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ORIGINAL ARTICLE

Biochemical study for the effect of henna (*Lawsonia inermis*) on *Escherichia coli*

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Abstract Leaf samples of *Lawsonia inermis* (*Li*) were examined for their antimicrobial potential. Broth extracts in different concentrations were prepared and bioassayed in vitro for the growth of *Escherichia coli*. The growth of *E. coli* pathogen was inhibited to various degrees by increasing the concentration of the herbal powder. In addition to the observed alterations which were detected electrophoretically in the protein pattern, were activities of the amylase enzyme and glycoprotein fractions. The protein pattern has one common band of Rf 0.47 and two characteristic bands of Rf 0.36 and Rf 0.42 for *E. coli* sample. The quantitative mutation was observed in the bacteria with different concentrations of *L. inermis* compared with the control. Some types of proteins in *E. coli* completely disappeared upon being S.I affected. The amylase pattern showed one common band with Rf 0.037 and two characteristic bands with Rf 0.18 and Rf 0.37 for *E. coli* sample. The obvious quantitative mutation observed in bacteria with different concentrations of *L. inermis* compared with *E. coli*. The glycoprotein pattern recorded one common band at R_1 with Rf 0.94 for *E. coli* sample and bacteria inoculated with different concentrations of *L. inermis*. These results confirmed the antibacterial activity of henna leaves and supported the traditional use of the plant in therapy of bacterial infections and disturbances that occurred at the biochemical level. The broth extract of the *L. inermis* leaves showed obvious antibacterial activity against *E. coli*.

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1. Introduction

Plants produce a diverse range of bioactive molecules, making them rich sources 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.

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 in 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 and 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 and Fessenden, 1998).

Leaves of the henna are strikingly most effective against the spectrum of the tested bacteria compared 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.

Uropathogenic *Escherichia coli* (UPEC) is responsible for approximately 90% of urinary tract infections (UTI) seen in individuals with ordinary anatomy. In ascending infections, fecal bacteria colonize the urethra and spread up the urinary tract to the bladder as well as to the kidneys, or the prostate in males. Because women have a shorter urethra than men, they are 14-times more likely to suffer from an ascending UTI (Nicolle, 2008 and Todar, 2007).

Quinones are a source of stable free radicals and are known to complex irreversibly with nucleophilic amino acids in proteins often leading to inactivation of the protein and loss of function (Kamei et al., 1998; Stern et al., 1996 and Thastrup et al., 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, seen to be carrying out the duties specified by the information encoded in genes. With the exception of certain types of RNA, most other biological molecules are relatively inert elements upon which proteins act. Proteins make up half the dry weight of an *E. coli* cell, whereas other macromolecules such as DNA and RNA make up only 3% and 20%, respectively. The set of proteins expressed in a particular cell or cell type is known as its proteome (Lodish et al., 2004 and Voet and Voet, 2004).

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 interesting and staining was 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, 1971 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 the investigated antimicrobial activity of the leaves of *Lawsonia inermis* against *E. coli*.

In the present work the effect of henna leaves extract (*Li*) on the *E. coli* bacteria 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 used in this study is the broth as this medium contained neither the bacteria (*E. coli*) nor the henna leaves powder (*L. inermis*). It is used as a signal for the growth of bacteria (*E. coli*) after and before being inoculated with *L. inermis*. Therefore, the molecular weight of the marker is expressed in the molecular weight of the broth.

2.1. Samples

2.1.1. Plant material

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

2.1.2. Preparation of nutrient agar

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

2.1.3. Preparation of nutrient broth

It was prepared by using US Food and Drug Administration, 1995 technique and steps of Downes and Ito, 2001.

2.1.4. Bacterial strains

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

2.1.5. Inoculation of E. coli

Different concentrations of herbal powder (1, 2, 3, 4, 5, 10 and 15 gm/100 ml nutrient broth) were used to obtain different concentrations of powdered henna leaves. Constant volumes of nutrient broth were inoculated with 1 ml of *E. coli* (bacterial suspension) under sterile conditions using Laminar flow, a sterile pipette and ethanol 70% and then incubated at 37 °C for 24 h (Wayne, 1998). Turbidity in the conical flasks was checked because the nature of the herbal solution turbidity was not clearly visible.

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

Table 1 The total soluble solids measurement for different concentrations of *Lawsonia inermis* (1%, 2%, 3%, 4% and 5%) with and without *E. coli*.

Concentration of henna nutrient broth (*E. coli*)	With bacteria	Without bacteria
1%	2.5	2
2%	2	2.2
3%	2.6	2.8
4%	3.8	4
5%	3.8	4.2
N.B	2.2	1.8

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

Concentration of henna nutrient broth (*E. coli*)	With bacteria	Without bacteria
5%	4.4	5
10%	6.8	7.1
15%	8.2	9.9
N.B	1.4	1

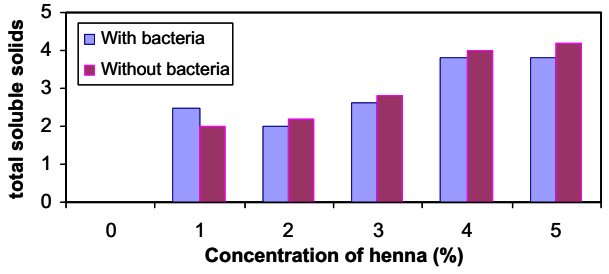

Figure 1 The diagram of total soluble solids measurement for different concentrations of *Lawsonia inermis* (1%, 2%, 3%, 4% and 5%) with and without *E. coli*.

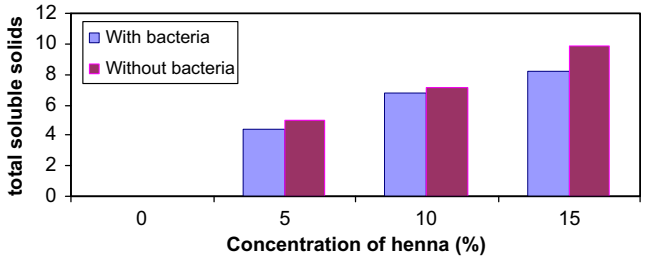

Figure 2 The diagram of total soluble solids measurement for different concentrations of *Lawsonia inermis* (5%, 10% and 15%) with and without *E. coli*.

2.1.6. The homogenate preparation

The bacterial growth on henna nutrient broth was collected by sterile pipette into eppendorf and centrifuged at 15,000 rpm for 5 min. The supernatant was then decanted and ground with 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 min. The supernatant obtained contains proteins which will be measured in each bacterial concentration of each bacterial pathogen samples (Bradford, 1976).

2.1.7. Protein reagent

Protein reagent was prepared by dissolving 100 mg 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 l.

2.1.8. Protein assay

One milliliter of protein reagent was added to the test tubes that 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 2 min at 595 nm (Bradford, 1976).

2.1.9. Preparation of the sample

The homogenate sample was mixed with the sample buffer which was prepared by the method mentioned earlier 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 proteins.

2.1.10. Protein electrophoresis

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

2.1.11. Resolving gel (10%)

Gel solution was 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 (1.5 M, pH 8.8). The total volume of the solution was 30 ml. To this solution 150 µl of 10% APS, freshly prepared, and 30 µl of TEMED were added prior to pouring into the gel plate assembly. The prepared gel plate was assembled to running conditions. At the end of the run the gels were stained overnight through a shaker and then photographed after destaining.

2.2. Amylase enzyme

The amylase enzyme activity determined (according to) (Siciliano and Shaw, 1976; Rammesmayer and Praznik, 1992) is summarized as follows:

2.2.1. Washing buffer

It consists of Tris/HCl (pH 7.1) which prepared by dissolving 6 gm of tris HCl in liter of dist H_2O.

2.2.2. Working buffer

It was prepared by mixing 50 ml of Tris/HCl pH 7.5 (6 gm/1 l) with 110 mg $CaCl_2$ followed by 0.5 gm soluble starch.

2.2.3. Staining solution

It was prepared by mixing 300 mg Pot. Iodide with 130 mg iodine dissolved in little amount of water followed by heating and then filled up to 100 ml with dist. H_2O.

2.2.4. Fixative solution

It was prepared by mixing 9 ml of ethyl alcohol with 11 ml acetic acid (20%).

2.3. The glycoprotein

Native protein gel was stained for glycoproteins pattern using Elftman (1959) and Kasten (1960) method through Periodic acid (Oxidizing solution), Schiff's reagent and Sodium bisulfite (storage solution).

(1) Native protein gel was treated with alcoholic periodic acid for 2 h.
(2) Gel was washed with 90% alcohol for 5 min.
(3) Hydrated quickly to water.
(4) Treated with Schiff reagent for 10 min.
(5) The gel was transferred through sulfite solution, 3 changes: 1.5–2 min for each gel.
(6) Gel was washed by running water for 5 min.
(7) The gel was stored in storage solution.

2.4. 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 *L. inermis* inoculated with *E. coli* as well as *E. coli* sample using 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: $D = 1–S$.

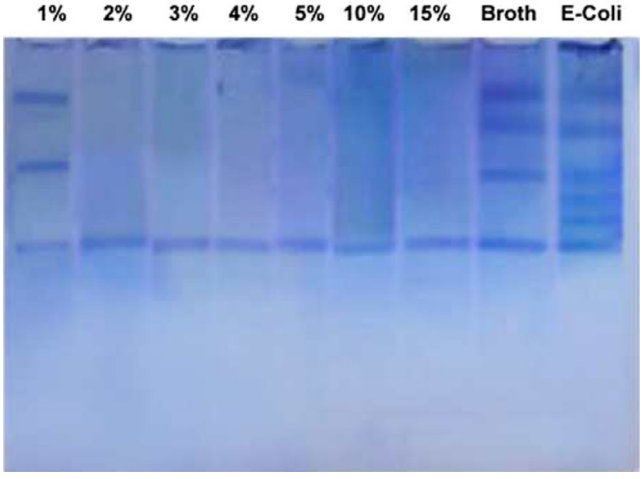

Figure 3 Photograph and diagrammatic illustration of electrophoretic protein pattern of *E. coli* and *E. coli* inoculated with different concentrations of *Lawsonia inermis*.

Table 3 Protein pattern of *E.coli* and *E.coli* inoculated with different concentrations of *Lawsonia inermis*.

Rows	1%		2%		3%		4%		5%		10%		15%		Broth		*E.coli*	
	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf
R1	30.76 (7.14)	0.11	–	–	–	–	–	–	–	–	–	–	–	–	32.77 (12.9)	0.11	19 (9.7)	0.11
R2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
R3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	27.69 (10.9)	0.19	21.75 (11.1)	0.19
R4	40.67 (9.44)	0.31	–	–	–	–	–	–	–	–	–	–	–	–	20.24 (7.97)	0.31	19.13 (9.76)	0.31
R5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12.59 (6.42)	0.36
R6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12.4 (6.33)	0.42
R7	28.57 (6.63)	0.47	100(11)	0.47	100 (8.18)	0.47	100 (8.28)	0.47	100 (9.8)	0.47	100 (5.64)	0.47	100 (7.46)	0.47	19.3 (7.6)	0.47	15.13 (7.72)	0.47

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

Table 4 Protein similarity index (SI) and Genetic distance (Gd) between *E.coli* and *E.coli* inoculated with different concentrations of *Lawsonia inermis*

		S.I								
		E.coli	Concentrations of inoculated henna powder with *E.coli*							
			1%	2%	3%	4%	5%	10%	15%	Broth
G.D	*E.coli*	–	0.67	0.28	0.28	0.28	0.28	0.28	0.28	0.8
	1%	0.33	–	0.5	0.5	0.5	0.5	0.5	0.5	0.86
	2%	0.72	0.5	–	1	1	1	1	1	0.4
	3%	0.72	0.5	0	–	1	1	1	1	0.4
	4%	0.72	0.5	0	0	–	1	1	1	0.4
	5%	0.72	0.5	0	0	0	–	1	1	0.4
	10%	0.72	0.5	0	0	0	0	–	1	0.4
	15%	0.72	0.5	0	0	0	0	0	–	0.4
	Broth	0.2	0.14	0.6	0.6	0.6	0.6	0.6	0.6	–

3. Results

3.1. Protein pattern

The protein profile pattern of *E. coli* and *E. coli* inoculated with different concentrations of *L. inermis* (1%, 2%, 3%, 4%, 5%, 10% and 15%) is showed in Fig. 3 and the data were presented in Table 3. Inspection of Fig. 3 and Table 3 reveals that six types of protein fractions produced from *E. coli* at R_1, R_3, R_4, R_5, R_6, R_7 with Rf ranged between 0.11–0.47. When *E. coli* injected with 1% *Li* three types of protein fractions produced at R_1, R_4, R_7 with Rf ranged between 0.11–0.47. On the other hand, when *E. coli* inoculated with 2%, 3%, 4%, 5%, 10% and 15% *Li* produced only one type of protein fraction at R_7 with Rf 0.47.

Comparing the effect of 1% *Li*, it was observed that the 2nd band at R_3, 4th band at R_5 and 5th band at R_6 disappeared from *E. coli* (Qualitative mutation) rather than that injected by 1% *Li*. On the other band, the concentrations (2%, 3%, 4%, 5%, 10% and 15%) of *Li* inoculated with *E. coli* compared with *E. coli*, it was observed that the 1st band at R_1, 2nd band R_3, 3rd band at R_4, 4th band at R_5 and 5th band at R_6 completely disappeared from *E. coli* (Qualitative mutation).

In the case of broth, it produced four bands at R_1, R_3, R_4 and R_7 with Rf that ranged between 0.11–0.47. Thus, the broth showed the disappearance of two bands from *E. coli*, in 4th band at R_5 and 5th band at R_6 from *E. coli*.

From this data, one observed the presence of one common band at R_7 with Rf 0.47 and two characteristic bands observed at R_5 with Rf 0.36 and at R_6 with Rf 0.42 for *E. coli* sample.

The quantitative mutation observed at R_7 for 2%, 3%, 4%, 5%, 10% and 15% recorded the highest value of amount percent 100 due to the fact that it is the only band which is still there after *E. coli* is inoculated with 2%, 3%, 4%, 5%, 10% and 15% *Li*. On the other hand, 1% recorded amount percent 28.57 from the total proteins secreted and broth recorded amount percent 19.3 from the total proteins secreted compared with *E. coli* which recorded amount percent 15.13 from the total proteins secreted. So *E. coli* injected with 2%, 3%, 4%, 5%, 10% and 15% *Li* recorded six or seven duplicate amounts compared to *E. coli* and *E. coli* injected with 1% *Li* recorded duplicate amount compared by *E. coli*.

The protein similarity index between *E. coli* and *E. coli* injected with 1% *Li* recorded (0.67), On the other hand (2%, 3%, 4%, 5%, 10% and 15%) *Li* recorded a low value (0.28) and between *E. coli* and broth recorded (0.8) as shown in Table 4.

3.2. Amylase enzyme

The amylase pattern of *E. coli* and *E. coli* injected with different concentrations of *L. inermis* are recorded in Fig. 4 and the data presented in Table 5. Inspection of Fig. 4 and Table 5 reveals that 3 types of amylase fractions produced from *E. coli* at R_1, R_2 and R_3 with Rf ranged between 0.037–0. 37. When *E. coli* was injected with 1%, 2%, 3%, 4%, 5%, 10%, 15% *Li* and broth produced only one type of amylase fraction at R_1 with Rf 0.037.

Comparing the effect of 1%, 2%, 3%, 4%, 5%, 10% and 15%, we observed that 2nd band R_2 and 3rd band at R_3 completely disappeared from *E. coli* (Qualitative mutation).

From these data, one observed the presence of one common band at R_1 with Rf 0.037 and 2 characteristic bands were observed at R_2 with Rf 0.18 and at R_3 with Rf 0.37 for *E. coli* sample.

The obvious quantitative mutation observed at R_1 for 1%, 2%, 3%, 4%, 5%, 10%, 15% *Li* and broth recorded the highest value of amount% 100 due to it is the only band which still

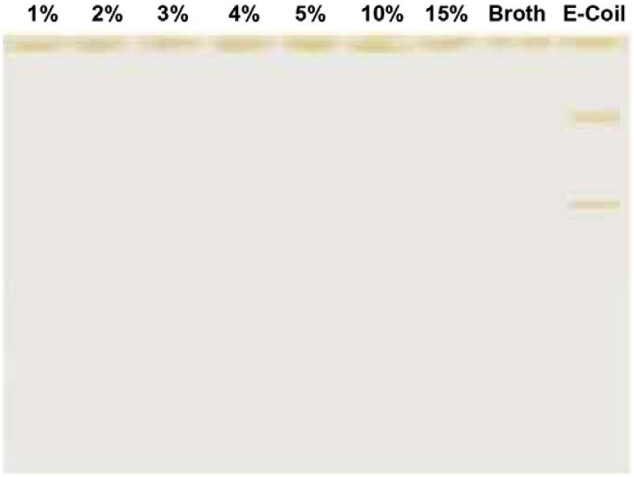

Figure 4 Photograph and diagrammatic illustration of electrophoretic amylase pattern of *E. coli* and *E. coli* inoculated with different concentrations of *Lawsonia inermis*.

Table 5 The amylase pattern of *E. coli* and *E. coli* inoculated with different concentrations of *Lawsonia inermis*.

Rows	1%		2%		3%		4%		5%		10%		15%		Broth		*E. coli*	
	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf
R_1	100 (10.5)	0.037	100 (11.6)	0.037	100 (11.6)	0.037	100 (12.3)	0.037	100 (14.5)	0.037	100 (12.2)	0.037	100 (11.2)	0.037	100 (11.3)	0.037	41.3 (11.1)	0.037
R_2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	35.7 (9.59)	0.18
R_3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	23 (6.18)	0.37

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

after *E. coli* inoculated with 1%, 2%, 3%, 4%, 5%, 10% and 15% *Li* comparing with *E. coli* recorded amount% 41.3 from the total proteins secreted. So *E. coli* injected with 1%, 2%, 3%, 4%, 5%, 10% and 15% *Li* and broth recorded duplicate amount compared by *E. coli*.

The amylase similarity index between *E. coli* and *E. coli* injected with 1%, 2%, 3%, 4%, 5%, 10%, 15% *Li* and broth recorded a low value (0.5) as shown in Table 6, indicating that the broth has no effect on amylase fractions because it is used only as a signal for growth of bacteria on it and effect of *Li* on the bacterial growth.

3.3. Glycoprotein pattern

The peroxidase pattern of *E. coli* and *E. coli* inoculated with different concentrations of *L. inermis* (1%, 2%, 3%, 4%, 5%, 10% and 15%) recorded in Fig. 5 and the data are presented in Table 7. Inspection of Fig. 5 and Table 7 reveals that only one type of glycoprotein fraction is produced from *E. coli* at R_1 with Rf 0.94. When *E. coli* was injected with 1%, 2%, 3%, 10%, 15% *Li* and broth produced one type of glycoprotein fraction at R_1 with Rf 0.94.

Comparing the effect of 1%, 2%, 3%, 10% and 15%, one observed the absence effect of *Li* on glycoprotein fraction of *E. coli* and there was no difference among the usages of *Li* with glycoprotein fraction of *E. coli*.

From these data, oneobserved the presence of one common band at R_1 with Rf 0.94 this common band was the only type of glycoprotein which was produced from *E. coli*.

The glycoprotein similarity index between *E. coli* and *E. coli* inoculated with different concentrations 1%, 2%, 3%, 10%, 15% *Li* and broth recorded a high value (1) as shown in Table 8, indicating that the broth has no effect on lipoprotein fractions because it used only as a signal for growth of bacteria on it and effect of *Li* on the bacterial growth.

4. Discussion

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 on 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, and interfere with electrons of the respiratory chain on mitochondrial membranes. Plants with naphthoquinone content are widely used in China and South America, where they are applied to malignant and parasitic disease treatment (Babula et al., 2007).

Studies on the biological activity of naturally occurring naphthoquinones, such as plumbagone, lawsone and naphthazarin (Binutu et al., 1996; Gafner et al., 1996 and Brigham et al., 1999), and naphthoquinones obtained by synthesis (Bogdanov et al., 2001 and Riffel et al., 2002), had demonstrated that these molecules inhibited pathogenic fungi and bacteria.

Table 6 The amylase similarity index (SI) and Genetic distance (Gd) between *E. coli* and *E. coli* inoculated with different concentrations of *Lawsonia inermis*.

		Concentrations of inoculated henna powder with *E. coli*							
	E. coli	1%	2%	3%	4%	5%	10%	15%	Broth
E. coli	–	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
1%	0.5	–	1	1	1	1	1	1	1
2%	0.5	0	–	1	1	1	1	1	1
3%	0.5	0	0	–	1	1	1	1	1
4%	0.5	0	0	0	–	1	1	1	1
5%	0.5	0	0	0	0	–	1	1	1
10%	0.5	0	0	0	0	0	–	1	1
15%	0.5	0	0	0	0	0	0	–	1
Broth	0.5	0	0	0	0	0	0	0	–

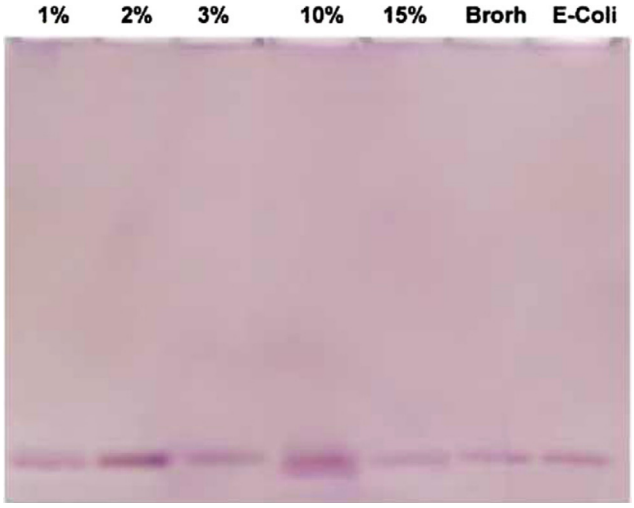

Figure 5 Photograph and diagrammatic illustration of electrophoretic glycoprotein pattern of *E. coli* and *E. coli* inoculated with different concentrations of *Lawsonia inermis*.

The mechanism of antibacterial activity of several naphthoquinones is to produce reactive oxygen species by one-electron reduction (Jarabak and Jarabak, 1995), that formed adducts with DNA, proteins, and other cell components (Zhang et al., 1994; Ollinger and Brunk, 1995), and that it also inhibited electron transport (Haraguchi et al., 1986).

The cytotoxicity of naphthoquinones is usually associated with the following: (i) the formation of adducts to cellular components (i.e., proteins and DNA); (ii) the inhibition of cellular processes, such as DNA synthesis and the electron transport chain; and (iii) redox cycling of carbonyl groups generating superoxide anion or hydrogen peroxide (Ollinger and Brunk, 1995) observed in Table 9.

4.1. Protein pattern

A genetic modification facilitates the process of constructing a desirable producer strain. A central element of metabolic models is the biomass, mainly composed of proteins, lipids, DNA and RNA.

Outer membrane proteins are indispensable components of bacterial cells and participate in several relevant functions of the microorganisms. Changes in the outer membrane proteins composition might alter antibiotic sensitivity and pathogenicity (Baneyx, 1999).

In the protein pattern, the similarity index between *E. coli* and *E. coli* inoculated with 2%, 3%, 4%, 5%, 10% and 15% *L. inermis* recorded a low value (0.28) and genetic distance (0.72) but with 1% *Li* recorded a high value (0.67) and genetic distance (0.33) indicating that most of the protein fractions in *E. coli* at high concentrations of *L. inermis* disappeared completely. The disappearance of protein bands may be attributed to the effect of high concentrations of *L. inermis* which inhibited the synthesis and expression process of *E. coli* proteins.

4.2. Amylase enzyme

It is difficult for *E. coli* to secrete products such as recombinant enzymes, because the *E. coli* has a double membrane structure and so some of the products are accumulated in a periplasmic space demonstrating that recombinant α-amylase can be released from recombinant *E. coli* during cultivation by applying a pulsed electric field (PEF). When a PEF was applied for 30 min, the amount of released α-amylase was about 30% of

Table 7 The glycoprotein pattern of *E. coli* and *E. coli* inoculated with *Lawsonia inermis* at different concentrations.

Rows	1%		2%		3%		10%		15%		Broth		*E. coli*	
	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf
R1	100 (7.42)	0.94	100 (8.76)	0.94	100 (8.66)	0.94	100 (11.9)	0.94	100 (6.84)	0.94	100 (6.46)	0.94	100 (7.29)	0.94

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

Table 8 The glycoprotein similarity index (SI) and Genetic distance (Gd) between *E. coli* and *E. coli* inoculated with *Lawsonia inermis* at different concentrations.

G.d	S.I						
		Concentrations of inoculated henna powder with *E. coli*					
	E. coli	1%	2%	3%	10%	15%	Broth
E. coli	–	1	1	1	1	1	1
1%	0	–	1	1	1	1	1
2%	0	0	–	1	1	1	1
3%	0	0	0	–	1	1	1
10%	0	0	0	0	–	1	1
15%	0	0	0	0	0	–	1
Broth	0	0	0	0	0	0	–

Table 9 Percentage inhibition of broth dilution test.

Herbal solution in mg/ml	25	35	45	55	65	75	85	95	105
E. coli (EC)	0	40 (4.46)	68 (3.37)	92 (2.44)	100	–	–	–	–

Undermarked readings indicate the corresponding MIC concentrations in mg/ml.
EC: 55–65.

the total amount of α-amylase produced in the cells. As a result of PAGE and activity staining analyses, it was confirmed that the released proteins were not all of the intracellular proteins, and the α-amylase, which was identical with intracellular α-amylase, was released by applied PEF cultivation. PEF treatment could be useful for easy release of periplasmic protein with selectivity (Shiina et al., 2008).

The purified protein rapidly digested amylose, starch, amylopectin, and maltodextrins. It also digested glycogen, but much more slowly. It was specific for the alpha-linkage, being unable to digest cellulose. The principal products of starch digestion included maltotriose and maltotetraose as well as maltose, verifying that the protein was an alpha-amylase rather than a beta-amylase. The newly discovered gene has been named amyA. (Raha et al., 1992).

In the amylase pattern, the similarity index between *E. coli* and *E. coli* inoculated with different concentrations of *L. inermis* or with broth recorded a low value (0.5) and genetic distance (0.5), indicated the strong effect of *L. inermis* to disappear of *E. coli* amylase bands (Qualitative mutation).

4.3. Glycoprotein

Glycosylation is the enzymatic process that links saccharides to produce glycans, attached to proteins, lipids, or other organic molecules (Varki, 2009) Most glycosylation takes place in the endoplasmic reticulum and golgi apparatus, there are a large family of nucleotide sugar transporters that allow nucleotide sugars to move from the cytoplasm, where they are produced, into the organelles where they are consumed (Handford et al., 2006 and Gerardy-Schahn et al., 2001).

Glycosylation is now being increasingly reported in prokaryotes, the capabilities for carbohydrate structure determination of bacterial glycoproteins and the emergence of glycoproteomic strategies that have evolved from proteomics and genomics for the functional analysis of bacterial glycosylation (Paul and Anne Dell, 2006).

There are two main types of protein glycosylation: *N*-glycosylation, in which the oligosaccharide is attached to an asparagine residue, and *O*-glycosylation, in which the oligosaccharide can be attached to a serine, threonine or tyrosine residue. The glycans found on prokaryotic glycoproteins are far more diverse in terms of sugar composition and structure than those found in eukaryotic organisms. More recently, non-S-layer glycoproteins have been discovered in bacteria, particularly in the medically relevant pathogens (Benz and Schmidt, 2002; Szymanski and Wren, 2005 and Upreti et al., 2003).

In the glycoprotein pattern, the similarity index between *E. coli* and *E. coli* inoculated the highest value (1) and genetic distance (0) indicated the absence effect of *L. inermis* to disappear of glycoprotein bands.

5. Conclusion

Medical plants are commonly used by local inhabitants for their invitro antibacterial activity. Use of a simple method for extraction of *L. inermis* leaves has proven to be successful in the estimation of antimicrobial activity against *E. coli* which is responsible for urinary tract infections.

The data confirmed the effective role of *L. inermis* to cause high disturbances of protein, amylase and glycoprotein fractions of *E. coli* and a high effect of *L. inermis* on bacterial growth at a higher or a lower concentration of *L. inermis*.

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