2000 (M38-P)]. The results demonstrated that extract of pomegranate was the highest activity as antifungal against for isolated dermatophytes. The mean of MIC_{50} and MIC_{90} were 0.8 to 1.6 mg/ml, respectively. Among of the crude extracts, extract of prickly pear was the least activity. *M. canis and T. rubrum* were the more sensitivity to plant extracts whereas *T. mentagrophytes and T. verrucosum* were the more resistant than other isolates. These results concluded that all crude extracts showed fungicidal activity against isolated dermatophytes. This study suggested that extracts of those plants could be used as a natural biological agents for a treatment of dermatophytosis.

Key words: Dermatophytes; Medicinal plants; NCCLS.

Dermatophytes belong to three genera (*Trichophyton*, *Epidermaphyton* and *Microsporum*) which cause superficial infections skin, nail and hair in humans and animals. These organisms have ability to metabolize a keratinous materials in human and animal body (Weitzman and Summerbell, 1995; Kane and Summerbell, 1999; Hay, 2000). A dermatophytosis have increased in the last decades. Espinel-Ingroff *et al.,* (1991) reported that 80% of the skin lesions of human transmit from animals and are often recalcitrant to antifungal agents. Recently a several drugs have used to treatment of dematophytosis, such as itraconazole and posaconazole that have potent activity against systemic fungal infections (Pfaller *et al.,* 1997). Barchies *et al.,* (2001) found that posaconazole a more activity against genus *Microsporum* than itraconazole and investigated using of these drugs for treatment of skin fungal diseases. Numerous researches proved that there a several plants were used to treatment of dermatophytosis such as *Euphorbia thymifolia* (Sharkar, 1986), crude extract garlic (Sharma *et al.,*2011) and Henna (Bosoglu *et al.,* 1998). The medical plant have many advantages such as safety, available and inexpensive.

The aim of this study was to evaluate a activity of henna (*Lawsonia-inermis*), handal (*Citrullus colocynthis*), indian fig (*Opuntia ficus-indica*), and pomegranate (*Punica granatum*) as

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antifungal agents against dermatophytes isolated from patients in Saudi Arabia.

MATERIALS AND METHODS

Dermatophytes isolates

T.violacium (4 isolates), *T. rubrum* (6 isolates), *T. verrucosum* (5 isolates), *T. mentagrophytes* (4 isolates), *T.scholnenii* (one isolates), *T.concentricum* (1 isolates), *M.canis* (11 isolates), *M.audouni*(4 isoaltes) , and *E. flocosum* (3 isolates) were purchased from department of Botany and Microbiology, collage of science, King Saud University**.**

Culture media

Roswell park memorial institute (RPMI 1640) medium broth with L-glutamine, phenol red and without sodium bicarbonate(Sigma), 3-(N-morpholino) propanesulfonic acid (MOPS) buffer PH 7 (Sigma), and Sabouraud Dextrose Agar (SDA) (Oxoid) were used and prepared according to Rippon (1974) and Geramishoar *et.al.*, (2002).

Crude extracts

Four antifungal medicinal plants were supplied as fresh leaf or fruit. A raw materials were extracted from henna (Lawsonia-*inermis*), handal (*Citrullus colocynthis*), indian fig (*Opuntia ficus-indica*), and pomegranate (*Punica granatum*) according to Taylor et al., (1996) and Betancur-Galvis et al., (2002). A stock solutions (200 mg/ml) were prepared then were sterilized by membrane filters (0.22 µm) (Chrom Teach Company). The stock solutions were stored at a temperature -70°C. Serial twofold drug dilutions were prepared according to the National Committee for Clinical Laboratory Standards (NCCLS 2000).

Preparation of fungal inoculum

Stock fungal inoculum suspensions of the dermatophytes were prepared from 7 to 15 day old cultures grown on PDA at 28°C. Mature colonies were covered with approximately 10 ml of sterile saline (0.89% NaCl) and the suspensions were made by gently shaking the colony with the tip of a Pasteur pipette. The resulting mixture of conidia and hyphal fragments were transferred to sterile tubes. Heavy particles of the suspension were allowed to settle for 10 to 15 min at room temperature, and the upper homogeneous suspension was used for further testing. The optical densities of the suspensions were read at 530 nm by a spectrophotometer and adjusted to 0.15 - 0.17 to yield 0.6×10^6 to 1.4×10^6 spores/ml. The suspensions containing conidia were diluted with RPMI 1640 medium to obtain the final desired inoculum size of approximately 0.4×10^4 to 5×10^4 spores/ml. The macrodilution tubes were incubated at 30°C for 10 to 15 days. The MICs endpoint for henna (Lawsonia-*inermis*), handal (*Citrullus colocynthis*), indian fig (*Opuntia ficus-indica*), and pomegranate (*Punica granatum*) were defined as the lowest concentration that produced prominent inhibition of growth relative to the drug-free growth control according to Tatsumi *et al.,* (2001).

In *vitro* susceptibility testing methods

The broth macrodilution method was performed according to NCCLS M38-A guidelines (NCCLS, 2000) and as described by (Espinel-Ingroff *et al.,*1997; Espinel-Ingroff *et al.,* 1999)

Statistics

A completed random design (CRD) was applied in this experiment. Statistical analysis of MICs data was performed by analysis of variance (SAS, 2002).

RESULTS

The minimum inhibitory concentration (mg/ml) of the crude plant (Henna, Handle, Prickly pear and Pomegranate) extract required to inhibit the growth of 50 % (MIC_{50}) of dermatophytic isolates are shown in table 1. The MIC_{50} of crude henna extract ranged from 0.1 to 3.2 mg/ml. 0.1 mg/ml of crude was enough to inhibit *T. rubrum* and *M. canis* while inhibition of *T. sholnenii* and *M. audonii* required 3.2 mg/ml. In the crude handle extract, 0.05 mg/ml inhibited growth of 50 % of *T. rubrum* whereas *T. verrucosum* and *T. mentagrophyte*s were inhibited by 3.2 mg/ml. MIC_{50} of Prickly pear for inhibition the *T. rubrum* and *M. canis* was similar the MIC_{50} of henna whilst MIC_{50} required to inhibit *M. canis* reached 0.8 mg/ml in pomegranate.

The $MICs_{90}$ of the crude plant (Henna, Handle, Prickly pear and Pomegranate) extracts ranged between 0.1 to 6.4 mg/ml (Table 2). To inhibit 90 % of *T. verrucosum*, *T. metagrophytes*, *T. scholnenii*, *T. concentricum* and *M. audonii* required high concentrations of crude extracts in all plant under study. In all extracts, the lowest concentrations were with *M. canis* and *T. rubrum*.

Table 1. Minimum Inhibitory Concentration of the crude plant extract (Henna, Handle, Prickly pear and Pomegranate) required to inhibit the growth of 50 % (MIC 50) of dermatophytic isolates

Isolates	Henna		Handle		Prickly pear		Pomegranate	
	Range (mg/ml)	MIC50 (mg/ml)	Range (mg/ml)	MIC50 (mg/ml)	Range (mg/ml)	MIC50 (mg/ml)	Range (mg/ml)	MIC50 (mg/ml)
T.violacium	0.1-3.2	0.8	0.2-6.4	1.6	0.8-12.8	3.2	0.025-0.8	0.2
T. rubrum	0.0125 0.4	0.1	0.06-0.2	0.05	0.06-0.4	0.1	0.05-1.6	0.4
T. verrucosum	0.2-6.4	1.6	0.4-12.8	3.2	0.4-12.8	3.2	0.2-6.4	1.6
T. mentagrophytes	0.2-6.4	1.6	0.4-12.8	3.2	0.4-12.8	3.2	0.2-6.4	1.6
T. scholnenii	0.4-12.8	3.2	0.2-6.4	1.6	0.8-12.8	3.2	0.1-3.2	0.8
T. concentricum	0.2-6.4	1.6	0.2-6.4	1.6	0.8-12.8	3.2	0.2-6.4	1.6
M .canis	0.0125-0.4	0.1	0.025-0.8	0.2	0.0125-0.4	0.1	0.06-0.2	0.05
M. audonii	0.4-12.8	3.2	0.2-6.4	1.6	0.8-12.8	3.2	0.1-3.2	0.8
E. flocosum	0.1-3.2	0.8	0.1-3.2	0.8	0.4-12.8	3.2	0.025-0.8	0.2
Means		1.4		1.5		2.5		0.8

Table 2. Minimum Inhibitory Concentration of the crude plant extract (Henna, Handle, Prickly pear and Pomegranate) required to inhibit the growth of 90 % (MIC 90) of dermatophytic isolates

Isolates	Henna		Handle		Prickly pear		Pomegranate	
	Range (mg/ml)	MIC90 (mg/ml)	Range (mg/ml)	MIC90 (mg/ml)	Range (mg/ml)	MIC90 (mg/ml)	Range (mg/ml)	MIC90 (mg/ml)
T.violacium	0.1-3.2	1.6	0.2-6.4	3.2	0.8-12.8	6.4	0.025-0.8	0.4
T. rubrum	0.0125 0.4	0.2	0.06-0.2	0.1	0.06-0.4	0.2	0.05-1.6	0.8
T. verrucosum	0.2-6.4	3.2	0.4-12.8	6.4	0.4-12.8	6.4	0.2-6.4	3.2
T. mentagrophytes	0.2-6.4	3.2	0.4-12.8	6.4	0.4-12.8	6.4	0.2-6.4	3.2
T. scholnenii	0.4-12.8	6.4	0.2-6.4	3.2	0.8-12.8	6.4	0.1-3.2	1.6
T. concentricum	0.2-6.4	3.2	0.2-6.4	3.2	0.8-12.8	6.4	0.2-6.4	3.2
M .canis	0.0125-0.4	0.2	0.025-0.8	0.4	0.0125-0.4	0.2	0.06-0.2	0.1
M. audonii	0.4-12.8	6.4	0.2-6.4	3.2	0.8-12.8	6.4	0.1-3.2	1.6
E. flocosum	0.1-3.2	1.6	0.1-3.2	1.6	0.4-12.8	6.4	0.025-0.8	0.4
Means		2.9		3.1		5.0		1.6

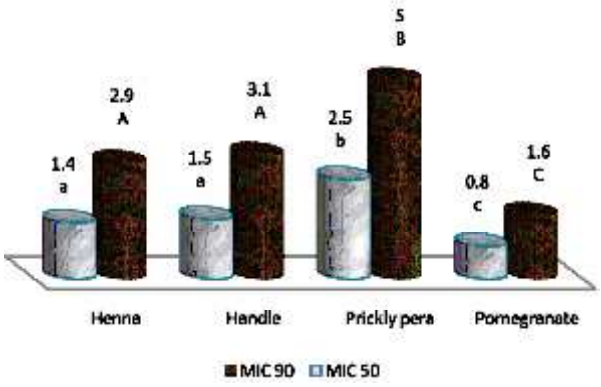

Fig. 1. Means of Minimum Inhibitory Concentration of the crude plant extract (Henna, Handle, Prickly pear and Pomegranate) required to inhibit the growth of 90 % (MIC 90) and required to inhibit the growth of 50 % (MIC 50) of dermatophytic isolates. Means with different letters notification are significant at $P< 0.05$

The mean $MICs_{90}$ and $MICs_{50}$ of each crude plant extracts with all isolated dermatophytes are shown in Figure 1. The mean $MICs_{90}$ (mg/ml) of Henna, Handal, prickly pear, and pomegranate were 2.9, 3.1, 5 and 1.6 whilst the $MICs_{50}$ were 1.4, 1.5, 2.5 and 1.6, respectively. The mean MICs 90 and MICs 50 have shown significant increase ($P<0.05$) in prickly pear whereas have shown significant reduce ($P<0.05$) in pomegranate.

DISCUSSION

In this study, all plant extracts demonstrated effective for inhibiting the growth of the isolated dermatophytes. The results agreed with a several studies. Sharma et al., (2011) found that a water extract of *L. inermis* have antifungal activity against some deramtophytes. Also Ekwealor et al., (2012) observed that the extract of *L. inermis* have antifungal activity against non dramtophytes isolated molds from onychomycosis. Habbal et al., (2005) reported that crude extract of henna does not have any activity against *Candida albicans*. Djaalab et al., (2012) concluded that hydroalcoholic extracts of *L. inermis* possess antifungal against the *T. rubrum*, *T. mentagrophyte*, *M. Canis* and *C. Albicans*. Also Dutta et al., (1998) found that a water extract of Punica granatum was effective in inhibiting the growth of *Trichophyton*, *Microsporum* and *Trichosporon* species.

In this study, the results indicated that *M. canis and T. rubrum* were more sensitivity to palnt extracts of Henna, Handal, prickly pear, and pomegranate whereas *T. mentagrophytes and T. verrucosum,* were more resistant than another isolates. Also The results demonstrated that the crude extract of pomegranate was the most effective in the inhibition 50% and 90% of growth while extract of prickly pear was the least. A average MIC of pomegranate, henna, handal and prickly pear were 1.6, 2.9, 3.1 and 5.0 mg/ml, respectively. These results agreed with results of Alkamel (2005) which showed that MIC of the aqueous extract of *C. Colocynthis* (handal) against *T. mentagrophytes* and *T. violacium* was 3.1 mg/ml. The results found that extract of handal a more activity against *T. rubrum* than other extracts while extract of pomegranate was a more activity against *M. canis*. Sharma *et al.,* (2011) reported that MICs of the henna water extracts required to inhibit growth of the *T. metagrophytes* , *T. rubrum*, *M. gypseum* and *M. Fluvum* were 6.25-12.5, > 12.5, >12.5 and 6.25-12.5 mg/ml, respectively.

CONCLUSION

The results concluded that crude plant extracts of henna (Lawsonia-*inermis*), handal (*Citrullus colocynthis*), indian fig (*Opuntia ficus-indica*), and pomegranate (*Punica granatum*) possess a ability to inhibit a growth of dermatophytes that isolated from human patients. This study support the previous researches about the biological importance of natural sources as antimicrobial agents.

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