

BIOACTIVITY CHARACTERIZATION OF PURIFIED RECOMBINANT HYPOTHETICAL PROTEIN CODED BY OPEN READING FRAME-112 OF *STREPTOMYCES*.

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ABSTRACT

This study was aimed to investigate the Open Reading Frame-112 (ORF-112) gene, which encoded for a hypothetical 218 amino acids protein in *Streptomyces* bacteria. A complete ORF-112 gene was synthesized, with addition of a 6xHis-Tag at the N-terminal location. The synthesized DNA nucleotides were sub-cloned into bacterial expression plasmid pBAT4. The pBAT4-ORF-112 plasmid transformed in bacterial cells BL21(De3)pLysS, intended for protein over expression, induced by isopropyl β -d-1-thiogalactopyranoside (IPTG). The IMAC affinity chromatography technique was deployed for protein purifications. Highly-purified fractions of ORF-112 were achieved by using affinity Ni^{++} -columns. The purified ORF-112 protein was tested for possible biological activity. The SDS-PAGE analysis exhibited a soluble 30 kDa size purified ORF-112 protein which showed a slight gel shifting from the predicted size. The virulent activity test on purified fractions of ORF-112 was measured using the Disk Diffusion Test and it disclosed a clear zone formed in response to fungi *Candida albicans* growth. The data implies that the ORF-112 protein has an acceptable protective effect against the fungus *C. albicans* as compared to positive control ketoconazole (KCZ) ($P < 0.05$) while the protein has a significantly lower protective effect against the fungus than Itraconazole (ICZ) ($P > 0.05$). The results clarify the hypothetical ORF-112 protein is a novel protein with protective response effect against fungi cells *C. albicans* on disk-diffusion test.

KEYWORDS: Bioactive compound; *Candida albicans* sensitivity; hypothetical protein-ORF-112; *Streptomyces*.

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تنقية وتوصيف الفعالية الحيوية للبروتين الافتراضي المشفر من Open Reading Frame-112 لبكتيريا *Streptomyces*

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المستخلص

يهدف البحث الى دراسة الجين Open Reading Frame-112 (ORF-112) ، والذي تم تشفيره لإنتاج البروتين الافتراضي المكون من 218 حامض أميني لبكتيريا *Streptomyces*. صنع جين ORF-112 كاملاً ، مع إضافة 6xHis-Tag في الموقع الطرفي N-terminal ثم أستنسخ (cloning) جين ORF-112 المُصنَّع في بلازميد pBAT4 بهدف التعبير الجرثومي للجين. تم تحويل (Transformation) البلازميد المُكَلَّون pBAT4-ORF-112 الى خلايا بكتيريا BL21(De3)pLysS *E.coli* ليُعَبَّر عن البروتين عبر استعمال مركب isopropyl B-d-1-thiogalactopyranoside (IPTG) ثم إستُخِدِمَت تقنية الكروماتوجرافي الإنجذابي IMAC affinity chromatography technique لتنقية البروتين. لقد تم الحصول على بروتين ORF-112 عالي النقاوة بإستخدام أعمدة Ni^{++} . أُخْتَبِر البروتين المنقى ORF-112 للكشف عن وجود فعالية حيوية محتملة بإستخدام Disk Diffusion Test. كشف تحليل SDS-PAGE وجود بروتين ORF-112 القابل للذوبان بحجم 30 كيلو دالتون والذي أظهر تغيراً طفيفاً في حجمه عن الحجم المتوقع عند ترجيله في الهلام. كما أظهرت نتائج Disk Diffusion Test منطقة تثبيط واضحة Clear zone تشكلت إستجابة لنمو فطر *Candida albicans*. تشير البيانات إلى أن بروتين ORF-112 له تأثيراً وقائياً واضحاً ضد الفطر *C. albicans* مقارنة مع المضاد الفطري Ketoconazole ($P > 0.05$) في حين أظهرت النتائج تأثيراً وقائياً أقل للبروتين ضد الفطر مقارنة مع المضاد الفطري Itraconazole ($P > 0.05$). تشير نتائجنا إلى أن بروتين ORF-112 الافتراضي هو بروتين جديد له تأثير إستجابة وقائية ضد خلايا *C. albicans* في اختبار Disk- Diffusion Test.

الكلمات الرئيسية: المركبات ذات الفعالية الحيوية ؛ حساسية *Candida albicans*؛ بروتين افتراضي؛ *Streptomyces*

INTRODUCTION

The soil bacterium species of the *Streptomyces* belong to the Actinobacteria phylum, characterized by a broad spectrum of modes of action, such as antibiotic production and antimicrobial activities. They produce a variety of biologically active agents alone or amalgamation with other bioactive agents (3, 7). *Streptomyces* bacteria contain three types of plasmids, large (pSLA2-L), medium (pSLA2-M) and small (pSLA2-S). The unknown ORF-112 gene product (Accession no. NP_851534.1) encoded for a hypothetical 218 amino acid protein, which remains to be characterized, is mapped at the large plasmid (pSLA2-L) (14). Protein affinity chromatography techniques have been utilized for endogenous and/or recombinant protein purification, which allow us to extend our understandings in term of: a) perform detailed functional analysis, b) determine 3D structures, and could also be employed as a target for drug-screening studies (1). Proteins are very diverse group of macromolecules, many newly unverified coding regions of ORF exist in the sequence databases, stays annotated as Hypothetical Proteins' (HPs) of uncharacterised protein. Bioinformaticians often anticipate new HPs from compatible open reading frame (ORF) genome regions, but still experimental proof is required for an actual protein expression, both prokaryotes and eukaryotes (9) and functional studies will be demonstrated through in cell biology research using molecular biology techniques (10). Therefore, we have deployed poly-histidine tag in order to purify proteins with ease and as a primary attempt to predict the hypothetical protein ORF-112 function, with limited resources at our research laboratory facility. It is well-known that the hexa histidine (6xHis) tag add 1 kDa to the recombination protein ORF-112 size, separated with a glycine-linker between the coding region and the tag in order to avoid any structural hinder with the parental protein (28). In current study, we wanted to explore the biological activity of recombinant HP protein ORF-112, using bacterial expression system. We have been successful in using Ni⁺⁺-metal affinity chromatography to enrich the 6xHis-recombinant ORF-112 protein. Then to

characterize the protein size and purity on SDS-PAGE. The pooling fractions of the purified protein were used to measure bioactivity feature on disk diffusion assay.

MATERIALS AND METHODS

Bacterial isolate identification

Streptomyces isolate was a gift from the University of Baghdad, College of Science, Department of Biotechnology. The isolate was validated and confirmed by examining the gene 16S rRNA using conventional PCR technique as previously described (13, 23). Briefly, the PCR device run for 30-35 cycles, denaturing stage 94 °C/5 min., annealing stage 50°C/1 min., extension stage 72°C/1 min, and the final extension 72°C/7 min (23). The primer sequence used for PCR were 5'-TCACGGAGAGTTTGATCCTG-3' for the forward primer and 5'-GCGGCTGCTGGCACGTAGTT-3' for the reverse primers (13).

Genomic and plasmid DNA extraction

For genomic and plasmid DNA extraction the *AccuPrep*TM Genomic DNA Extraction Kit (Bioneer, South Korea) and *AccuPrep*[®] Plasmid Mini Extraction Kit (Bioneer, South Korea) were used according the manufacturer's instructions. For later use, the purified DNA plasmids were aliquoted and stored at -20 °C. To determine the DNA concentration and DNA quality, the spectrometer Genova Nano (Jenway, UK) was used.

Gene synthesis and plasmid cloning of ORF-112

The ORF-112 gene synthesized with 6x-His-tag located at the N-terminal of ORF-112 sequence and expressed as a single transcript (GeneScript, USA), flanked with the restriction sequences *NcoI* at 5'-end and *NotI* at 3'-end, which is used for sub-cloning into the bacterial plasmid pBAT4. Briefly, the synthesized gene ligated with pBAT4 vector by using T4 DNA ligase followed by transformation of recombinant plasmid pBAT-6x-HIS-ORF-112 into competent *E.coli* DH5- α . To identify any defect in cloning process, the blue-white screening (*lac* selection) was used by culturing the transformed bacteria on LB plates containing 100 μ g/ml Ampicillin, 100 μ g/ml X-gal and 1mM IPTG then incubated over night at 37°C (20). The pBAT4

cloned vectors were extracted from positive *E. coli* DH5- α cells (white colonies) following instructions of AccuPrep[®] Plasmid Mini Extraction Kit (Bioneer, South Korea) and was sequenced by Sanger DNA sequencing (GenScript, USA). Cloned ORF-112 gene was digested by *NcoI* and *NotI* at the sites 84 bp and 794 bp respectively and electrophorized on agarose gel electrophoresis 1%. The pQE40-6x-His-Tag-DHFR (Mouse Dihydro Folate Reductase) supplied by the Ni⁺⁺-NTA Spin Kit (Qiagen/ Germany) was applied as a positive control, expressing a 24.5 kDa DHFR protein.

Transformation and induction of bacterial cells *E. coli* BL21(De3)pLysS

The *E. coli* BL21(De3)pLysS (Promega, USA) cells was utilized for recombinant protein expression. The bacterial transformation of the plasmid pBAT4-6x-His-ORF-112, plasmid pQE40-6x-His-DHFR (positive control) and negative controls; non-induced (NI) and non-transformed (NT) were introduced into competent *E. coli* BL21 cells with heat shock method. Both plasmids contain Ampicillin resistant gene (100 μ g/ml). The transformant colonies of *E. coli* BL21 cells were incubated for 3-4 h under 37 °C in falcon tubes with LB medium containing (100 μ g/ml) Ampicillin, until the optical density reached 0.6 at 600 nm. Next, the BL21 cells were induced (except for negative control) with 1 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) (8) and incubated under the same conditions for another 60 min. Finally, all samples were centrifuged at 4 °C for 25 min at 5000 rpm and the pellet and supernatant were kept for subsequent analysis at - 80 °C (19).

Purification of His-Tag recombinant proteins

The recombinant proteins 6x-His-ORF-112, 6x-His-DHFR (positive control) and negative controls (NT and NI) were purified on the Ni⁺⁺-NTA Spin Kit (Qiagen/ Germany), according to the manufacturer's instruction. Briefly, the cell pellets thawed, re-suspended in 700 μ L lysis buffer/binding buffer with 3 μ L/mL nuclease and then incubated with agitation at RT. The cell lysates were sonicated on ice with three to five seconds pulses at high intensity. The lysate centrifuged at 12000 x g for 15-30 min at RT to pellet the

cellular debris. The protein content of ORF-112, positive and negative controls were resolved using 12.5% Sodium Dodecyl Sulfate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) with standard protein marker (11 kDa-260 kDa) on a separate lane. The gel stained with Coomassie blue-R staining solution for 4 h, and then de-stained overnight (21, 2).

Biological assessment of ORF-112

Kirby-Bauer Disk-diffusion test

The Muller-Hinton agar plates were prepared according to previous method with slight modifications (26). The disks of 6 mm diameter were papered and soaked with 20 μ L of sample solutions followed by dryness of the disks at 37°C/4 hours and were placed in petri dishes approximately 5 mm apart and stored at -20 °C. The disks prepared with protein fractions enriched with ORF-112 were screened for the organism's sensitivity ranges. The organisms used were *Staphylococcus aureus* ATCC 25923, *Acinetobacter Baumannii* and *Candida albicans* wild-type standard strains. The concentrations of ORF-112 purified samples were of 2 mg/L (applied 2 ng/ μ L). For positive controls, two commercial anti-fungal drugs were used with recommended concentrations 16 mg/L (applied 16 ng/ μ L) for Ketoconazole (KCZ) (29), (5) and 16.1 mg/L (applied 16.1 ng/ μ L) for Itraconazole (ICZ) (12), (31). Glycerol (10%) and negative control sample with elution buffer of the IMAC purification kit were used as negative controls. After incubation, the zone of inhibition was measured for each disk.

Statistical analysis

Software data including means, standard deviations, standard errors and T-test were coded in Microsoft Excel 2010 software (Microsoft Corporation, Redmond, WA) and analysed using IBM-SPSS Statistics software version 26.

Prediction of ORF-112 protein functional

annotation: calculator v 3.4 (www.protcac.sourceforge.net) and Predict Protein (PP (www.predictprotein.org)) were used to predict ORF-112 by submission of amino acids FASTA of ORF-112 protein (NP_851534.1). Protein calculator estimates parameters based on a one-dimensional protein sequence,

molecular weight-based calculations, estimated charge-based calculations, estimated UV absorption-based calculations, atom and residue counting and solvent content calculations for X-ray crystallography. Predict Protein (PP) calculates secondary structure and returning families of related proteins.

RESULTS AND DISCUSSION

Bacterial isolate identification: The purity and concentration of the DNA extracted from the bacterial isolates were ranged from 45 to 63 ng/μL, and the purity of the genomic and plasmid DNA ranged from 1.71 to 2.09 (based on the relative ratio of the absorbance at 260/280 nm). The isolate was designed as *Streptomyces* spp. after recovering 500 bp

amplicon from the PCR run designed for the 16S rRNA gene, confirming the positive band size according to the previous identification procedure (23) (Figure 1A).

Construction of pBAT4-6x-His-ORF-112

The gene synthesis and sub-cloning construction of pBAT4-6x-His-ORF-112 was sequenced using Sanger DNA sequencing (GenScript, USA) (Figure 2). As shown in Figure 1B and 1C, a correct fragments size were released after restriction digestion with *NcoI* and *NotI* which resulted the 6x-His-ORF-112 band 710 bp and the plasmid backbone pBAT4 was also correct 4368 bp band size (Figure 1B and 1C).

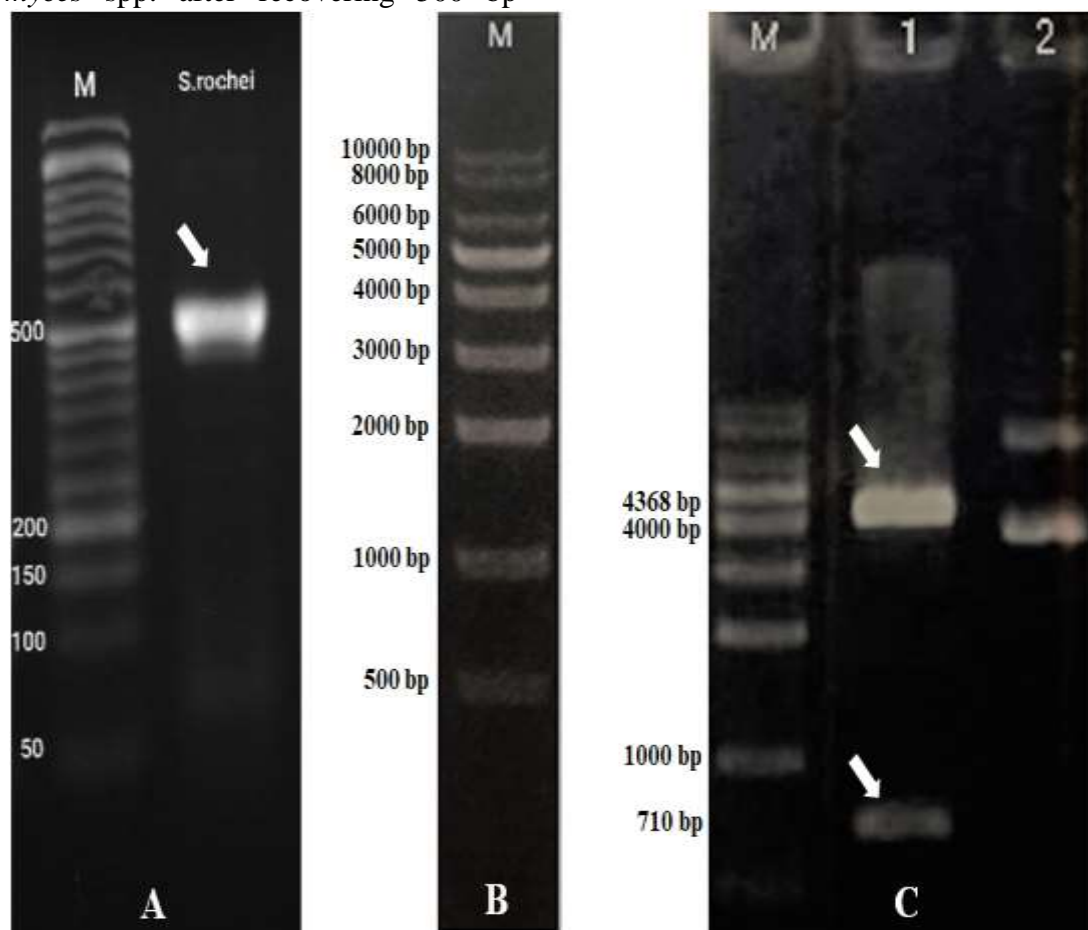


Figure 1. PCR product of 16S rRNA gene and the restriction digestion of constructed pBAT4-6x-His-ORF-112.

(A) PCR product confirming the *Streptomyces* spp. (13). Lane M: 50 bp ladder marker. Lane 2: The 500 bp band represents the 16S rRNA of *Streptomyces* spp. (B) Lane M: KB Ladder. (C) Lane M: DNA KB Ladder, Lane 1: The restriction digestion of constructed pBAT4-6xHis-ORF-112 by *NcoI* and *NotI* at the sites 84 bp and 794 bp respectively to yield two linear bands comprising 710 bp His-Tag-ORF-112 and 4368 bp the backbone of the plasmid, lane 2: non-digested plasmid pBAT4-6xHis-ORF-112 showing three bands representing the three topological structures of plasmid (L, OC, and S).

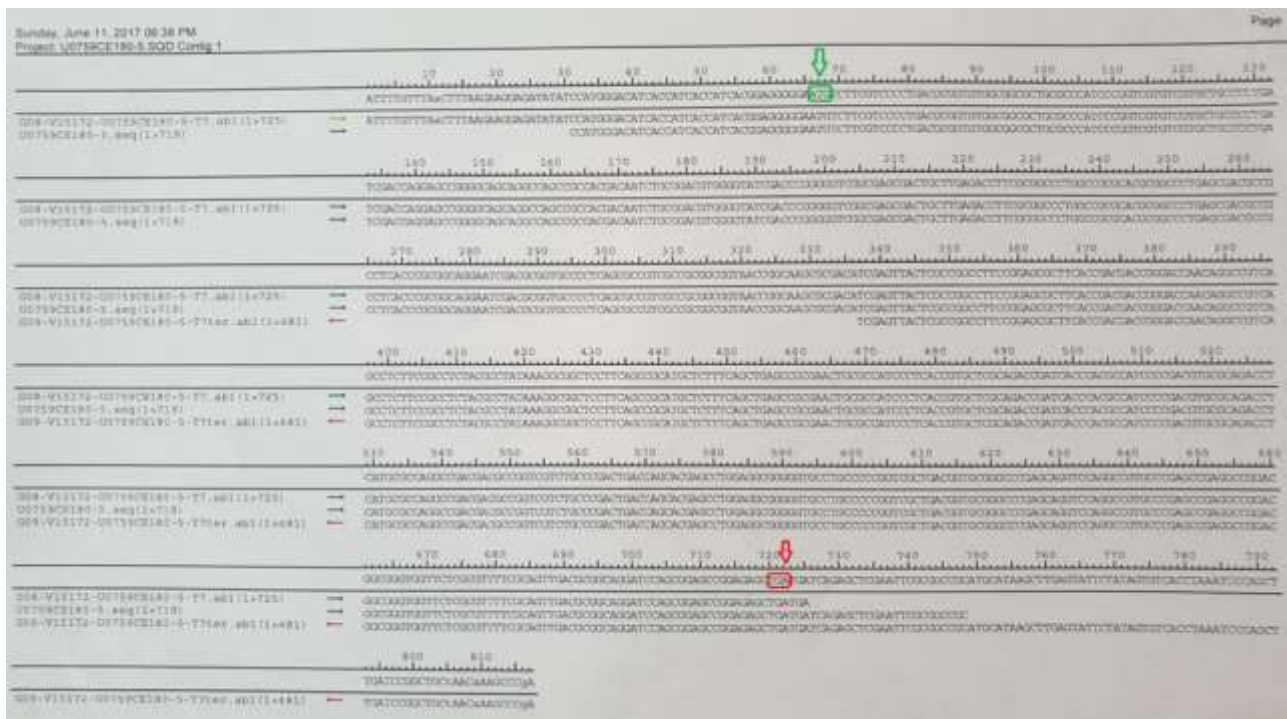


Figure 2. The DNA sequence of cloned ORF-112 (656 bp) and flanking regions (GenScript, USA). The green arrow represents the start codon of ORF-112 located at 66-68 bp while the stop codon is located at the position 720-722 bp of the sequence (red arrow) representing the correct sequence and size ORF-112 (656 bp).

Expression of the heterologous recombinant 6x-His-ORF-112 : To verify the expression of ORF-112 protein, the protein bands were confirmed and separated according to their molecular weight with 12.5% SDS-PAGE. The ORF-112 band was clearly visible as one band with molecular weight about 30 KDa (Figure 3A, Lane 2 & Figure 3B, Lane 4-5). The negative control (NT) sample confirmed that the ORF-112 is not present and does not grow on LB medium in the presence of Ampicillin (Figure 3B, lane 2). The non-IPTG induced sample (NI) did not express the corresponding band size for ORF-112 (Figure

3B, lane 6), while the positive control DHFR express DHFR protein at size 30 KDa (Figure 3B, Lanes 3). As shown in Figure 3, both ORF-112 and DHFR proteins are present in a molecular weight size 30 kDa, as the 6xHis-tag add 1 kDa size on both proteins (28). In all our purification attempts, an additional 48 kDa protein contaminant appears in the SDS-PAGE gels (all lanes), including the negative control, that suggest a common sticky protein appears in all fractions. It was found that this contaminant protein is difficult to wash away or to get rid of it by additional cleaning steps (not shown).

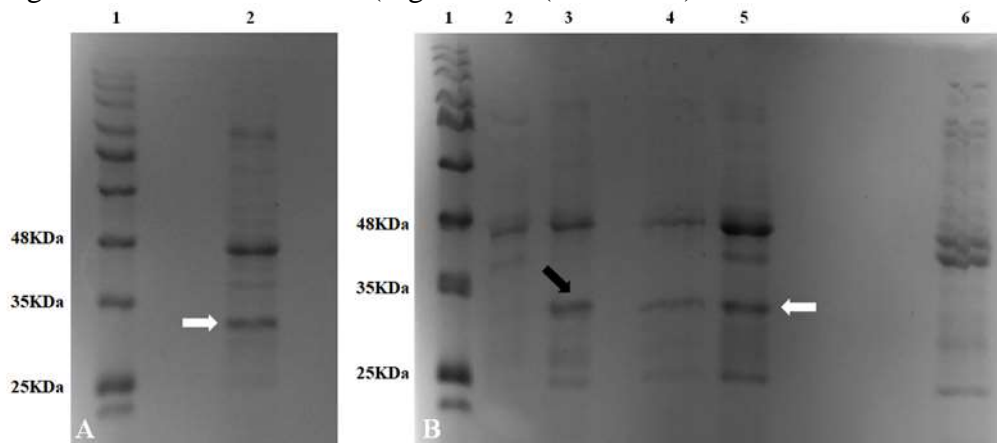


Figure 3. SDS-PAGE 12.5%.

(A) Lane1: pre-stained protein ladder 11KDa-260KDa, Lane 2: ORF-112 protein 30 KDa (white arrow). (B) Lane 1: pre-stained protein ladder 11KDa-260KDa, Lane 2: -ve control Non-Transformed, Lane 3: DHFR (+ve control) 30 KDa (Black arrow), Lane 4 and 5: induced ORF-112 30 KDa protein (white arrow), Lane 6: Non-Induced ORF-112.

Biological activity of ORF-112

The disk-diffusion test for bacterial strains *Staphylococcus aureus* and *Acinetobacter baumannii*, were negative as no zone of inhibition observed (data not shown). The yeast *C. albicans* gave different zone of inhibition sizes in response to the activity of purified ORF-112 protein (Figure 4). The means of zone of inhibition of ORF-112 are shown in (Table 1), including positive controls and two negative controls (Addition of 20 μ L of each reagents and protein in respective spot/wells). For positive control, two anti-fungal drugs applied (Itraconazole and Ketoconazole). For negative control, glycerol (G) and negative control elution buffer (N) were utilized. These data are a sum of three independent results. Regarding biostatistics analysis the zone of inhibition means of ORF-112 were acceptable as compared to Ketoconazole means ($P < 0.05$), but the protein gave markedly lower means than those of Itraconazole ($P > 0.05$). The standard error of ORF-112, KCZ and ICZ values were 0.83,

1.10 and 1.24 respectively (Table 1) (Figure 5).

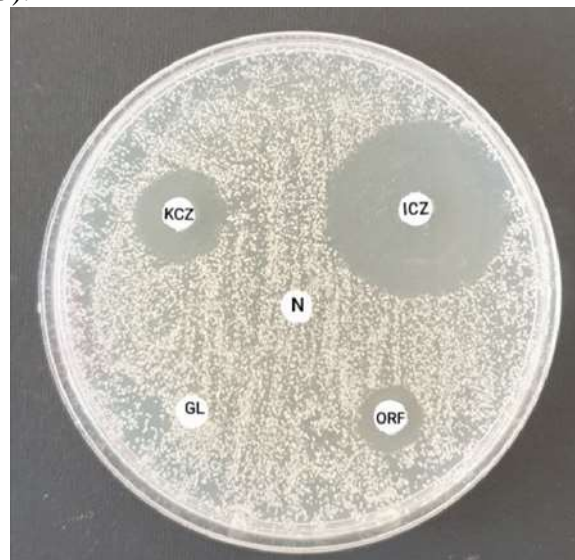


Figure 4. Kirby-Bauer Disk-diffusion test used to determine the biological effect of ORF-112 protein against *Candida albicans*. In comparison to positive controls Ketoconazole (KCZ) and Itraconazole (ICZ); and two negative controls Glycerol (GL) and Elution buffer (N). This is a representative image from 12 repeats

Table 1. Zones of inhibition of ORF-112 compared to positive controls and negative controls †

Groups	A. Purified Protein	B. Positive Control Drugs		C. Negative Control Reagent	
Reagents and Proteins	ORF-112‡	1. KCZ#	2. ICZ*	1. G*** (100%)	2. PSB***
Concentration (ng / μ L)	2	16.0	16.1	20 μ L / spot	20 μ L / spot
Zone of inhibition (mm) range of <i>Candida albicans</i>	9-15	14-24	36-40	0	0
Mean	13.25	18.83	36.83	0	0
Standard Error	0.83	1.10	1.24	0	0
P value group A and B-1	< 0.05			-	
P value group A and B-2		> 0.05		-	

†Data are presented as Mean \pm SEM; ‡Open Reading Frame-112 protein; #Ketoconazole, *Itraconazole; ** Glycerol; *** Protein Sample Buffer

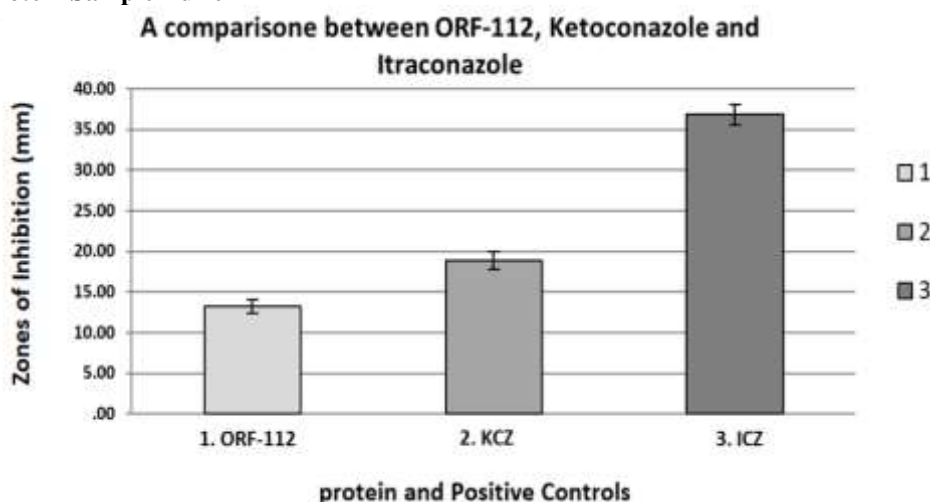


Figure 5. Means of zone of inhibition induced by three groups (ORF-112, Ketoconazole and Itraconazole).

Positive control represented by two anti-fungal drugs applied (Itraconazole and Ketoconazole). These data are a sum of 12 repeats. Standard deviation and statistical significance were calculated using SSPS V.26.

Prediction of ORF-112 protein functional annotation: ORF-112 Predict Protein calculation encoded residue composition, secondary structure, Solvent accessibility, transmembrane helix prediction, disulphide bridges, and finally trans-membrane beta barrels structures (data not shown). For protein calculator the predicted results were; M.W. = 24.5, isoelectric point (pI) 8.85 and charge over pH range (data not shown). The current study highlights the conceivable biological activity of the novel ORF-112 purified protein *in vitro*. The function of ORF-112 protein was validated by performing the disk diffusion test against three chosen microorganisms; Gram's negative bacteria *A. baumannii*, Gram's positive *S. aureus* and *C. albicans* yeast. The results disclosed that purified fraction of ORF-112 did not have any biological activity toward both G -ve and G +ve bacteria, but it has a positive impact on *C. albicans* and produce a zone of inhibition range (9-15 mm), while the positive controls; KCZ and ICZ gave a range for zone of inhibition 14-24 mm and 36-40 mm respectively (Table 1). The zone of inhibition mean induced by ICZ was markedly above the findings recorded in previous study (11) which was 13 mm. The ketoconazole results were compatible with findings of (24) which gave zone of inhibition mean 22.3 mm. Surprisingly, the zone of inhibition means of KCZ and ICZ showed high difference measurements (18.83 mm and 36.83 mm respectively). When comparing positive controls means to ORF-112 protein mean (13.25 mm) the KCZ has a non-significant ($P < 0.05$) and slightly higher protective effect but the mean of ICZ was significantly higher than zone of inhibition mean of ORF-112 ($P > 0.05$) (Table 1 and Figure 5). The variance in zones of inhibition of the tested protein and positive controls can be clarified as the level of ORF-112 after freeze/dry vacuum concentration (2 mg/L) was eight times lower in contrast to the positive controls concentrations of both anti-fungal drugs (16 mg/L) thus, the low zone of inhibition is expected due to the low concentration of purified protein, in taking to consideration the recommended minimum inhibitory concentrations (MIC) of ICZ is 16.1 mg/L

(0.0161 $\mu\text{g}/\mu\text{L}$) and for KCZ is 16 mg/L (0.016 $\mu\text{g}/\mu\text{L}$).

Gel shifting

The predicted size of the ORF-112 protein and DHFR proteins was 24.5 KDa for both proteins (protein calculator/ DNA to protein by HSLs). Surprisingly the SDS-PAGE electrophoresis demonstrated about 30 KDa band for both purified proteins. It's not uncommon that proteins run aberrantly on SDS-PAGE, it can often be explained as gel shifting (18). There are different reasons cause of what so called "gel shifting" on SDS-PAGE electrophoresis such as: a) ratio of SDS binding to protein, b) numbers and positions of disulfide linkages, c) isoelectric point (pI) / pH of the target protein, d) type of protein (transmembrane or cytosolic), e) acidic amino acids constituent of protein, f) post translational modifications (PTM). The structure of proteins can influence both, the concentrations of detergent loading, and the rates of migration of polypeptide-SDS-PAGE (18). Usually the inside of protein consists of hydrophobic amino acids (AAs) while the outside consists of hydrophilic AAs (30). Determining molecular weight (MW) of protein through PAGE in the existence of sodium dodecyl sulfate (SDS) is a technique that is widely used in biomedical research. This condition is accomplished not by the complete unfolding of proteins but by the aggregation of SDS molecules at hydrophobic protein locations to initiate "reconstructive denaturation" (27). A provision for SDS-PAGE MW estimation is a rational quantity of detergent binding within proteins. For instance (4) suggested that, the maximum SDS-binding concentrations are generally estimated at 1.4 g SDS / g protein. It is known that disulfide bonds decrease SDS binding to proteins by up to 2-fold (22). The pI of proteins affects mobility too, because the presence of many charged AAs leads to disruption of their binding to SDS micelles (15). Normally each amino acid binds to one micelle. In correlation to the effect of previous factors the predicted percentage of ORF-112 content of hydrophobic AAs (52 %) which was higher than hydrophilic AAs (48%), the pI was 8.85 and ORF-112 has five cysteine residues. A disruption of proteins binding to SDS micelles

may be occurred resulting in retarded migration on the gel. Another point is the percentage of acidic amino acids within protein. A study conducted by (6) found that the discrepancy concerning the predicted and SDS-PAGE shown MW revealed a linear correlation with the percentage of acidic AA that matches the equation $y = 276.5x - 31.33$ (x represents the percentage of acidic AA; y represents the average Δ MW per AA). They suggested that the nucleolar Def protein in zebrafish is composed of 753 AA with a predicted MW of around 86.8 kDa. However, they noticed the endogenous Def protein migrated as a protein of nearly 100.0 kDa in an SDS-PAGE gel, approximately 13 kDa larger than the predicted MW, they also found that, the protein that contains a high percentage of acidic residues (E+D) gave a linear correlation with the average Δ MW (6). This equation was applied to the hypothetical protein ORF-112 and DHFR which showed a percentage for acidic residues aspartate (D) + glutamate (E) 10.6% and 12.1% respectively to give an interesting result to ORF-112 and DHFR, the Δ MW were 2.899 KDa and 3.345 KDa, respectively, and that explains the presence of a higher molecular weight bands on SDS-PAGE gels than those predicted by bioinformatics applications i.e. the ORF-112 and DHFR molecular weight showed an extra 3 KDa on the gel. The post-translational modification (PTM) can affect the migration of proteins. Usually amino acids (AAs) Lysine (K), arginine (R), tyrosine (Y), threonine (T) and serine (S) go through PTM. Studies found that PTM may produce a retarded gel mobility shift of proteins as glycosylation (25), methylation, acetylation, sumoylation and ubiquitination (32). It is generally expected that the actual MW is always higher than theoretical one (17) due to addition of extra Daltons to the protein, for example; phosphorylation (+80 Da), sulfation (+80 Da), nitration (+45 Da), O-glycosylation (>203 Da), and acylation (>200 Da), Ubiquitin (+8800 Da) and formylation is similar to acetylation (but with a smaller, 28 Da mass shift) (16). Regarding the previous studies of PTM and oligomerization they usually resulting in differences in protein interactions to SDS micelles to produce a gel

shift to protein. The ORF-112 protein bioinformatic analysis showed the presence of abundant ratios of R (11.5%), T (5%) and S (7.8%) in their structures that can be subjected to PTM and finally cause retardation of protein movement.

CONCLUSION

In conclusion, the purified ORF-112 is a novel protein which has a biological activity toward *C. albicans* but it did not show any activity to G+ve (*staphylococcus aureus*) and G-ve (*Acinetobacter Baumannii*). Therefore, we have concluded that the novel ORF-112 protein to be directly involved in defence mechanism toward fungus. Although, we do not know the mechanism of action, but our data reveal a novel biological action of ORF-112. Before we could speculate on the mode of action ORF-112, more studies in future required exploring further functional studies.

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