Isolation, Biochemical Identification and Antioxidant Activity of Locally Isolated *Lactobacillus* spp in Garmian Area

Hana K. Maikhan[1] & Saman M. Mohammad-Amin[2]

[1,2] College of Education, University of Garmian, Erbil, Iraq
Correspondence: Saman M. Mohammad-Amin[2], College of Education, University of Garmian, Erbil, Iraq. Email: saman.amin@garmian.edu.krd

Received: June 3, 2017 Accepted: August 23, 2017 Online Published: September 1, 2017

doi: 10.23918/eajse.v3i1sip128

Abstract: The study was conducted in the Biology research laboratory In Garmian University during the period of summer to autumn 2016, which aimed to the isolation and biochemically identification of *Lactobacillus sp.* and comparing their antioxidant activity with *Lactobacillus reuteri* ATCC 23272, as well as both of the isolates and reference strain with ascorbic acid which used as a control. Out of 25 samples were collected from different sources such as vaginal swabs, infant stool, sheep and cow's milk, six strains of *Lactobacillus* sp were Identified according to their colony morphology on MRS agar along with microscopic examination. Further identification strategies are carried out by biochemical analysis including catalase, oxidase, and API 50 CHL kit system through which strains as *L. pentosus, L. acidophilus, L. collinoides, L. fermentum, L. salivarius* and *L. brevis* were identified. The results of antioxidant activity which carried out by 2,2-DiPhenyl-2-Picryl hydrazyl hydrate (DPPH) assay for all six isolated strains revealed that the best antioxidant activity was recorded by *L. pentosus* strain IC_{50}=0.0464 which showed no-significantly differences with the ascorbic acid antioxidant activity IC_{50} = 0.0186, on the other hand, *Lactobacillus reuteri* ATCC 23272 exhibited antioxidant activity about IC50=1.998, in addition, other isolated strains *L. acidophilus, L. fermentum, L. salivarius, L. collinoides* and *L. brevis* demonstrates good antioxidant activity with IC_{50}= 0.2311, 0.2792, 0.3707, 3.744 and 5.766 respectively.

Keywords: *Lactobacillus* spp., Biochemical Tests, API 50 CHL, Antioxidant Activity

1. Introduction

Reactive Oxygen Species or free radical referred as strong reactive molecules such as hydrogen peroxide H_2O_2, hydroxyl radical OH, single activated oxygen O_2 and superoxide anion (O^-_2) which produced as a reaction from exogenous factors and believed to causes health disorder. There is evidence that the ROS playing an important role in the intestinal diseases (colon cancer and inflammatory bowel disease) (Valko et al., 2001). The antioxidant- dependent drug has been used as a treatment of complex diseases borne the idea with researchers to use natural antioxidant. Probiotics have been tested to their antiradical activities by various tests included ferric reducing antioxidant power, 2,2- Diphenyl-1-picrylhydrazy (DPPH) in its radical form, linoleic acid assay superoxide, hydrogen peroxide radical scavenging assay and nitric oxide (Venkatachalam et al., 2012). Wan et al., (2014) reported that *Lactobacillus* has the ability to exhibit certain reducing power, scavenging superoxide anion and total antioxidant capacity. The DPPH assay is one of the natural product

antioxidant studies. DPPH has the absorption band at 515 nm and disappears during the reduction by using antiradical compound. Isolating and identifying different Lactobacillus from different natural sources has augmented during the last three decade, and the fast way for achieving this criteria was carried out with phenotypically tests (Boyd et al., 2005), furthermore none of the available biochemical kits are accurate for identification of some strains of Lactobacillus as recommended by (Klein et al., 1998), however, in some other study API 50 CHL system strips was used to identifying and classification of Lactobacillus in different sources till their species level (Alvarez-Olmos et al., 2004). The current study was aimed to isolate and identifying different locally *Lactobacillus* species in order to evaluate their antioxidant activity, furthermore become as an alternative form for naturally treating different digestive system disorder.

2. Material and Methods

2.1 Specimens Collection and Isolation of Lactobacillus spp.

Fifty different specimens were suspected to contain *Lactobacillus* species collected from sources included; healthy infant stool 10 samples (1 month-6 month), healthy vaginal swabs 7 samples , breast milk 5 samples, cow milk 5 samples , cow yogurts 5 samples , sheep milk 5 samples , sheep yogurts 5 samples , deer milk 2 sample and finally goat milk 6 samples . Vaginal samples were taken from the posterior position of the fornix of the vagina from healthy women within 24-26 years by sterile swabs and directly cultured on MRS agar then incubated anaerobically at 37 °C for 18-24 hours (Pascual et al., 2006). One ml from each milk samples suspended in 100 ml of MRS broth and incubated at 37 °C for 18-24 hours under microaerophilic condition. After the complete incubation time, a loopful of each broth culture were streaked twice on the surface of MRS agar plate, one of them incubated aerobically and the other incubated anaerobically at 37 °C for 18-24 hours (Sneath et al., 2009).

One gram of both infant stool and yogurt were mixed with 100ml of the MRS broth and incubated under the microaerophilic condition at 37 °C for 18-24 hours then sub-cultured twice on MRS agar plates and incubated aerobically and anaerobically at 37 °C for 18-24 hours (Davoodabadi et al., 2015). *Lactobacillus reuteri* ATCC 23272 from American Type Culture Collection has been used in this study to comparison its antioxidant activity with the antioxidant activity of the locally isolates of *Lactobacillus sp.* The microorganism was stored in lyophilized MRS lactobacilli medium and propagated from the lyophilized form during inoculation in the MRS broth at 37 °C for 24 hours under aerobic condition and sub-cultured on the MRS agar plate at 37 °C for 18-24 hours.

2.2 Identification of Suspected Isolated Strains

The suspected colonies were grown on MRS agar plates identified according to their morphological characters, microscopic examinations, gram stain, and biochemical test which including catalase activity, oxidase test, and API 50 CHL Kit (Biomerex, France) (Hoque et al., 2010).

2.2.1 Morphological Examination

Morphological examination of the isolated bacterial strains included the study of the bacterial colony morphology (shape, color, texture, colony diameter and its margin), in furthermore, on the modified mMRS agar the suspected *Lactobacillus* strains must produce an organic acid and able to change the

color of the agar from blue to yellow.

2.2.3 Microscopic Examination

It was performed by staining the suspected isolates colony by grams stain and examined microscopically to see and confirm their bacilli shape and the purple color of the bacilli shaped bacteria under X40 and X100 consequently.

2.3 Biochemical Tests

2.3.1 Catalase Test

This test was performed by transferring a single colony of the isolated strain with a sterile loop to the surface of a cleaned glass slide and adding one drop of 3% H_2O_2. The formation of the bubble during seconds specifies their positive result (Nelson et al., 1995).

2.3.2 Oxidase Test

It was conducted through transferring a single colony of the isolated strain with a sterile loop to a filter paper saturated with oxidase reagent Tetramethyl–p-phenylenediamine dihydrochloride (TMPD) within 30 sec. Changing the color to purple indicated their ability to oxidizing the reagent (Mahon et al., 2015).

2.4 Identification by API 50 CHL Systems

Complete identification of the isolated strains of *Lactobacillus* at the level of species was carried by the fermentation of 49 carbohydrates through using API 50 CHL Kit (Biomerex, France) which consists of API 50 CH strips and 11 tubes of API 50 CHL medium. This test was carried out according to the instructions of the manufacturer as following and the results were obtained after incubation at 37 °C for 24-48 hours.

The results were obtained from carbohydrate fermentation was read depending on the color change of the wells of the strips from violet to complete yellow as a positive result (+), the changing of the color to green was marked as a variable (V), while no change in color was denoted as a negative result (-).Aesculin hydrolysis (well number 25) was revealed as a change to black color and denoted by the positive result (Nigatu, 2000). Later these results compared with the leaflet (API 50 CHL, V5.1) provided by the company for species identification. All isolates of *Lactobacillus* were identified, cultivated on MRS agar for 18-24 hours at 37 °C then subcultured twice and stored at 4 °C until further use.

2.5 Antioxidant Activity of Isolated Strains

According to the protocol described by Vardapetyan et al., (2012), the antioxidant ability of *Lactobacillus spp.* to the reduction of the DPPH solution carried out as follows:

10 mg of freeze-dried cell was dissolved in 1ml of absolute ethyl alcohol to prepare the tube number 1 in a serial dilution, then 500 µL was transferred from the 1st tube into the 2nd tube and the volume was completed into 1000 µL by absolute ethyl alcohol, so on until we reached into the last tube .As a result we were get different concentration of *Lactobacillus* sp.:10mg \ ml, 5mg \ ml, 2.5 mg \ ml,

1.25 mg\ ml and 0.625 mg\ ml. 0.002 g of 2,2-DiPhenyl-2-Picryl hydrazyl hydrate (DPPH) was dissolved in 100 ml of absolute ethanol and it was kept in dark place, and 2.5 ml of DPPH was transferred into each tube, as well as, 0.5 ml from these concentrations: :10mg \ ml , 5mg \ ml , 2.5 mg \ ml , 1.25 mg\ ml and 61.3 mg\ ml were transferred into the tubes were contained DPPH and left for 30 minutes in dark place. Finally, the absorbance of these tubes was read by spectrophotometer at wavelength 517 nm.

Ascorbic acid with different concentrations (0.9, 1.9, 3.9, 4.9, 6.9 µg/mL) were used as reference, as well as, *Lactobacillus reuteri* ATCC 23272 was also used to compare their antioxidant activity with our locally isolated strains

The percentage of reduced DPPH was calculated as this equation:

$$DDPH\ scavenging\ activity = 100 \times (Ac - As)/Ac$$

A_c: the absorbance of blank

A_s: the absorbance of the sample

IC_{50} and the relation between concentration and absorbance of each diluted tube were analyzed by PRISM 6 program.

3. Results and Discussion

3.1 Isolation and Identification of Isolated Strains

Out of fifty specimens were collected from different mentioned sources, only six isolates distinguished as (IN1, IN2. IN3, VA, CO and GO) appear to verify the general characters of *Lactobacillus* were grown on MRS agar medium with microaerophilic, small, spherical, smooth and white to creamy in color characters, furthermore, the isolated colonies having the ability for changing the color of the medium from blue to greenish-yellow with the production of acidity odor. Microscopic examination showed gram positive, rod and cocci shaped bacteria (coccobacilli) arranged as single or chain, non-spore forming (Figure1). Biochemically characterization of all six isolates strains as shown in (Table 1) revealed negative results with catalase test, in which can't produce of bubble when in mix with 3% H_2O_2, this was considered as one of the most important indicators for recognizing *lactobacillus* sp. (Hassan & Peh, 2014), in addition all isolate strains exhibited negative activity with oxidase test when inoculating with TMPD. Collectively and according to the obtaining phenotypic results and Sherlock microbial identification system analysis, all these six isolated strains are genuses of Lactobacillus (Arici, et al., 2004).

Table (1): Biochemical and phonotypical characterization of all isolated strains

Source	Abbreviated	Oxidase Test	Catalase Test	Shape	Gram test
Infant stool	IN1	-	-	Rod shape	+
Infant stool	IN2	-	-	Rod shape	+
Infant stool	IN3	-	-	Rod shape	+
Vaginal Women	VA	-	-	Coccobacilli	+
Cow milk	CO	-	-	Coccobacilli	+
Goat milk	GO	-	-	Rod shape	+

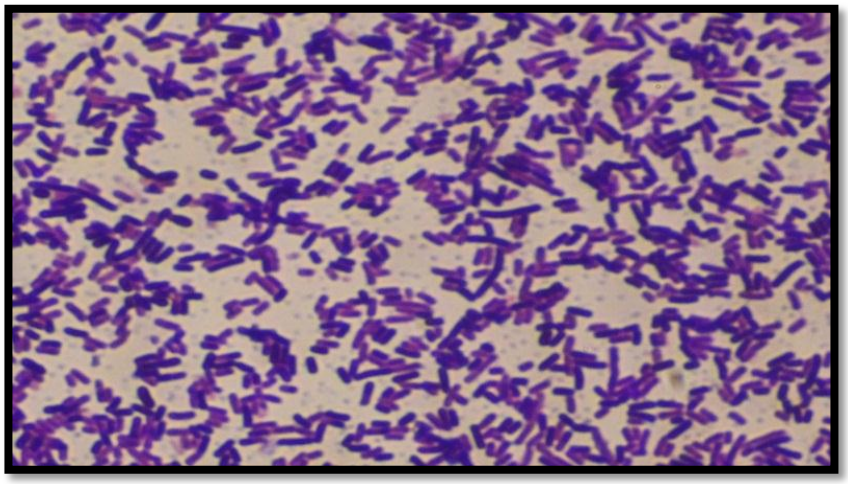

Figure (1): Microscopically examination of isolated strain from infant stool (IN1) viewing bacilli in shape

3.2 Identification of *Lactobacillus Species* By API 50CHL Kit

The results of the carbohydrate fermentation in API 50 CHL system by *Lactobacillus* and strains were able to ferment different carbohydrate sources, as shown in (Figure 2) was presented in (Table 2). The results of API 50 CHL were analyzed according to the biochemical profiles registered in the API web® database (bioMerieux) revealed that each isolated strains with exact species belong to the *Lactobacillus* spp, as summarized in (table 3), this results were in agreement with that approved previously by (Ogunshe, 2008, Ozgun & Vural, 2011; Ngongang et al., 2016), in case of identification of *Lactobacillus* till its species. On the other side (Arici, et al., 2004) they also found *L. brevis* and *L. fermentum* in them study from infant feces. (Ozgun & Vural, 2011) described that the molecular study such using PCR techniques was the best and valuable way to identifying the newly isolated strains, as well API 50 Ch system also can be used to identifying the isolated strain depending on the phenotypically and their biochemical characteristics.

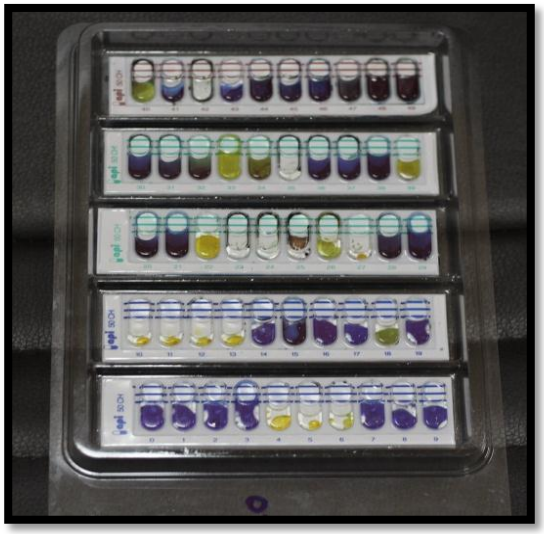

Figure (2): Carbohydrate fermentation in API 50 CHL system by *Lactobacillus spp*. after 24-48 hours of incubation at 37 ^{0}C

Table (2): Carbohydrate fermentation in API 50 CHL kit system tubes by all locally isolated strains

Isolated strains	CTR	GLY	ERY	DAR	LAR	RIB	DXY	LXY	ADO	MDX	GAL	GLU	FRU	MNE	SBE	RHA	DUL	INO	MAN	SOR	MDM	MDG	NAG	AMY	ARB
IN1	-	v	-	-	+	+	+	-	-	-	+	+	+	+	-	v	-	-	+	+	-	v	+	+	+
VA	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	v	v
CO	-	-	-	-	v	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	v	-	-
IN2	-	-	-	-	v	+	v	-	-	-	+	+	+	v	-	-	-	-	-	-	-	-	-	-	-
IN3	-	-	-	-	v	v	v	-	-	-	+	+	+	+	-	-	-	-	v	-	-	-	+	+	+
GO	-	-	-	-	v	v	-	-	-	-	+	+	+	+	-	v	-	-	+	+	-	-	+	-	v

Isolated strains	ESC	SAL	CEL	MAL	LAC	MEL	SAC	TRE	INU	MLZ	RAF	ADM	GLY	XLT	GEN	TUR	LYX	TAG	DFUC	LFUC	DARL	LARL	GNT	2KG	5KG
IN1	+	+	+	+	+	+	+	+	-	-	v	+	+	+	+	v	-	-	-	-	-	-	v	-	-
VA	v	+	+	+	v	-	+	+	-	-	-	-	-	-	+	-	-	v	-	-	-	-	-	-	-
CO	-	v	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
IN2	-	-	-	+	+	v	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	v	-	-
IN3	+	+	+	+	v	+	+	v	v	-	v	-	-	-	+	-	-	-	-	-	v	-	+	-	-
GO	v	v	-	+	+	+	+	+		-	+	-	-	-	-	-	-	-	-	-	-	-	v	-	-

CTRL, Control ;GLY, Glycerol; ERY ,Erythritol ; DAR, D-arabinose ; LARA, L-arabinose ; RIB, Ribose ; DXYL, D-xylose ; LXYL ,L-xylose ; ADO,Adonitol ; MDX ,ß methyl-D-Xyloside ; GAL, Galactose ; GLU, Glucose ; FRU, Fructose ; MNE, Mannose ; SBE, Sorbose; RHA, Rhamnose ; DUL, Dulcitol; INO,Inositol; MAN, Mannitol ; SOR , Sorbitol ; MDM, Methyl-D-mannoside ; MDG, Methyl-D-glucoside ; NAG, N-Acetyl-Glucosamine ; AMY, Amygdalin ; ARB, Arbutin ; ESC, Esculin ; SAL, Salicin ; CEL, Cellobiose ; MAL, Maltose ; LAC, Lactose ; MEL, Melibiose; SAC, Sucrose ; TRE, Trehalose ; INU, Inulin ; MLZ, Melezitose ; RAF, Raffinose ; ADM, Starch ; GLYG, Glycogen ; XLT, Xylitol ; GEN, ß Gentiobiose ; TUR, D-turanose ; LYX, D-lyxose ; TAG, D-tagatose ; DFUC, D-fucose ; LFUC, L-fucose ; DARL, D-arabitol ; LARL, L-arabitol ; GNT, Gluconate ; 2KG, 2-Keto-Gluconate ; 2KG, 5-Keto-Gluconate

Table (3): overall identification of all six isolated *Lactobacillus* sp. after complete carbohydrate fermentation on API 50 CHL kit

Isolated strains	Identified by API 50 CHL
IN1	*L. pentosus*
IN2	*L. fermentum*
IN3	*L. brevis*
VA	*L. acidophilus*
CO	*L. collinoides*
GO	*L. salivarious*

3.3 Antioxidant Activities of Isolated Stains

The results of the antioxidant activity were studied to all locally isolated strains of *Lactobacillus* which presented as (IC_{50} mg\ml, which expressed the concentration where inhibited 50% of the DPPH activity), and for both vitamin C as a control and *Lactobacillus reuteri* ATCC 23272 are shown in (Table 4). These results were obtained from spectrophotometer at 517nm and plotted against the concentration of the samples. The absorbance of DPPH decreases with an increase in the DPPH radical scavenging activity. The highest radical scavenging activity was obtained by *L. pentosus (*IC_{50} = 0.04645 mg/ml), which indicated very high strong activity and look like the activity of the control (vitamin C) which recorded (IC_{50} =0.0186 mg/ml), while all *L. acidophilus*, *L. fermentum* and *L. salivarious* also recorded the better results than *L. reuterii* ATCC 23272 as summarized in (Table 4). The obtained results recorded better antioxidant activity than the results were obtained by (Nyanzi *et al*., 2015) especially for Lactobacillus *acidophilus* with (IC_{50} = 4.24mg\ml), as well as, these results was in agreement with that of (Risan *et al.*, 2016) that the antioxidant activity was dependent on both the concentration and the dosage therefore, increasing the dose as well as causing increasing the scavenging activity. Number of studies such (Abu-Bakr et al., 2012) reported that the *Lactobacillus spp*. antioxidant activity recorded from whey by fermenting the skim milk using different strains and isolates.

Table (4): The antioxidant activity of all isolates strains of *Lactobacillus* and *Lactobacillus reuteri* ATCC 23272

No.	**Isolated strains**	**IC_{50}(mg\ml)**
1	Control Vitamin C	0.01862
2	*L. pentosus*	0.04645
3	*L. brevis*	5.766
4	*L. acidophilus*	0.2311
5	*L. collinoides*	3.744
6	*L. fermentum*	0.2792
7	*L. reuterii* ATCC 23272	1.998
8	*L. salivarious*	0.3707

References

Abubakr, M.A.S.; Hassan, Z.; Imdakim, M.M.A., & Sharifah, N. R. S. A. (2012). Antioxidant activity of lactic acid bacteria (LAB) fermented skim milk as determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ferrous chelating activity (FCA). *African Journal of Microbiology Research*, 6(34), 6358-6364. doi: 10.5897/AJMR12.702

Alvarez-Olmos, M.I., Barousse, M.M., Rajan, L., Van Der Pol, B.J., Fortenberry, D., Orr, D., & Fidel Jr, P.L., (2004). Vaginal lactobacilli in adolescents: presence and relationship to local and systemic immunity, and to bacterial vaginosis. *Sexually Transmitted Diseases*, 31(7), 393-400.

Arici, M., Bilgin, B., Sagdic, O., & Ozdemir, C. (2004). Some characteristics of *Lactobacillus* isolates from infant feces. *Food Microbiology*, 21(1), 19-24. https://doi.org/10.1016/S0740-0020(03)00044-3

Boyd, M.A., Antonio, M.A., & Hillier, S.L. (2005). Comparison of API 50 CH strips to whole-chromosomal DNA probes for identification of *Lactobacillus* species. *Journal of Clinical Microbiology*, 43(10), 5309-5311. doi: 10.1128/JCM.43.10.5309-5311.2005

Davoodabadi, A., Dallal, M.M.S., Lashani, E., & Ebrahimi, M.T. (2015). Antimicrobial Activity of *Lactobacillus* spp. Isolated From Fecal Flora of Healthy Breast-Fed Infants Against Diarrheagenic *Escherichia coli*. *Jundishapur Journal of Microbiology*, 8(12). doi: 10.5812/jjm.27852

Hassan,P., & K.K Peh. (2014). Isolation and identification of Lactobacillus acidophilus. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6,1.

Hoque, M.Z., Akter, F., Hossain, K.M., Rahman, M.S.M., Billah, M.M., & Islam, K.M.D. (2010). Isolation, identification and analysis of probiotic properties of *Lactobacillus* spp. from selective regional yoghurts. *World Journal of Dairy & Food Sciences*, 5(1), 39-46.

Klein, G., Zill, E., Schindler, R., & Louwers, J. (1998). Peritonitis Associated with Vancomycin-ResistantLactobacillus rhamnosus in a Continuous Ambulatory Peritoneal Dialysis Patient: Organism Identification, Antibiotic Therapy, and Case Report. *Journal of Clinical Microbiology*, 36(6), 1781-1783.

Mahon, C.R., Lehman, D.C., & Manuselis Jr, G. (2014). Textbook of diagnostic microbiology. Elsevier Health Sciences.

Nelson, G.M., & George, S.E. (1995). Comparison of media for selection and enumeration of mouse fecal flora populations. Journal of Microbiological Methods, 22(3), 293-300. https://doi.org/10.1016/0167-7012(95)00004-5

Ngongang, E.F.T., Tiencheu, B., Achidi, A.U., Fossi, B.T., Shiynyuy, D.M., Womeni, H.M., & François, Z.N. (2016). Effects of Probiotic Bacteria from Yogurt on Enzyme and Serum Cholesterol Levels of Experimentally Induced Hyperlipidemic Wistar Albino Rats. *American Journal of Biology and Life Sciences*, 4(6), 48.

Ngongang, E.F.T., Tiencheu, B., Fossi, B.T., Achidi, A.U., Shiynyuy, D.M., Womeni, H.M., & François, Z.N. (2016). Isolation and Identification of Cholesterol Lowering Probiotic Bacteria from Palm Wine (Raffia mambillensis). *Journal of Microbiology Research*, *6*(5), 93-102. doi:10.5923/j.microbiology.20160605.01

Nigatu, A. (2000). Evaluation of numerical analyses of RAPD and API 50 CH patterns to differentiate Lactobacillus plantarum, Lact. fermentum, Lact. rhamnosus, Lact. sake, Lact. parabuchneri, Lact. gallinarum, Lact. casei, Weissella minor and related taxa isolated from kocho and tef. *Journal of Applied Microbiology*, 89(6), 969-978. doi: 10.1046/j.1365-2672.2000.01202.x

Nyanzi, R., Shuping, D.S., Jooste, P.J., & Eloff, J.N. (2015). Antibacterial and Antioxidant Activity of Extracts from Selected Probiotic Bacteria. *Journal of Food Research*, 4(5),122. http://dx.doi.org/10.5539/jfr.v4n5p122

Ogunshe, A.A. (2008). Bioinhibition of diarrhogenic Gram-positive bacterial pathogens by potential indigenous probiotics. *Asian Pacific Journal of Tropical Medicine*, 1(2), 7-11.

Ozgun, D., & Vural, H.C. (2011). Identification of Lactobacillus strains isolated from faecal

specimens of babies and human milk colostrum by API 50 CHL system. *Journal of Medical Genetics and Genomics*, 3(3), 46-49.

Pascual, L.M., Daniele, M.B., Pájaro, C., & Barberis, L. (2006). *Lactobacillus* species isolated from the vagina: identification, hydrogen peroxide production and nonoxynol-9 resistance. *Contraception*, 73(1), 78-81. https://doi.org/10.1016/j.contraception.2005.06.066

Risan, M.H., Abdulmohimin, N., & Mohammad-Amin, S.M. (2016). Production, Partial Purification and Antitumor Properties of Bioactive Compounds from Locally Isolated Actinomycetes (KH14). *Iraqi Journal of Biotechnology*, 15(3), 51-64.

Sneath, P.H.S.; Mair, N.S.; Sharpe, M. E., & Holt, J. C. (2009). *Bergey's Manual of Systematic Bacteriology*. Baltimore, Williams and Wikins.

Valko, K., Du, C., Bevan, C., Reynolds, D.P., & Abraham, M.H. (2001). Rapid method for the estimation of octanol/water partition coefficient (log Poct) from gradient RP-HPLC retention and a hydrogen bond acidity term (Sigma alpha2H). *Current Medicinal Chemistry*, 8(9), 1137-1146. https://doi.org/10.2174/0929867013372643

Vardapetyan, H.R., Blbulyan, S.S., Tiratsuyan, S.G., & Trchounian, A.H. (2012). Elucidation of Antiradical and Antibacterial Properties of Hypericum Perforatum Extracts. ՀՀ ԳԱԱ Զեկույցներ, 112(3), 282-290.

Venkatachalam, R.N., Singh, K., & Marar, T. (2012). Phytochemical screening in vitro antioxidant activity of Psidium guajava. Free Radicals and Antioxidants, 2(1), 31-36. https://doi.org/10.5530/ax.2012.2.7

Wan, Y., Xin, Y., Zhang, C., Wu, D., Ding, D., Tang, L., Owusu, L., Bai, J., & Li, W. (2014). Fermentation supernatants of *Lactobacillus delbrueckii* inhibit growth of human colon cancer cells and induce apoptosis through a caspase 3-dependent pathway. Oncology letters, 7(5), 1738-1742. https://doi.org/10.3892/ol.2014.1959