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The synergistic effect of secondary metabolites of locale isolates of lactic acid bacteria with some plant extract against Aspergillus Niger

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***Abstract*---**Food is important for survival organisms. Food preservation is an important method preserving food. There are many issues on it, to avoid these problems and reduce side effects when using traditional methods of food preservation. The use of probiotics has been resorted to. Lactic Acid Bacteria (LAB) is among the distinct groups of bacteria as probiotics. It produces many antimicrobial contents, including antifungal. Therefore, secondary metabolites were extracted for some locale LAB isolates (Pediococcus sp., Enterococcus sp. and Lactobacillus sp.) in synergy with aqueous extracts of Syzygium and Cinnamon and Aspergillus niger was treated with these extracts. The results of statistical analysis showed a synergistic antifungal activity of these extracts against A.niger The highest synergistic effect was of Lactobacillus sp (L2) extract that synergy with Syzygium extract with a significant difference at probability 0.05 and it was % 96 with growth area 50.24 mm2 as compared with area growth of control 1674.66 mm2, while the synergistic activity of bacterial extracts with Cinnamon extract were less than with Syzygium extract. As the highest inhibition against A. niger was reached for Lactobacillus sp (L2) that synergistic with Cinnamon extract, the percentage was %57 with growth area as 706.5 mm2 compared with control.

***Keywords*---**lactic acid, plant extract, synergistic activity, antifungal activity, bacteria extract.

Introduction

Over the last decades, Researchers have focused more emphasis on the health advantages of microbes species which inhibiting in mammals, including humans. The reason is because the gut microbiota is considered to be the animal's greatest bacterial reservoir(Reuben et al. 2019). Food-borne infections linked to the use of fresh, minimally processed agricultural goods have caused widespread outbreaks and health issues. As the side effects of using artificial antibiotics and preservatives incorrectly have become significant. Traditional sterilization procedures in the food industries include the use of high-temperature thermal or chemical sanitizers treatment. However, dangerous bacteria are not completely organoleptic and eradicated properties decline as a result .So, numerous research aims to use antibacterial substances derived from microbes to enhance shelf life and antibacterial properties (Ren et al. 2018).Lactic Acid Bacteria are microbes that used for preventing the growth of pathogens. (LAB) is a varied group that has been identified as possess the ability to develop integrated bio refineries.

LAB are Gram-positive rods or cocci that are non-motile, non –sporulating, non-respiring but aerotolerant, catalase-negative and acid-tolerant. During its development LAB creates a variety of antimicrobial metabolites. These organic acids and metabolites including, benzoic acids, phenyllactic, hydroxy phenyllactic, fatty acids, volatile chemicals (such as diacetyl, acetone), hydrogen peroxide, cyclic dipeptides, reuterin, hydrogen peroxide, and/or proteinaceous substances, have antifungal action (Leyva Salas et al. 2017). The use of LAB to decrease level or completely replace antifungal chemical preservatives is certainly an option worth considering, so these micro - organisms are known to be safe (as demonstrated by (QPS) Qualified Presumption of Safety or Recognized as Safe - GRAS- status), have a long and varied history use, and clearly show antifungal properties.

In the food industry, lactic acid bacteria have a prolonged history of use. Some species are found naturally in the human microbiome and have beneficial effects for human health. Some organisms are naturally occurring in the human microbiota, because of their lengthy history of usage and significant biotechnology potential, various methods have been developed to take use of their engineering benefits for human health (Plavec and Berlec 2020). Their usage in bio-preservation of food is seen as a method to preventing pathogenic microbes from growing (O. Y. Ramos et al. 2020).

Medicinal Plant

For decades, plant compounds have been used to prevent illnesses and treat ailments. natural resource, like medicinal plants, have produced several pharmacologically active drugs. (Heydari et al. 2019) .In this study we searched the effect of crud extract of Syzygium aromaticum and Cinnamomum cassia synergistic with secondary metabolic product of LAB against Aspergillus niger.

Fungus used in experiment

Fungi are a varied group of organisms that play an important role in the functioning of environments. Through symbiotic relationships. It require specific ecosystem properties for suitable habitat, so the communities of fungi may be disturbed when their habitat polluted with chemicals or becomes eutrophic (Newbound, Mccarthy, and Lebel 2010). In our study we used Aspergillus niger. It can be found in a wide variety of agricultural products (e.g., different types of onion, fruits, corn and nuts) under a wide temperature rate (6–47 °C) and pH (1.5–9.8). So, it has made a significant contribution to our research by acting as a model organism. The broad importance of Aspergillus spp. pushed it to the forefront of fungal studies in the medical and industrial communities. The studies of Aspergillus increased because availability of genomic sequences of Aspergillus species (De Vries et al. 2017).

Methods

Sample Collection

Forty eight isolates of Lactic Acid Bacteria were collected from different sources in AL-Ramadi city (raw milk of cows, sheep and goats, canned food, fermented meat, canned cheese and dairy, pickles, vegetables and fruits). in iddition from fecal of children who depend on breastfeeding for their nutrition, whose ages don't exceed 4 mounths and have never been a mother or child take antibiotic. Samples were collected using sterile nylon bags and brought to the laboratory.

Isolation and Identification of Lactic Acid Bacteria

After samples collection, they were labeled. Serial dilution were done (102 to 109 time) (Janssen et al. 1997). Incubated about 48 h. at 35 C0 (Das and Prasad 2010). Typical strains of LAB were picked up randomly and purify by streaking for two or three time, followed by some examinations (Wassie and Wassie 2016), which will mention later. After isolated of bacterial strains we carried out Gram stain, catalase, oxidase reaction, salinity tolerance test and IMVIC tests. Then Vitek device used to insure the identification of the isolates which identified as Lactobacillus acidophilus , Enterococcus faecalis and Pediococcus acidolactici then we reduced the samples based on similarity of phenotypic , microscopic characteristics and biochemical tests stored in MRS Agar slant tubes to use in the experiment.

Extracting LAB Metabolites

About 100 ml of MRS broth was fermented after inoculation with 1ml of activated isolates overnight. Then we carried out a cooling centrifuge of 10000 rpm for 10 minutes in order to get rid of cells and obtain the metabolites leaching, then they were gradually precipitated until saturation with ammonium sulfate (80%),according to (Vijay Simha et al. 2012). The precipitate was obtained after cryogenic centrifugation and packed in to sterile tubes.

Estimation of Total Proteins

The proteins were quantitatively calculated using a device semi auto-analyzer, which is based on principle of Biuret reaction (copper salt in an alkaline medium). Protein sample forms a blue colored complex when treated with cupric ions in alkaline solution. The intensity of the blue color is proportional to the protein concentration. According to the following equation:
Total protein con. (g/dl) =Absorbance of sample / Absorbance of standered * con. Of standered.
Con . of standered was 6 g/dl. Wave lenghth = 546 nm.

Collection of medicinal plants

The plant samples (Syzygium and Cinnamomum) were taken from AL-Fajr grass in AL-Ramadi city. They were classified scientifically and their return was confirmed by Desertification Center at the University of AL-Anbar.

Preparation of Plant Extract

Plant samples they were cleaned of dust and impurities by washing them by plain water several times, then with distilled water and letting them dry at room temperature. Plants extracts were prepared according to (Kassab and Bauomy 2014).

Getting Fungus

The fungus used in the study Apergillus niger was obtained from the fungi laboratory in the faculty of science, department of biological sciences. diagnosed with biology.

Inhibitory Potency of Plant

plant extract was mixed with autoclaved sterilized PDA medium at a ratio 1:10, then fungal colonies (Aspergillus) was inoculated in the center of plates, then incubated at 25 C0. Fungal growth was observed daily for 5 days (Behdani 2019) and the percentage of inhibition was according to the following equation (Al-Ani 2021) :

$$I\% = T- C/ T*100$$

Where I% is inhibition percentage, T is fungi growth rate in comparation, C is fungi growth rate in the treatment.

Anti- fungal activity of synergism of plant and LAB'S extract

Plant extracts (Syzygium spp. and Cinnamomum spp.) were added to PDA medium, which had previously been sterilized and cooled to an acceptable degree so as not to effect the activeness of the extracts. The ratio of adding was 1:10. Mixing was in a circular motion, well and slightly in Petri dishes. The dishes were left to harden and then inoculated by cork borer with the fungi used in the study, where the inoculated was in the center of the plat. Dishes were placed in the

incubator at 25 C0 and were monitored on a daily basis. in order to observe the activity of plant extracts separately against the fungi. The inhibitory activity of the plant extracts was subsequently measured.

As a control, Aspergillus niger was inoculated on PDA medium without any addition of extract. After that, growth results of A.niger on mixed medium were compared with the growth on controls dished . Inhibition rate was calculated as mentioned above. For antifungal activity test of bacterial extracts. The prepared and sterilized pre-prepared PDA medium (left to warm slightly in order not to affect the activity of bacterial extracts) was inoculated with bacterial extracts (each extract of each isolate were added separately PDA) that had previously prepared. The ratio of mixing were 1:10 , inoculation with A.niger used were carry out in the same method above. Control dishes were used as in the previous method above. Inhibtion effect was measured as away was mentioned above. To test the antifungal activity which, produced from the synergy between plant and bacterial extracts. The plant extracts and bacterial extracts were mixed well in a ratio of 1:1 in sterile petri dish and mixed well in a circular motion until homogeneity. Then the autoclaved and slightly warmed PDA (Poteto Dextrose Agar) medium was added to the mixture of extracts and they were mixed well in a ratio 1:10 in a circular motion until the mixture was perfect homogeneous. Medium was left to harden .After solidation . The medium was inoculated with Aspergillus niger by cork borer separately and inoculated at 25C0.Growth was monitored on a daily basis and compared with control cultures...After 5 days the inhibition effect was calculated according to a aforementioned equation after measuring growth diameters. Control culture were us used as mentioned above. All of these experimented were carried out with3 replications.

Results and Discussion

One hundred and forty-one samples were collected from different sources in AL-Ramadi city (fruits , vegetables, raw milk of sheep, cow and goats , canned food and from stool of infants whose age did not exceed 40 days and their mothers had not previously taken any antibiotics)They were cultured on MRS agar supplemented with Tween 80 . Microscopic examinations were carried, where the isolates were Gram-positive , as they were appeared in purple with different shapes under the microscope including rods , as in Figure 1. and cocci, as in Figure 2.

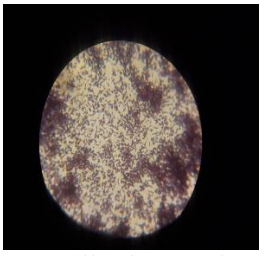

Figure 1. Bacilli shape of LAB under (100X)

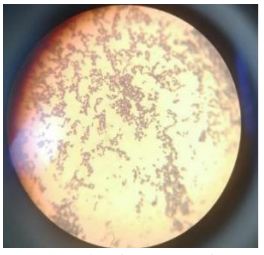

Figure 2. Cocci shape of LAB under (100X)

Biochemical tests were conducted, as the isolates were positive for Indole test, positive for Methyl Red test, negative for Vogase Proskaure test, negative of Citrate test. All isolates were able to grow in media with NaCl (4% ,6% and 8%).The number of isolates were reduced to 37 and then to 10 isolates , depending on the similarity of phenotypic and microscopic characteristics and the results of biochemical tests. Randomly then selected and diagnosed with Vitek 2 system. The isolates diagnosed were Lactobacillus acidophilus, Pediococcus acidolactici and Enterococcus faecalis. All of these isolates were precipitated with ammonium sulfate at saturation rate of %80-%90, which explains the low molecular weight of the precipitates and then centrifuged at a cooling rate of 1000 rpm for 15 min. The filtrate was neglected and the precipitate was recovered with 3 ml distilled water. The sedimentation process did not comply with study of (Islam et al. 2020), as the isolates were deposited at a saturation rate %70 with ammonium sulfate. The precipitate were collected for each isolate and protein quantity was measured for each automated analyzer, which principle of its action depends on Biurate reaction to estimate total protein concentration according to the following equation:

Total protein con.(g/dl)=Ab. Of sample/ Ab. Of standard * con. of standard
Where standard con. = 6 g/ dl , Ab. = 546 nm.

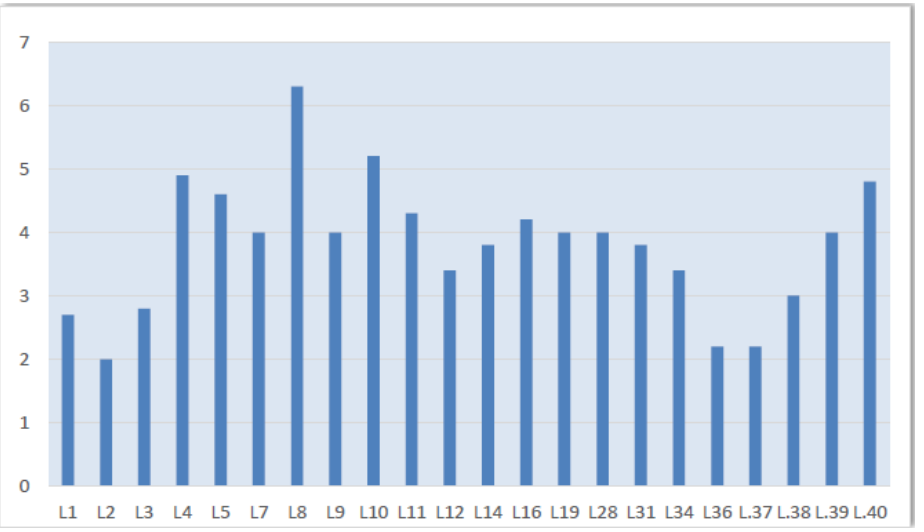

Figure 3. Proteins concentration

On the other hand, aqueous extraction of medicinal plants (Syzygium and Cinnamon) were performed as previously described by the preparation method. Then the plants extracts were centrifuged and filtrated by Millipore (0.45). Bacterial extracts activity of each isolate were tasted against Aspergillus niger. The inhibitory activity of each plants extracts were tested separately . Then the fungus was treated with synergistic bacterial extract with clove and synergistic extract with Cinnamon. The study demonstrated that there was synergistic inhibitory activity of bacterial and plants extracts against Aspergillus niger .As shown in the Table 1.

Table 1
Growth area and inhibition percentage of treatments against A .niger

Treatmet	Con. (mm^2) /%	(L1) (mm^2) /%	(L2) (mm^2) /%	(L3) (mm^2) /%	(L4) (mm^2) /%	(L5) (mm^2) /%	(L6) (mm^2) /%	(L7) (mm^2) /%	(L8) (mm^2) /%	(L9) (mm^2) /%	(L10) (mm^2) /%
Bacterial extract	1674.66/0	803.84/51	530.66/68	1133.54/32	153.86/90	803.84/51	28.26/98	254.34/84	907.46/45	1256/24	78.5/95
Syzygium extract	542.16/72	-	-	-	-	-	-	-	-	-	-
Cinnamon extract	803.48/51	-	-	-	-	-	-	-	-	-	-
Bacterial+Syzygium extract(synergy)	-	78.5/95	28.26/98	112.04/93	50.24/96	314/81	12.56/99	200.96/87	28.26/98	200.96/87	28.26/98
Bacterail+ Cinnamon extract(synergy)	-	28.26/98	28.26/98	530.66/68	50.24/96	706.5/57	12.56/99	200.96/87	706.5/57	706.5/57	50.24/96
LSD (0.0..5)	-	188.17/17	388.6/6	3337.6/2.9	122.11/2.3	166.7/3.3	276.4/5.7	278.5/2.8	134.6/207	267.4/3.2	411.38/5.6

Where L1(L.acidophilus), L2(Lactobacillus sp), L3(Pediococcus sp.), L4(Lactobacillus sp),
L5(Lactobacillus sp), L6(Pediococcus sp.), L7(Pediococcus acidolactici), L8(E.faecalis),
L9(Lactobacillus sp), L10(Lactobacillus sp)

The results t of inhibitory activity of the extract of Lactobacillus acidophilus (L1) and the other treatments against A.niger showed a significant difference (at the probability 0.05%) .The stud study proved that the extract of L.acidophilus(L1) had an important role in inhibiting the growth area to 1519.79 mm2 compared with the growth area of control treatment 1674.66 mm2, with inhibition percentage at % 0.9, where the extract L.acidophilus of (L1) synergistic with clove had significant inhibitory ability against A.niger with a percentage of 43% and decrease in the growth area to 452.16 mm2, when A.niger was treated with L.acidophilus (L1) extract and Cinnamon the growth area of it decreased from 1674.66 2mm as control to 1133.45 2mm, with an estimated inhibitory rate of 32%.

The results of statistical analysis also showed a synergistic inhibitory activity of Lactobacillus sp. (L4) which isolated from carrots with plants extracts and for all treatments against A.niger with significant difference, that the inhibitory effect of Lactobacillus sp (L4) extract and clove extract against A.niger reached 77% with growth area of 379.74 mm2 compared to the control. There was also a significant difference at the probability 0.05 in the synergistic inhibitory activity of Lactobacillus sp (L4) extract and Cinnamon extract against A.niger with a percentage estimated at 32% and growth area about 1133.54 mm2 compared

with control. There was also the synergistic effect of Lactobacillus sp (L5) extract with natural plants extracts had a significant effect against A.niger, as the ratio of synergistic effectiveness of Lactobacillus sp (L5) extract with clove extract was 43% , with growth area of 452.16 mm2 compared with control as 1674.66 mm2, while the significant effect of Lactobacillus sp (L5) was synergy with Cinnamon extract with an inhibition rate of 24% and a growth area of 1256 mm2 compared with control.

Also the extract of Lactobacillus sp (L9) had a synergistic inhibitory activity with clove extract, with a significant difference of 43% and area growth was 452.16 mm2 as compared with area growth of control 1674.66 mm2. Although there was a synergistic inhibition effect of Lactobacillus sp (L9) extract with Cinnamon extract, but it did not rise to the significant. The significant effect of Lactobacillus sp (L2) extract was similar to that of Lactobacillus sp (L9) extract, as there were a synergy activity of Lactobacillus sp (L2) with clove extract at probability 0.05 and there was 51%, this percentage increased when A.niger was treated with extracts of Lactobacillus sp (L9) and clove to reach at 96%. Despite the synergy inhibitory effectiveness of Lactobacillus sp (L9) with Cinnamon extract, which reached to 57%, but it did not rise to significant. The same is the case with extracts of Lactobacillus sp (L10), Enterococcus faecalis (L8) extract and for Pediococus sp (L6) as well.

The results obtained proved that the inhibitory activity of Pediococcus acidolactici(L7) against Aspergillus niger with significant difference(at probability 0.05) by 17%, where the A.niger growth was slightly reduced from 1674.66 mm2 as control to 1384,74 mm2 .The antifungal activity against A.niger was somewhat increased when the synergy between P.acidolactici (L7)extract and clove extract, whereby the growth decreased from 1674.66 mm2 as control to 314 mm2 with synergistic activity at 81% ,while the results of the synergy between Pediococcus acidolactici (L7) extract and Cinnamon extract against Aspergillus was less effective than it with the results of the synergy between Pediococcus acidolactici(L7) extract and clove extract and by 32% and it did not rise to the level of significance, where the Aspergillus growth decreased to 1133.54 mm2 after being treated with the synergistic extract between Pediococcus acidolactici (L7) and Cinnamon. The results of statistical analysis showed a synergistic activity with a significant difference of Pediococcus sp (L3) and for all treatments against A.niger.

As the growth decreased from 1674.66 mm2 as control to 452.16 mm2 with an inhibitory activity estimated at about 43%, when it was the synergistic effect between the extract of Pediococcus sp (L3) and the extract of Cinnamon there was a significant effect against A.niger ,the growth decreased from 1674.66 mm2 as control to 1256 mm2 with an estimated inhibitory activity of about 24%. There was also significant effect in inhibiting the growth of A.niger, especially when it was treated with the synergistic extract between the extract of Lactobacillus sp. (L2)and clove extract, that may be due to the activity of Syzygium(clove) as antifungal, because it contains a variety of active organic compounds such as eugenol, which is damage the cell membrane by its effect on ergosterol (a substance specifically found of cell wall fungi and its major sterol component) and it responsible for the safety and function of the cell, where clove impair the

synthesis of ergosterol and thus create a vacuole in the fungal cell membrane(Pinto n.d.). The activity of clove may be partly due to the hydrophobicity responsible for breaking down the bilayer of lipids in the fungal cell wall, which leads to a change in the permeability and leakage of the cell contents (Burt 2004), thus this may allow the entry of active antifungal metabolites of Lactic Acid Bacteria such as reuterins, which inhibit DNA synthesis by suppressing ribonuclease activity (Axelsson et al. 1989) and consequently cell death.

There was a synergistic inhibitory activity between Cinnamon extract and extracts of some isolates against Aspergillus and there was a restriction of its growth, where the maximum activity of synergistic effect of Cinnamon and extract of Lactobacillus sp L2 was about %57. This may be due to the ability of Cinnamon extract to effect on fungal cell by causing an accumulation of reactive oxygen species ROX level (which are by products of oxygen metabolism and they play an important role in cellular performance ,they found at low and fix levels in cells) inside the cells ,which effects the integrity of the cell membrane (Zhang et al. 2021). The interaction of the Cinnamon extract with enzymatic reactions of the synthesis fungal cell wall effects on the growth and formation of the fungus, which causes a morphological and structural change, in turn ideas to a disturbance of the cytoplasmic membrane and consequently the loss of cytoplasmic contents (Wu et al. 2019).

Synergy between Cinnamon extract and bacterial extracts is a more inhibiting ability against fungi, as the effect of Cinnamon on the cell wall and its breaking allows the contents and metabolic products of bacterial extracts to reach in to the cellular content, perhaps including organic acids, which access to the cytoplasm increases its acidity, in turn effects cell metabolism by breaking down enzymes or inhibiting proteins synthesis and destroying genetic material (Gao et al. 2019) . Possibly breaking the cell wall allows H2O2, which is one of inhibitory metabolites of LAB, where it effects by disrupting the structure of cellular proteins (Pradhan and Kadyan 2020). From the foregoing, it is clear that the results obtained for Pediococcus sp. are consistent with what was reached by Vaitkevičienė (Vaitkevičienė et al. 2019),where he proved that Pediococcus acidolactici did not show any activity against Aspergillus niger and A.terreus, while it showed medium and low effectiveness against A.verciolor and A.fumigatus ,as well as a low and medium effect against Pancillium sp..

Also the results of our study are parallel to what Yanina obtained at (Yanina et al. 2018), where he abled to isolate phanyllactic acid from Pediococcus acidolactici and tested it on many fungi that grew on bread, including Aspergillus niger ,A.japonicus ,Pancillium sp. And some yeast like S. cervisiae. Yanina proved the inability of Pediococcus acidolactici to inhibit A. niger CH1and A. niger CH3, while it was able to inhibit A. niger CH2, A. japonicus and all Pancillium, while it did not inhibit S. cerevisiae. Nevertheless, it was found that Pediococcus acidolactici LAB5 showed a wide range of inhibition against fungi. This is what Chandra proved (Chandra 2013), this isolate was able to prevent the growth of all types of fungi, including the phytopathogenic (Cladosporium herbarum), Colletotirchum acutatum,Fusarium sp. And Aspergillus sp., but it failed to inhibit Alternaria alternate and Alternaria solani .In order to detect and prove the presence of active

molecules in bacterial extracts , which they had antifungal activity , Pediococcus acidolactici(L7) extract was purified by Ion Exchange Chromatography using CMC as ion exchanger , which purify cationic proteins. There were 28 fractions were obtained using CMC column…The absorbance of each fraction was measured at 280 nm wave-length, and it was found that there was one peak that extended from fraction 14 to 17, as in the Figure 4.

CMC is a chitosan derivative. It has a better water solubility than chitosan. CMC is an important compound because of its high water solubility, it has no cytotoxicity, and excellent 96 bioactivity (Zhou et al. 2010). The result obtained using CMC ion exchanger indicates that this exchanger is distinguished by a high separation of proteins …Perhaps what confirms this is its ability to separate proteins which obtained by a precipitation process at a saturation limit of %80-% 90, and this indicates that the separated proteins have a relatively small molecular weight .The most important characteristic of CMC ion exchanger is the idea of charge-dependent linkage on the osmotic force of the recovery solution, which facilitates obtaining different types of linked proteins .The result of proteins separation under study is in agreement with the scientist Sunying Zhou (Zhou et al. 2010) and closely to the results of Karin. Bronnenmeier and his group (Bronnenmeier et al. 1995),where mentioned that the main peak of xylanase activity that was eluted at 0.2 M (NaCl) can be easily differentiated the enzymes exhibiting interaction with Avicel, CMC, and barley b-glucan. As in Figure 5.

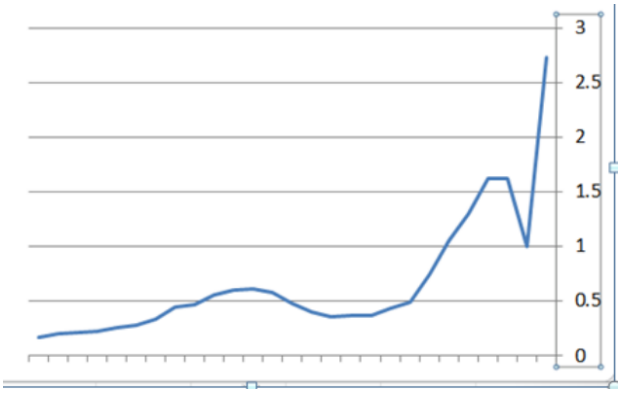

Figure 4. Protein concentration

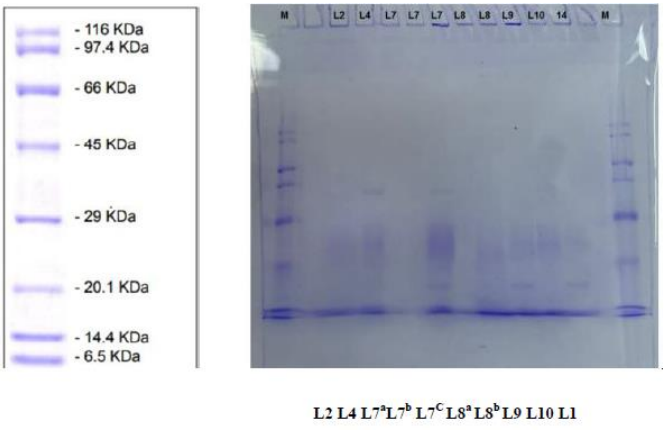

Figure 5. Electrophoresis in polyacrylamide gel in the presence of SDS PAG for calculation of molecular weight of bacterial extract. Where (A) leader proteins. (B)Electrophoresis for estimated the molecular weight of LAB extracts, where L2=L2extract, L4=L4extract, L7a = primary supernatant of L7 cells removed, L7b = proteins purified by ion exchange, L7C= L7extract, L8a=primary supernatant of L8extract, L8b=L8 extract, L9=L9 extract, L10=L10 extract, L14=L1extract

The electrophoresis results were obtained were in agreement with the study of Saleh (Saleh and El-Sayed 2004), who was able to estimate the largest and smallest molecular weight spectra of bacteriocin isolated from Bifidobacterium lactis at 89 KDa and 25 KDa respectively. Our results were also parallel to the results of Islam(Islam et al. 2020) ,who was able to isolate and determination of bacteriocin that produced from Lactobacillus sp. and he was estimated them about 40KDa ,15KDa and 30 KDa on three species of Lactobacillus. Also the results of our report agreed with the study of Chu (Chu et al. 2019),who estimated the molecular weight of alkaline phosphatase secreted by Lactic Acid Bacteria at about 43 KDa by SDA PAGE. CMC column included the use of a buffer potassium phosphate solution (0.05 M) free on EDTA at a concentration of (0.0001M) with PH= 6 was recovered using NaCl. at (0.5 M) in a separation column of dimensions (3*16 cm), the flow rate was 1 ml per min. and a rate was 2 ml per fraction. This is similar to the study of Rhee (Rhee and Park 2001), that he identified Anti-mutagenic Activity of glycoproteins extracted from L.plantarum ,he extracted with ammonium sulfate and then purified those proteins with DEAE-cellulose, then he splitted the purified proteins to three fractions and performed an electrophoresis gel of them …The results were that the three fractions showed single band, so all the fractions have the same glycoprotein.

Conclusion

The bacterial species belonging to Lactic Acid Bacteria that were isolated from different local sources have varying ability to inhibit fungi, according to the species of isolate. The ability of natural compounds that were extracted from medicinal plants (Syzygium and Cinnamon) to cause inhibition of the growth of fungi studied in the experiment. There was different synergistic efficacy against

fungi, when synergizing bacterial extracts with medicinal plants extracts that were used in the study , according to the type of bacterial extracts.

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