

## Antifungal Activity of *Lawsonia inermis* L. (Henna) Against Clinical Candida Isolates

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### ABSTRACT

*Lawsonia inermis* L. (henna) is a much branched shrub or small tree, cultivated for its valuable leaves although stem, bark and roots have also been used in traditional medicine for over 9,000 years. In this study, the antifungal activity of *L. inermis* was determined against clinical Candida isolates. The study was carried out using 192 clinical isolates of Candida from six different species; 135 *Candida albicans*, 19 *Candida parapsilosis*, 17 *Candida glabrata*, 13 *Candida tropicalis*, 5 *Candida krusei*, 3 *Candida kefyr*. The anticandidal activities of *L. inermis* (henna) as paste form was determined by agar diffusion technique. The highest antifungal activity of henna was obtained against 68 (35.4%) clinical Candida isolates ( $\geq 20$ mm inhibition zone) and moderate activity also detected against 73 (38.0%) isolates (5-15 mm inhibition zone). Fifty-one (26.5%) isolates were resistance (no inhibition zone) to henna paste.

**Key Words:** Henna, Candida Species, Antifungal Activity

## Kınanın (*Lawsonia inermis* L.) Klinik Candida İzolatları Üzerine Antifungal Aktivitesi

### ÖZ

Kına (*Lawsonia inermis* L.), yaprakları, sürgünleri ve kökleri 9000 yıldan beri geleneksel tedavi için kullanılan, çok dallı çalı veya küçük ağaç formunda bir bitkidir. Bu çalışmada kınanın, klinik olarak izole edilen Candida türleri üzerine antifungal etkisi araştırılmıştır. Çalışma 6 farklı türe ait 192 klinik izolatla gerçekleştirilmiştir. İzolatların 135'i *Candida albicans*, 19'u *Candida parapsilosis*, 17'si *Candida glabrata*, 13'ü *Candida tropicalis*, 5'i *Candida krusei* ve 3'ü *Candida kefyr* türlerine aittir. Antikandidal aktivite, agar difüzyon tekniğiyle belirlenmiştir. En yüksek aktivite 68 (%35.4) *Candida albicans* izolatlarında 20mm den büyük inhibisyon zonuyla elde edilmiştir. Orta derecede aktivite ise yine aynı türe ait izolatlarda 73(%38.0) oran ve 5-15 mm inhibisyon zonuyla elde edilmiştir. İzolatlardan % 26'sı kınaya karşı dirençli (inhibisyon zonu oluşmamış) bulunmuştur.

**Anahtar Kelimeler:** Kına, Candida türleri, Antifungal Etki

### 1. Introduction

*Lawsonia inermis* syn. *Lawsonia alba* known as henna is a flowering plant, it is take place in the genus Lawsonia (Siddique et al.,2003; Arun et al.,2010). It is native for some subtropical and tropical regions of Africa and Southern Asia in semi-arid zones.

Henna (*Lawsonia inermis*) is cultivated commercially throughout Pakistan, India, Iran, Libya and Sudan for its valuable leaves (Saadabi, 2007).

Henna plant is deciduous, has a perennial shrub which is reaching a height of up 2,5-5m. The plant leaves are small, lanceolate,

dark-green, opposite and have short petioles. The leaves of plant contains a red orange color component, lawsone (2-hydroxy-1, 4-Napthoquinone). Lawsone (2-hydroxy-1, 4-Napthoquinone) is easily bonding with protein, and thus it has been used to dye skin, hair and fingernails (Siddique et al., 2003; Rahiman and Taha, 2011). The plant traditionally use for its red or black coloring to hands, feet and hair in some occasions such as weddings etc. (Saadabi, 2007).

For the cooling effect of henna, the paste form is used to bring down fever. Henna is beleived as a medicinal plant, because of its antibacterial effects especially on gram positive bacteria, antifungal activity against dermatophytes, wound healing, antitumoral effects, hypotensive, astringent and sedative effects. We have seen it as a folk medicine in using against headache, jaundice and leprosy. Several studies are being carried towards it activates like cytotoxic, hypoglycaemic, antimicrobial, antibacterial, antioxidant, trypsin inhibitory, wound Healing, analgesic, anti-corrosin, anti-inflammatory, anti-parasitic, tuberculostatic, hepatoprotective, anti-tumoral activity (Berenji et al., 2010; Elmanama et al., 2011; Karpe et al., 2011; Rayavarapu et al., 2011). The paste form or decoction of henna leaves are also used as a prophylactic against skin inflammation (Siddique et al., 2003).

Phytochemical characterision of *Lowsania inermis*, included the leaves contain about 0.5-1.5% lawsone, the antimicrobial agent responsible for the dyeing propertied of the plant (Charoensup et al., 2017). The plant has contain various compounds like; gallic

acid, coumarins, naphthalene derivates, flavonoids, sterols, triterpenoids, tannins, saponins, glycosides, and xanthones (Muhammed and Muhammed, 2005; Chaudhary et al, 2010)

Generally, studies on the antimicrobial activity of *L. inermis* has focused on this plant leaves extraction which is prepared with methanol, ethanol, and chlorofom extracts which is prepared by hot (soxhlet)sequential extraction method (Jeyaseelan et al., 2012; Chowdhury et al., 2014).

To the best of our knowledge there have been no reports in the available literature that explaining the paste form of henna leaves as using folk medicine. In our study, we investigated of anticandidal activity of powdered leaves, in the form of a paste as it is original usage in folk medicine against clinical isolates of *Candida* species.

## 2. Experimental

### 2.1. Plant material

The powdered leaves form Henna was purchased from local herbalist. The dry powder of henna was mixed with sterile saline solution to make a paste form (Henna paste; people traditionally use this form in many countries for feet, hands and hair) and was sterilized at 121 °C for 15 minute in autoclave.

### 2.2. *Candida* Isolation and Identification

The study was carried out using 192 clinical isolates of *Candida* (isolated from blood, urine, wound, oral cavities, sputum and

other specimens) representing six different species; 135 *Candida albicans*, 19 *Candida parapsilosis*, 17 *Candida glabrata*, 13 *Candida tropicalis*, 5 *Candida krusei*, 3 *Candida kefyr*. These fungal species were obtained from Research Hospital, Atatürk University, Erzurum.

The identification of isolates were performed by standard taxonomic procedures (such as including germ tube production, typical microscopic appearances, colony morphology and etc.) and was confirmed by the API 32C AUX identification system for yeasts. The yeasts were maintained on Sabouraud glucose agar slants, stored at 4 °C, until used in the study. *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019), were used as reference strains.

### **2.3. Antifungal activity**

The antifungal activity of the henna was determined by agar diffusion technique. Isolates of *Candida* were subcultured onto Sabouraud dextrose agar and were incubated at 37 °C for 48 h. The colonies after 48 h cultures were suspended in 5 ml of a sterile-saline solution. The turbidity of the inoculum was adjusted McFarland 1 standard and 0.1 ml of broth inoculum was swabbed on the surface of the petri plates containing solidified Sabouraud Dextrose agar. Well of 4 mm depth and 6 mm diameter, were made in the center of the agar plate. The well was then filled with the henna paste and allowed to diffuse at room temperature for an hour. Agar plates were incubated at 37 °C for 48 h and the diameter of the zones of inhibition

around the well were measured. In this study, Fluconazol discs (25 mg, Oxoid) were used as positive control.

### **3. Results and Discussion**

The world is filled with the richness of medicinal plants. Medicinal plants have an important role for curing a number of diseases. The therapeutic use of medicinal plant is becoming popular because of its minimal side effects when we comparing with antibiotics. It is explained by World Health Organization (WHO) medicinal plants would be the greatest source and the origin of contents of a large variety of drugs. Therefore, medicinal plants should be investigated to understand their activities, safety and efficiency properties for people using (Rayavarapu et al., 2011). And henna is one of the most popular plant known with these features, and is now the subject of several scientific studies (Barbieri et al., 2017).

Medicinal plants are widely used as a drug in health care system. *Lawsonia inermis* L. is recognized in traditional system of medicine. It consists of several phytochemicals like flavonoids, steroids, coumarins, xanthenes and triterpenoids. A large number of studies reported that henna is used for headache, lumbago, bronchitis, syphilitis, scabies, sores, amenorrhea, diarrhea, bleeding disorder, diuretic, skin diseases, anti-amoebiasis, antibacterial, antifungal, sedative, astringent, anti-hemorrhagic, and hypotensive effect (Borade et al., 2011).

Also Henna is used for cosmetic utilization of the coloring material in its leaves. Henna body art is popular by applying henna paste to the skin: the lawsone in the henna coloured the skin and makes red brown stain (Al-Rubiay et al., 2008). The plant powder leaves are used both in cosmetic dye and as a remedy for wounds, boils, and mycotic infection, successfully (Sharma et al., 2011). In this investigation we used henna paste against *Candida* species (Total of 192 yeast isolates) which is isolated from various clinical specimens (blood, urine, wound, oral cavities, sputum and other specimens). A

total of six different species of *Candida* were isolated, of which *C. albicans* was the most common (70.3%). After *C. albicans*, secondly common species was *C. parapsilosis* (9.8%), followed *C. glabrata* (8.9%), *C. tropicalis* (6.8%), *C. krusei* (2.7%) and *C. kefyr* (1.5%). Most of our isolates had been recovered from urine (30.7%) and blood (25.0%). *Candida* species were also isolated from oral cavities, wound, sputum and others specimens (cerebro spinal fluid, peritoneal and pericardial fluid, vaginal swabs, ear swabs, stool, IV catheter) Table-1.

**Table 1.** Distribution of *Candida* species according to their origin

Species (n)	Urine	Blood	Oral cavities	Wound	Sputum	Others
<i>C. albicans</i> (135)	43	21	9	20	20	22
<i>C. parapsilosis</i> (19)	4	9	-	1	-	5
<i>C. glabrata</i> (17)	8	5	1	-	-	3
<i>C. tropicalis</i> (13)	2	10	-	-	-	1
<i>C. krusei</i> (5)	2	3	-	-	-	-
<i>C. kefyr</i> (3)	-	-	2	1	-	-
Total (192)	59	48	12	22	20	31

In present study, henna paste are used for screening the antifungal activity against clinical isolates of *Candida* species by using agar diffusion methods which is used for quick screening of natural products. The

zones of inhibition were calculated for each isolates. The zone of inhibition exhibited by the effect of henna paste on *Candida* species is shown in Table-2, and illustrated in Figure-1.

**Table 2.** Antifungal activity of henna paste against clinical candida isolates

Species (n)	Inhibitory diameter zone (mm)													
	5 mm		10 mm		15 mm		20 mm		25 mm		30 mm		R	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
<i>C. albicans</i> (135)	7	5.1	42	31.1	5	3.7	36	26.6	5	3.7	5	3.7	35	25.9
<i>C. parapsilosis</i> (19)	1	5.2	4	21.0	1	5.2	4	21.0	-	-	3	15.7	6	31.5
<i>C. glabrata</i> (17)	-	-	7	41.1	1	5.8	3	17.6	-	-	-	-	6	35.2
<i>C. tropicalis</i> (13)	-	-	2	15.3	1	7.6	8	61.5	-	-	-	-	2	15.3
<i>C. krusei</i> (5)	-	-	2	40.0	-	-	2	40.0	-	-	-	-	1	20.0
<i>C. kefyr</i> (3)	-	-	-	-	-	-	1	33.3	1	33.3	-	-	1	33.3
Total (192)	8	4.1	57	29.6	8	4.1	54	28.1	6	3.1	8	4.1	51	26.5



**Fig.1.** Inhibition zone of henna paste

The highest antifungal activity was carried out against 46 (34.0%) *C. albicans* and the other species followed as: 7 (36.8%) *C. parapsilosis*, 3 (17.6%) *C. glabrata*, 8 (61.5%) *C. tropicalis*, 2 (66.6%) *C. kefyr*, 2 (40.0%) *C. krusei* strains (with 20-30 mm inhibition zone) and also moderate activity was detected against 54 (40.0%) *C. albicans*, 6 (31.5%) *C. parapsilosis*, 8 (47.0%) *C. glabrata*, 3 (23.0%) *C. tropicalis*, 2 (40.0%) *C. krusei* strains (5-15 mm inhibition zone). However, henna paste had any antifungal activity against 35 (25.9%) *C. albicans*, 6 (31.5%) *C. parapsilosis*, 6 (35.2%) *C. glabrata*, 2 (15.3%) *C. tropicalis*, 1 (20.0%)

*C. krusei*, 1 (33.3%) *C. kefyr* strains (Table-2).

Henna is accepted as a medicinal plant because of its attributed strong fungicidal, antibacterial, analgesic, virucidal, antiparasitic and several other features (Babu and Subhasree, 2009).

Lawson (2-hydroxynaphthoquinone) is the most important constituent of the plant. The lawson content of henna leaves were found to be 0,76-0,86g/100g of dried crude material (Charoensup et al, 2017). Henna also contains flavonoids, sterols, tannins, saponins, tannic acid, gallic acid and etc. Antimicrobial activity may be due to these numerous free hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall. The inhibition activity may be observed these constituents affects to enzyme sites of microorganisms (Al-Rubiay et al., 2008). Inhibitory action of henna was shown a wide range of microorganisms such as gram negative, gram positive bacteria and

dermathophytes (Babu and Subhasree, Arun P., Purushotham K.G., Jayarani J. and Kumari V. 2010). In vitro antibacterial activity and flavonoid contents of *Lawsonia inermis* (henna). International Journal of Pharm Tech Research, 2, 1178-1181.

Most of the studies showed that the leaves of *L. inermis* were found to exhibit strong fungitoxicity (Babu and Subhasree, 2009). There is a strong activity on treat skin infections such as tinea and it may be attributed to naphthoquinones, which is including lawson (Arun et al., 2010).

Saadabi et al. (2007) showed that, the growth in culture media of the different clinical fungal isolates was suppressed when the water leaf extract of henna was used in different concentrations. Bark extract of *L. inermis* was showed fungitoxic effect against ringworm fungi (Sowjanya and Chary, 2012). Lawsonsone has been shown to be effective against oral *C. albicans* isolated from patients with HIV/AIDS (Babu and Subhasree, 2009). Our findings were related with these previous studies which were showed the antifungal activity of henna.

#### 4. Conclusion

Henna paste showed the highest antifungal activity against 68 (35.4%) clinical *Candida* isolates ( $\geq 20$ mm inhibition zone) and moderate activity against 73 (38.0%) clinical *Candida* isolates (5-15 mm inhibition zone). Fifty-one (26.5%) isolates were resistance (no inhibition zone) to henna paste.

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