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Reveal of Antimicrobial Effect of *Lawsonia inermis* Extract on *Candida albicans* Using Molecular and Biochemical Techniques

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Abstract

Leaf samples of *Lawsonia inermis*(*Li*) was examined to their antimicrobial potential. Broth extracts in different concentrations were prepared and bioassayed in vitro for the growth of *Candida albicans.* The growth of *Candida albicans* pathogen was decreased. In addition to the observed alterations which detected electrophoretically in the protein pattern and activity of the catalase enzyme. The protein pattern has one common band of Rf 0.28 and one characteristic band of Rf 0.48 for *Candida albicans* sample. The obvious quantitative mutation was observed in the fungi with 5, 10, 15% of *Lawsonia inermis* compared with the control. Some types of proteins in *Candida albicans* were completely disappeared after inoculated with 5, 10, 15% *Li.* The catalase pattern showed one common band at R_1 with Rf 0.067 and the characteristic bands observed at R_5 with Rf 0.42 and at R_6 with Rf 0.47 $_{for}$ *Candida albicans* inoculated with broth. These results confirmed the antifungal activity of henna leaves and supported the traditional use of the plant in therapy of bacterial infections. Disturbances occurred at the biochemical level. The broth extract of the *Lawsonia inermis* leaves showed obvious antifungal activity against *Candida albicans.*

1. INTRODUCTION

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today were obtained from natural sources or semi-synthetic derivatives of natural products and used in the traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products.

Henna (*Lawsonia inermis*) has been shown to have strong fungicidal as well as anti-inflammatory, analgesic, antibacterial, virucidal, antiparasitic, anticancer and possible anti-sweating properties. The chemical constituents of this plant include: naphthalene derivatives, quinoids, b-sitosterol glycoside, xanthones, flavonoids, galic acid, coumarins, and lawsoniasides. Lawsone (2-hydroxy-1,4-naphthoquinone) is responsible for henna's fungicidal activity. They would like to hypothetically suggest that coloring parts of the body which are in high risk of fungal infections (for example foot) with henna might be useful in preventing mycosis. In addition gargling with henna solution might be useful for individuals with high risk of mouth infection. Washing other mucous membrane of body might also be preventive for the cases like vaginal infection. A support of the hypothesis might be tinea pedis (athlete's foot) infection rate in India where is well known for the coloring of hands and feet with henna. Tinea pedis infection is a common adolescent infection which is comparatively rare in this country. In practice, rare incidence of sensitivity might appear. Thus

adulterant henna or natural henna with impurities should be avoided, as in most cases allergic reactions are caused by the additives and not due to pure henna. The source of henna which is going to be used must be checked for its microbial content, heavy metal contamination and impurities (***Habbal et al., 2007 and Polat et al., 2009***).

Lawsone has been shown to be effective against oral *Candida albicans* isolated from patients with HIV/AIDS has reported activity against *Candida albicans* using Omani henna. During antifungal screening of higher plants, the leaves of *Lawsonoia inermis* were found to exhibit strong fungitoxicity where naphthoquinones were found to be the active factor. Henna paste applied to skin, the lawsone molecules migrate from the henna paste, traverse the outermost skin layer stratum corneum and stain the layer. It is powerful antioxidant. Molecule formula is given to be C10H6O3 and its total molecular weight of 174.16. Pure lawsone is an orange powder, insoluble in water, with a melting point higher than 192ºC and optical absorption maximum of 452 nm (***Habbal, 2005; Prasirst, 2004 and Tripathi, 1978***).

Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market were obtained from natural or semi synthetic resources ***(Sukanya et al., 2009)***.

The antimicrobial activity of the henna sample was generally more evident in the leaves of the plant rather than the seeds, the latter having only demonstrated a limited antibacterial activity and at higher concentrations. The anti *Candida albicans* activity is self evident as it demonstrated sensitivity to the leaves but not the seeds ***(Fessenden, 1998).***

. It is the presence of quinones in henna which gives that material its dyeing properties. The switch between diphenol (or hydroquinone) and diketone (or quinone) occurs easily through oxidation and reduction reactions. The individual redox potential of the particular quinone hydroquinone pair is very important in many biological systems ***(Fessenden, 1998).***

Leaves of the henna are strikingly most effective against the spectrum of the tested fungi comparing to the seeds. This is probably due to the inherent characteristics of the fully grown plant and the maturity of its chemically active constituents such as quinones. Such constituents would not have been found in seeds. Although fresh leaves demonstrated bacteriostatic activity in general, this was less evident when compared with the effect of dry leaves. It is possible that the drying effect on the plant causes the active ingredients to be more concentrated than those in the green leaves, where water and other constituents are still present.

Henna powder was significantly inhibited (either completely or partly) at different concentrations used. The growth of two fungal isolates of *Candida albicans*(1 from male and another from female) and observed that two fungal isolates of *Candida albicans* were completely inhibited at 5% Henna concentration (***Nemmat et al.,2005***).

Crude extracts of fresh and dry leaves and seeds were investigated for their antimicrobial activity against Candida *albicans* was used for testing the antifungal activity of the henna sample. Henna dry leaves demonstrated the best in-vitro antimicrobial activity so henna possesses in-vitro antibacterial activity against a wide spectrum of bacterial strains and *C. albicans* (***Habbal et al., 2005***).

Candida albicans is a diploid fungus and a causal agent of opportunistic oral and genital infections in humans. Systemic fungal infections (fungemias) have emerged as important causes of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). In addition, hospital-related infections in patients not previously considered at risk (e.g., patients in an intensive care unit) have become a cause of major health concern ***(dEnfert, 2007; Ryan and Ray, 2004).***

Quinones are a source of stable free radicals and know to complex irreversibly with nucleophilic amino acids in proteins often lead to inactivation of the protein and loss of function (***Kamei, 1998; Stern, 1996 and Thastrup, 1985***).

For that reason the potential range of quinone antimicrobial effects is great. Portable targets in the microbial cell are surface exposed adhesions, cell wall polypeptides, and membrane bound enzymes. Quinones may also render substrates unavailable to the microorganism. In addition they show to inhibit cell growth in culture.

Proteins are the chief actors within the cell, say to be carrying out the duties specify by the information encod in genes. With the exception of certain types of RNA, most other biological molecules are relatively inert elements upon which proteins act. Other macromolecules such as DNA and RNA make up only 3% and 20%, respectively.The set of proteins express in a particular cell or cell type is known as its proteome ***(Lodish et al., 2004 and Voet et al., 2004).***

Naphthoquinones are wide-spread phenolic compounds in nature. They are products of bacterial and fungal as well as high-plants secondary metabolism. Juglone, lawsone, and plumbagin are the most widespread compounds. Naphthoquinones display very significant pharmacological properties. They are cytotoxic, they have significant antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory, and antipyretic properties. Pharmacological effects to cardiovascular and reproductive systems have been demonstrated too. The mechanism of their effect is highly large and complex. They bind to DNA and inhibit the processes of replication, interact with numerous proteins (enzymes) and disturb cell and mitochondrial membranes, interfere with electrons of the respiratory chain on mitochondrial membranes. Plants with naphthoquinone content are widely used in China and the countries of South America, where they are applied to malignant and parasitic diseases treatment (***Babula et al., 2007)***.

The antimicrobial activity of *Lawsonia inermis* is no longer in doubt. Many workers reportedly demonstrated such activity on a wide spectrum of microbes. We will review such activity under various microbial categories, namely bacteria, fungi, viruses and parasites. Henna contains Lawsone in about 0.5 to 1.5% of its ingredients. Lawsone(2-hydroxynapthoquinone) is the principal constituent responsible for the dyeing properties of the plant. However, henna also contains mannite, tannic acid, mucilage and gallic acid. These substances are present in henna in the form of a mixture. Antimicrobial activity may be due to numerous free hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall. They may get attached to enzyme sites rendering them inactive. Water extracts did not show any antibacterial activity. This may be due to the lack of the solvent properties which plays an important role in antibacterial efficacy (***Harborne and Baxter, 1995 and Kelmanson et al.,2002***).

Polyacrylamide gel electrophoresis has become a standard tool in every laboratory in which proteins are analyzed and purified. Most frequently, the amount and location of the proteins were of interesting and staining were sufficient. However, it may also be important to correlate an activity of a protein with a particular band on the gel. Enzymatic and binding activities can sometimes be detected in situ by letting substrates or ligands diffused into the gel (***Gordon and Williamson, 1971***). The range of gel electrophoretic separation systems is limited by the pore size of the gels and diffusion of the antibody. The systems were also dependent on concentration and type of antigen or antibody to give a physically immobile aggregate. Analysis of cloned DNA has been revolutionized (***Southern, 1975***) by the ability to fractionate the DNA electrophoretically in polyacrylamide/ agarose gels.

The effectiveness of henna is caused through investigated the antimicrobial activity of leaves of *Lawsonia inermis* against *Candida albicans.* An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of

multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells (***Sies, 1997)***.

Catalase is a common enzyme found in nearly all living organisms which are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. Catalase has one of the highest turnover numbers of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second (***Chelikani et al., 2004 and Goodsell, 2004)***.

Hydrogen peroxide is a harmful by-product of many normal metabolic processes: to prevent damage, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules ***(Gaetani et al., 1996).*** The reaction of catalase in the decomposition of hydrogen peroxide is: $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

The enzyme catalase is produced by most aerobic microorganisms for the degradation of hydrogen peroxide ***(Lemberg and Legge, 1949).*** The result of normal respiration in most aerobically growing cells, hydrogen peroxide (H202), is highly toxic and can be bactericidal if not degraded ***(Lehninger, 1975)***. Catalase catalyzes the breakdown of H202 to molecular oxygen and water ***(Chance, 1949).*** It is highly intriguing how the relatively low levels of hydrogen peroxide produced safely by some bacteria are bactericidal to others, despite the relative abundance of mechanisms protecting bacterial cells from oxidative damage, such as H_2O_2-inactivating enzymes and antioxidants or DNA lesion repair systems ***(Cosgrove et al., 2007 and Goerlich et al., 1989***).

In the present work the effect of henna leaves extract *(Li)* on the *Candida albicans* fungi was tested using Vertical slab gel electrophoresis and U.V. / Visible spectrophotometer.

2. MATERIALS AND METHODS

The study was carried out in the Control labs, Kebab building of the nuclear research center, atomic energy authority, Inshas. The marker in this study is the broth due to this media contained neither the fungi (*Candida albicans*) nor henna leaves powder (*Lawsonia inermis*). It used as a signal for the growth of fungi (*Candida albicans*) after and before inoculated with *Lawsonia inermis*. Therefore the molecular weight of the marker expressed in the molecular weight of the broth.

2.1. Samples

3.1.1. Plant material

Henna plant *Lawsonia inermis* leaves samples used in this study were collected from Aswan city at winter 2006 where the soil is sandy. Fresh leaves were dried in shade then were ground to powder (***Crombie et al., 1990***).

3.1.2. Preparation of nutrient agar:

It was prepared by using (***Clesceri et al., 1998 and Horwitz, 2000***) technique and steps of (***Downes and Ito, 2001***).

3.1.3. Preparation of nutrient broth:

It was prepared by using ***U.S. Food and Drug Administration, 1995*** technique and steps of (***Downes and Ito, 2001***).

3.1.4. Fungal strain

Fungal pathogen included in this study was *Candida albicans* obtained from clinical isolates obtained at Microbiology department, faculty of Science, Helwan University. The fungi was cultured on nutrient agar medium, incubated at 37°C for 24h to obtain inoculums for testing (***Abdulmoneim Saadabi, 2007***).

Inoculation of *Candida albicans*

Different concentrations of herbal powder (5, 10 and 15gm/100ml nutrient broth) to obtain different concentrations of powdering henna leaves. Constant volumes of nutrient broth were inoculated with 1ml of *Candida albicans* (fungal suspension) under sterile conditions using Laminar flow, sterilr pipette and ethanol 70% then incubated at 37°C for 24h (***Wayne, 1998***). Turbidity in the conicals was checked because the nature of the herbal solution turbidity wasn't clearly visible.

After the beginning of incubation the total soluble solids was measured as function of incubation period using (***ATAGO Hand Performance, E-Type Series***). The data were shown in tables 1, 2 and growth curves were shown in figures 1, 2.

The homogenate preparation:

The fungal growth on henna nutrient broth was collected by sterile pipette into eppendorf and centrifuged at 15000 rpm for 5 minutes. The supernatant then decanted and ground cell debris with liquid nitrogen, water and 1%SDS in a mortar. The homogenous solution was collected in new eppendorf and centrifuged at 6000 rpm for 5 minutes. The supernatant which obtained contains proteins which will be measured in each fungal concentration of fungal pathogen sample (***Bradford, 1976***).

2.2. Protein Reagent

Protein Reagent prepared by dissolving 100mg of Coomassie brilliant blue G-250 in 50 ml 95% ethanol. To this solution 100 ml of 85% (w/v) phosphoric acid was added. The resulting solution was then diluted by deionized water to a final volume of 1 liter.

2.2.1. Protein Assay:

One milliliter of protein reagent was added to the test tubes those contained 0.1 ml of each sample as well as the standard solution. The contents of the tubes were mixed by vortexing. The absorbance measured after two minutes at 595 nm (***Bradford, 1976***).

2.2.2. Preparation of the sample:

The homogenate sample mixed with the sample buffer which prepared by method mentioned before with different percentages depending on concentration of the total protein in each sample. The protein concentration in each well must be in the range between 60 – 80 µg protein.

2.2.3. Protein Electrophoresis:

Methods and procedures were taken from the book Gel electrophoresis of proteins (***Hames, 1990***).

2.2.4. Resolving Gel (10%):

Gel solution prepared by mixing 12.3 ml distilled water, 9.9 ml of Acrylamide/Bis (30% T, 2.67% C) stock solution, and 7.5 ml Tris (1.5M, pH8.8). The total volume of the solution was 30 ml. To this solution 150µl of 10% APS, freshly prepared, and 30 µl of TEMED added prior to pouring into the gel plate assembly. The prepared gel plate assembled to running conditions. At the end of the run the gels were stained overnight through shaker then photographed after destaining.

2.2.5. Catalase enzyme:

Native protein gel stained by staining solution of catalase enzyme pattern used certain stain prepared according to ***(Gregory and Fridovich, 1974; Siciliano and Shaw, 1976; Baker and Manwell, 1977).*** The stained gel was stained by mixing 1 gm Potassium Iodide, 10 ml glacial acetic acid and 100 ml distilled water then washed by mixing 5 ml H_2O_2 completed to 150 ml with distilled water.

2.3. Data analysis:

Gel plate was photographed, scanned and then analyzed by using a gel pro Analyzer (Version 3.1 Media Cybernetics USA) for the analysis of tested samples. This program is a comprehensive computer software application designed to determine the relative fragmentation, the molecular weights and the amounts of peptide chains as well as scanned graphical presentation of the fractionated bands of each lane.

The similarity index (S.I.) compared patterns within different concentrations of *Lawsonia inermis* inoculated with *Candida albicans* as well as *Candida albicans* sample used the formula: S.I. = (2 Nab/Na + Nb) (***Nei and Li, 1979***) where :

Na and Nb are the number of bands in individuals a and b.

Nab is the number of shared bands between a and b.

The similarity values were converted into genetic distance (D) using the formula: D = 1 – S.

3. RESULTS

3.1. Protein pattern

The protein profile pattern of *Candida albicans* and *Candida albicans* inoculated with different concentrations of *Lawsonia inermis* (5, 10 and 15%) showed in figure (3) and the data were presented in table (3). Inspection of figure (3) and table (3) reveal that 6 types of protein fractions produced from *Candida albicans* at R_1, R_2, R_4, R_5, R_7, R_8 with Rf ranged between 0.2- 0.65. When *Candida albicans* injected with 5, 10, 15% *L1* produced 2 types of protein fractions at R_2, R_3 with Rf 0.28, 0.33.

Comparing the effect of 5, 10, 15% *LI*, it was observed that the 1st band at R_1 with Rf 0.2, 3rd band at R_4 with Rf 0.37, 4th band at R_5 with Rf 0.43, 5th band at R_7 with Rf 0.59, 6th band at R_8 with Rf 0.65 were disappeared completely from *Candida albicans* (Qualitative mutation) with appearing of new band at R_3 with Rf 0.33 for 5, 10, 15% *LI* injected with *Candida albicans*.

In case of broth, it was produced 4 bands at R_1, R_2, R_5 and R_7 with Rf ranged between 0.2- 0.59 so the broth showed disappearing of 2 bands at R_4 with Rf 0.37 and at R_8 with Rf 0.65 from *Candida albicans*.

In case of broth injected with *Candida albicans*, it was produced 8 types of proteins or bands at R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 with Rf ranged between 0.2- 0.65. so the broth injected with *Candida albicans* showed appearing of 2 new bands at R_3 with Rf 0.33 and at R_6 with Rf 0.48 compared by *Candida albicans*.

From this data, it was observed the presence of one common band at R_2 with Rf 0.28 and one characteristic band observed at R_6 with Rf 0.48 for broth inoculated with *Candida albicans*.

The quantitative mutation observed at R_2 for 5, 10, 15% recorded amount % ranged between 50.3- 56.1 compared with *Candida albicans* recorded amount % 18.4 from the total proteins secreted *Candida albicans* injected with 5, 10, 15% *Li* recorded 3 duplicate amount compared by *Candida albicans*.

The protein similarity index between *Candida albicans* and *Candida albicans* inoculated with 5, 10 and 15% *Li* recorded a low value (0.25), between *Candida albicans* and broth recorded a low value (0.8) and between *Candida albicans* and broth injected with *Candida albicans* recorded a high value (0.86) as shown in table(4).

By comparing all 3 concentrations of *Li* inoculated with *Candida albicans* and *Candida albicans* indicating the high effect of *Li* on protein fractions of *Candida albicans* and showed a high difference among the usage of *Li* with protein fractions of *Candida albicans*.

From this data indicating that the broth has no effect on protein fractions of *Candida albicans* but used only as a signal for growth of fungi on it.

3.2. Catalase enzyme

The catalase pattern of *Candida albicans* and *Candida albicans* inoculated with 15% *Lawsonia inermis* showed in figure (4) and the data were presented in table (5). Inspection of figure (4) and table (5) reveal that 5 types of catalase fractions produced from *Candida albicans* at R_1, R_2, R_3, R_4, R_7 with Rf ranged between 0.067- 0.54. When *Candida albicans* injected with 15% *L1* produced 1 type of catalase fraction at R_1 with Rf 0.067.

Comparing the effect of 15% *LI*, it was observed that the the 2^{nd} band at R_2 with Rf 0.17, 3^{rd} band at R_3 with Rf 0.24, 4^{th} band at R_4 with Rf 0.35and 5^{th} band at R_7 with Rf 0.54 were disappeared completely from *Candida albicans* (Qualitative mutation).

In case of broth injected with *Candida albicans*, it was produced 7 types of catalase fractions at R_1, R_2, R_3, R_4, R_5, R_6, R_7 with Rf ranged between 0.067- 0.54. So the broth injected with *Candida albicans* showed appearing of 2 new bands at R_5 with Rf 0.42 and at R_6 with Rf 0.47 in *Candida albicans*.

From this data, it was observed the presence of one common band at R_1 with Rf 0.067 and 2 characteristic bands observed at R_5 with Rf 0.42 and at R_6 with Rf 0.47 for broth inoculated with *Candida albicans*.

The quantitative mutation observed at R_1 15% recorded amount % 100 due to it is the only band which still after *Candida albicans* inoculated with 15% *Li* compared with *Candida albicans* recorded amount % 22.1 from the total proteins secreted so *Candida albicans* injected with 15% *Li* recorded 5 duplicate amount compared by *Candida albicans*.

The catalase similarity index between *Candida albicans* and *Candida albicans* inoculated with 15% *Li* recorded a low value (0.33) and between *Candida albicans* and broth injected with *Candida albicans* recorded a high value (0.83) as shown in table(6). This result indicates that the nutrient broth is a good media for growth of *Candida albicans* on it, showed the effect of *Li* on fungal growth so it had high S.I due to appear of 2 new types of catalase fractions comparing with catalase fractions of *Candida albicans*.

By comparing 15% *Li* injected with *Candida albicans* and *Candida albicans*, indicating the effect of *Li* on catalase fractions of *Candida albicans* and showed the difference among the usage of *Li* with catalase fractions of *Candida albicans*.

4. DISCUSSION

Protein profile of *Candida albicans:*

The *Candida albicans* cell wall maintains the structural integrity of the organism. The major components of the cell wall are fibrillar polysaccharides and proteins. Three classes of proteins are present in the candidal cell wall. One group of proteins attach to the cell wall via a glycophosphatidylinositol by an alkali-labile linkage. A second group of proteins with N-terminal signal sequences but no covalent attachment sequences are secreted by the classical secretory pathway. These proteins may end up in the cell wall or in the extracellular space. The third group of proteins lacks a secretory signal, and the pathway(s) by which they become associated with the surface is unknown. Potential constituents of the first two classes have been predicted from analysis of genome sequences. Experimental analyses have identified members of all three classes ***(LaJean Chaffin, 2008).***

In the protein pattern, the similarity index between *Candida albicans* and *Candida albicans* inoculated with different high concentrations of *Lawsonia inermis* recorded a very a low value (0.25) and genetic distance (0.75). This result showed that the *Lawsonia inermis* has a prominent role in preventing the emergence of *Candida albicans* protein bands (Qualitative mutation).

The quantitative mutation observed at R_2 for 5, 10, 15% recorded 3 duplicate amount compared by *Candida albicans*.

Pulsed-field gel electrophoresis techniques were used to Chromosome-sized DNA of two strains of *Candida albicans* has been resolved into Six bands were observed in chromosomal preparations of *C. albicans* using gel electrophoresis ***(Lasker et al., 1989).***

Catalase of *Candida albicans:*

The mechanism of protection against H2O2may be more efficient in photosynthetic organisms including algae and cyanobacteria than in other organisms because they not only consume oxygen during respiration but they also generate it during photosynthesis. Cyanobacteria were the first organisms that supplied dioxygen to the atmosphere by a system using the oxidation of water for electron donation to the reaction centre ***(Asada, 1993)***.Accompanied by an increase in the atmospheric concentration of dioxygen, effective H2O2-scavenging systems consisting of catalase and peroxidase activities were required in cyanobacteria to protect the cells from oxidative damage. The production of H2O2 is inevitable in cyanobacterial cells since superoxide dismutase has been present ***(Miyake, 1991).***The primitive scavenging system of H2O2 appears to be catalase (catalase±peroxidase), because many aerobic prokaryotes contain only catalase (catalase±peroxidase) and lack peroxide scavenging peroxidases. The absence of ascorbate in prokaryotes and fungi can account for the lack of ascorbate peroxidase in these organisms ***(Asada, 1993)***.

The expression of catalase against treatment with hydrogen peroxide took place very quickly and decreased slowly in the experimental condition adopted here assumed that the expression of the catalase in *Candida albicans* is regulated by various environmental conditions via motifs for transcriptional activation as in other yeast catalases ***(Nakagawa et al., 1999).***

Reactive oxygen species (ROS) can react with polyunsaturated fatty acids in cellular membranes, nucleotides, and sulfhydryl bonds in proteins ***(Machlin and Bendich, 1987)***, and have been related to tissue injury in yeast infections ***(Nishikawa et al., 1997)*** In *C. albicans*, mitochondria are capable of generating and releasing extracellular ROS ***(Danley et al., 1983)***.

In the catalase pattern, the similarity index between *Candida albicans* and *Candida albicans* inoculated with 15%of *Lawsonia inermis* recorded a low value (0.33) and genetic distance (0.67). Showed that the *Lawsonia inermis* has a prominent role in preventing the emergence of catalase bands of *Candida albicans*(Qualitative mutation). The quantitative mutation observed at R_1 15% recorded 5 duplicate amount compared by *Candida albicans*.

5. CONCLUSION

Medical plants commonly used by local inhabitants for its invitro antifungal activity. Used of simple method for extraction of *Lawsonia inermis* leaves had proven to be successful in the estimation of antimicrobial activity against *Candida albicans* which responsible for genital infections in humans.

The data confirmed the effective role of *Lawsonia inermis* to cause high disturbances for protein and catalase patterns of *Candida albicans* and a high effect of *Lawsonia inermis* on fungal growth at a higher concentrations of *Lawsonia inermis*.

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Table 1: The total soluble solids measurement for different concentrations of *Lawsonia inermis* (5, 10, 15%) with and without *Candida albicans*.

Concentration of henna nutrient broth(*Candida albicans*)	With bacteria	Without bacteria
5%	3	3.2
10%	4.8	4.8
15%	6.4	6.4
N.B	1.4	1

Table 2: The total soluble solids measurement for different concentrations of *Lawsonia inermis* (5, 10, 15%) with and without *Candida albicans*.

Concentration of henna nutrient broth(*Candida albicans*)	With bacteria	Without bacteria
5%	3	3.2
10%	4.6	4.8
15%	6.1	6.5
N.B	1.8	1

Table 3: The protein profile pattern of *Candida albicans* and *Candida albicans* inoculated with *Lawsonia inermis* at different concentrations.

Rows	5%		10%		15%		Broth+*Cand.*		*Cand.*		*Broth*	
	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf
R1	–	–	–	–	–	–	14.43(8)	0.2	16.17 (7.1)	0.2	25.33 (8.38)	0.2
R2	53.3 (7.55)	0.28	50.3 (7.63)	0.28	56.1 (7.57)	0.28	12.8 (7.09)	0.28	18.4 (8.08)	0.28	21.73 (7.19)	0.28
R3	46.7 (6.62)	0.33	49.7 (7.55)	0.33	43.9 (5.93)	0.33	10.52 (5.83)	0.33	–	–	–	–
R4	–	–	–	–	–	–	12 (6.65)	0.37	16.72 (7.34)	0.37	–	–
R5	–	–	–	–	–	0.25	13 (7.21)	0.43	15.83 (6.95)	0.43	28.3 (9.36)	0.43
R6	–	–	–	–	–	–	13.2 (7.3)	0.48	–	–	–	–
R7	–	–	–	–	–	–	12.5 (6.94)	0.59	18.1 (7.94)	0.59	24.64 (8.15)	0.59
R8	–	–	–	–	–	–	11.55 (6.4)	0.65	14.78 (6.49)	0.65	–	–

Rf: Rate of flow

Am: Amount

Note: The number between brackets expressed the real mass of protein

Table 4: The protein simiralrity index (SI) and Genetic distance (Gd) between *Candida albicans* and *Candida albicans* inoculated with *Lawsonia inermis* at different concentrations , Broth.

S.I

G.d	*Candida albicans*	Concentrations of inoculated henna powder with *Cand.*				
		5%	10%	15%	Broth	Broth+*Cand.*
Cand.	–	0.25	0.25	0.25	0.8	0.86
5%	0.75	–	1	1	0.33	0.4
10%	0.75	0	–	1	0.33	0.4
15%	0.75	0	0	–	0.33	0.4
Broth	0.2	0.67	0.67	0.67	–	0.67
Broth+*Cand.*	0.14	0.6	0.6	0.6	0.33	–

Table 5: The Catalase pattern of *Candida albicans* and *Candida albicans* inoculated with *Lawsonia inerm*

Rows	*Cand.*		15%		Broth+*Cand.*	
	Am%	Rf	Am%	Rf	Am%	Rf
R1	35.15 (22.5)	0.067	100 (23.8)	0.067	28.56 (16.2)	0.067
R2	20.62 (13.2)	0.17	–	–	19.22 (10.9)	0.17
R3	14.13 (9.04)	0.24	–	–	14.67 (8.32)	0.24
R4	14.96 (9.58)	0.35	–	–	7.77 (4.41)	0.35
R5	–	–	–	–	9.6 (5.44)	0.42
R6	–	–	–	–	8.92 (5.06)	0.47
R7	15.14 (9.69)	0.54	–	–	11.26 (6.39)	0.54

Rf: Rate of flow
Am: Amount
Note: The number between brackets expressed the real mass of protein.

Table 6: The catalase simiralrity index (SI) and Genetic distance (Gd) between *Candida albicans* and *Candida albicans* inoculated with *Lawsonia inermis* (15%).

	S.I		
G.d	*Candida albicans*	Concentration of inoculated henna powder with *Cand.*	
		15%	Broth + *Cand.*
Candida albicans	–	0.33	0.83
15%	0.67	–	0.25
Broth + *Cand.*	0.17	0.75	–

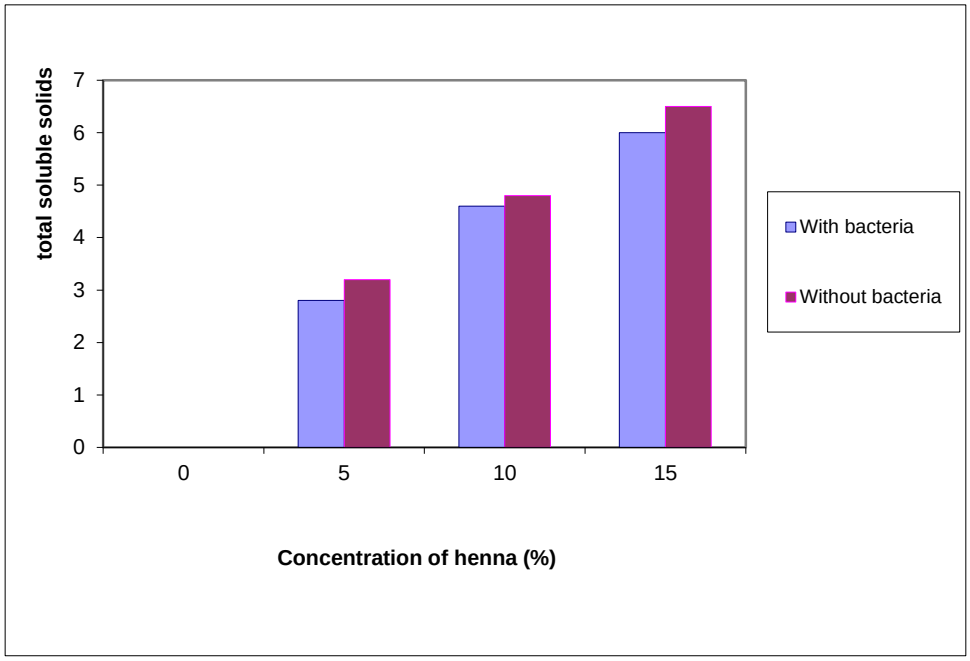

Fig 1: The diagram of total soluble solids measurement for different concentrations of *Lawsonia inermis* (5, 10, 15%) with and without *Candida albicans*.

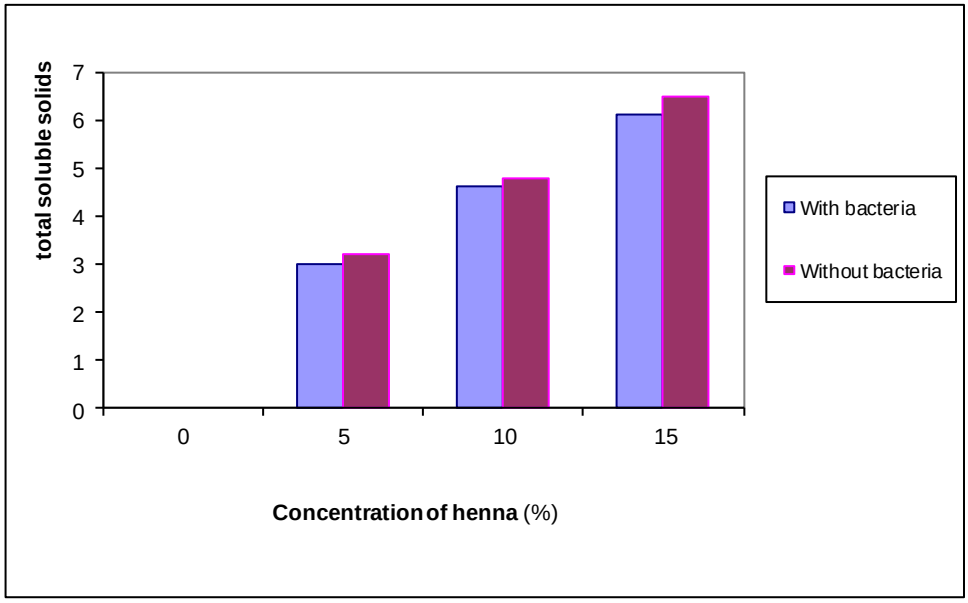

Fig 2: The diagram of total soluble solids measurement for different concentrations of *Lawsonia inermis* (5, 10, 15%) with and without *Candida albican*

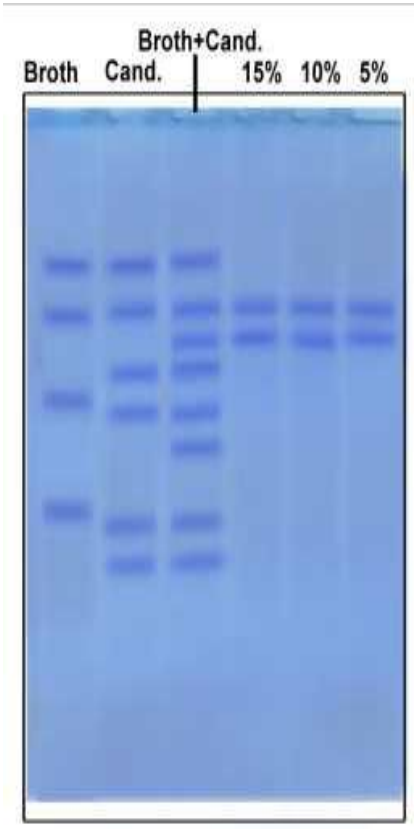

Fig 3: Photograph and diagrammatic illustration of electrophoretic protein pattern of *Candida albicans* and *Candida albicans* inoculated with 5, 10, 15% of *Lawsonia inermis.*

Fig 4: Photograph and diagrammatic illustration of electrophoretic catalase pattern of *Candida albicans* and *Candida albicans* inoculated with 15% of *Lawsonia inermis*.