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Antibacterial and Anti Diabetic Activities of Ethyl Acetate Fractions of *Lactobacillus* Isolated from Cow and Goat Milk

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Antibiotics are one of our most important weapons in fighting with bacterial infections and diabetics. Drugs were derived from natural sources that play an important role in the treatment and prevention of human diseases. Traditional fermented foods containing lactic acid bacteria such as milk, yogurt, curd, etc., have historically been consumed by human. Lactobacilli are well known friendly bacteria for their probiotic activities against pathogens. In this investigation cow and goat milk samples were selected and collected for the study. The gram stain was used for morphological identification of Lactobacillus sp. in the two samples. The two isolated Lactobacillus sp. from goat and cow milk samples were subjected to the extraction of secondary metabolites using the solvent ethyl acetate. The two extracts of Lactobacillus spp. those were isolated from cow and goat milk samples, showed antibacterial activity against Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa. The zone of inhibition was assessed by mm. The good results of zones were observed at 1000 and 2000 μg in cow milk and 2000 μg in goat milk extraction. These metabolites were also showed good results in antidiabetic activity using the alpha amylase inhibition activity. The IC50 value has showed moderate activity in the two crude extractions when compared to acarbose standard drug. Therefore, in this investigation the Lactobacillus sp. producing secondary metabolites was good source for the activity of antibacterial and antidiabetic activity.

Keywords: Antimicrobial; a-amylase inhibition; Milk; Lactobacillus spp; Secondary Metabolites.

The Multi-drug resistant bacteria are the cause of the disease which results in numerous clinical problems throughout the world. The use of bacterial antibiotic resistance has been considered difficulties nowadays, because of the extensive use of classical antibiotics in treatment of animal and human diseases[1, 2]. As a result of the various resistant strains has been appeared and extended causing difficulties and use of the antibiotics as growth promoters have been restricted. Therefore, the continuous improvement of new classes of the anti-microbial agents has become increasing significance to medicine[3, 4]. Diabetes, also known as diabetes mellitus (DM), is a set of illnesses brought on by the presence of excessive amounts of sugar in our blood cells, which causes high

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blood sugar levels. The 1.5 million people deaths because of diabetes and which result that 48% of death occurred due to DM in 2019 and which happened the age before 70 years[5]. The acarbose and miglitol are the inhibitors for diabetes which is using in clinical at present. Although the use of these inhibitors are causes some gastrointestinal problems[6]. Therefore, the new pharmacological active agents from medicinal plants, animals, microorganisms or their extracts can lead to efficient for many diseases[7].

The Probiotics are live bacteria or yeasts cells which make a health benefit to the host when it given sufficient proportions[8, 9]. The majority of probiotic species and strains are from the Lactobacillus and Bifidobacterium genus[10, 11]. Generally the Lactic acid bacteria (LAB) are safe bacteria and which is used to develop products with useful and probiotic properties due to their resistance to low pH and bile salts at the intestine[12]. The Lactic acid bacteria (LAB) which are occur naturally as an indigenous micro flora in raw milk and milk products such as cheese, vegetables, meat and beverages[13, 14]. The LAB are belongs to the group of Gram-positive, non-sporulating, facultative aerobic or anaerobic rods or cocci, which produce lactic acid as the most important fermentation products of the metabolism of carbohydrates[15, 16].

The arid type of LAB, that can produce antimicrobial substance such as hydrogen peroxide, bacteriocins, D-isomers of amino acids, acetaldehyde, CO_2, reuterin and diacetyl already have been isolated from different fermented products[17]. Many researchers have been reported that bacteriocins are produced by various *Lactobacilli*, *Leuconostoc*, *Lactococci*, *Enterococci* and *Pediococci*[18].

Lactobacillus sp. is a one of probiotics which is considered as a biological therapeutics and it is consider as a generally recognized as safe (GRAS) organisms[19]. Some studies have been confirmed that numerous antimicrobial mechanisms of *Lactobacillus* sp. such as production of inhibitory compounds, immune-stimulation, nutrient competition and competition for binding sites. Moreover these bacteria can also produce certain antimicrobial molecules viz, bacteriocins, hydrogen peroxide, ethanol and fatty acid which lead to inhibit the growth of pathogenic bacteria[20,21,22].

According to previous research the Lactobacillus sp. has the capability to inhibit numerous bacterial pathogens including *Escherichia coli*[23], *Streptococcus mutans*[24], *Pseudomonas aeruginosa*[25], *Staphylococcus aureus*[26], *Shigella spp.*[27], and *Clostridium difficile*[28]. Moreover recently some studies have been reported that probiotics are developing the symptoms of diabetes by regulating the increasing insulin sensitivity, intestinal microbiota composition, and mitigating autoimmune responses[29]. It has been reported that the favorable gut bacteria can able to decrease the blood glucose levels by regulating the release of hormones and enzymes[30]. Therefore, the present work aims to study the isolation and purification of *Lactobacillus* sp. from cow and goat milks, extraction of secondary metabolites and its antibacterial activity and alpha amylase inhibition activity.

MATERIALS AND METHOD

The two raw samples of cow and goat milk were collected from Koyambedu farm house, Chennai, Tamil Nadu, India. The samples were collected in the winter season of February 2022 only. The samples were carefully stored in the laboratory for further extraction and activities.

Isolation of bacterial culture

The collected two animals milk (cow and goat) were subjected to isolation and purification of *Lactobacillus* sp. An aliquot of 0.1 mL of each sample was introduced onto the nutrient broth and incubated for 24 hours at 37°C. The incubated tubes were examined for turbidity that indicating the bacterial growth. The turbidity samples were then inoculated onto LBS (*Lactobacillus* Selection) agar plates and incubated for 24 hours at 37°C and subculture onto LBS agar plates till to obtain pure culture. The subculture technique was followed up to obtaining pure culture. The isolates were subjected to Gram staining procedure. The gram stain was used for morphological identification of *Lactobacillus* sp.

Extraction and preparation of samples

The bacterial cultures were grown separately in large quantity in LBS broth medium

and incubated for three days at 37°C. The broth was centrifuged to separate the cells at 8000 rpm for 20 minutes. The clear supernatant containing the metabolites was collected. The pH of the supernatant was adjusted to 8.0 with 2 N of NaOH and extracted with an equal volume of ethyl acetate. The organic phase has dried over anhydrous sodium sulfate and concentrated in vacuo at 40°C using rotary evaporator. The resulting crude extract was dissolved in methanol to 28 mg/ml and stored at 4°C.

Anti bacterial activity

The preliminary screening for the antibacterial activity was carried out by the following procedure described by Elumalai *et al.*[31] in 2011. The antibacterial activity was investigated using the *Pseudomonas aeruginosa* (Gram Negative), *Escherichia coli* (Gram Negative), *Bacillus subtilis* (Gram Positive) and *Staphytococcus aureus* (Gram Positive) bacteria.

Disc-diffusion method

The disc diffusion method was used to determine antibacterial activity in the test samples. Mueller Hinton broth was used to produce the target organisms, in which they were kept for 24 hours. The diluted bacterial strains were cultured in the Mueller Hinton agar (MHA) medium containing petri dishes. On the culture medium, the prepared discs were placed. The sterilisation disc had been injected with test samples 500, 1000 and 2000µg. In order to determine the sensitivity of the microbial species tested, Streptomycin 20µg was used as a positive reference standard. Then, the inoculated petri dishes were incubated to 37 C for 24 hours. Measures of the diameter of the disc's Clear Zone were made and as far as its antibacterial activity is concerned, it expresses in millimeters.

Assay of Alpha-amylase inhibition

The á-amylase inhibitory activity of two sample extracts (from cow's milk and goat's milk) was performed according to standard methods with minor modifications[32]. In this the á-amylase solution of 100 ìL (0.1 mg/ml) was mixed with test samples at different concentrations, namely 10, 20, 40, 80, 160 and 320 ìg/ml. The standard (Acarbose) and control (no standard/test sample) was used for

Table 1. Characterization of isolates from cow and goat milk

No.	Collection area	Source	Gram's Reaction	Colony Morphology (Colour, Shape)
1	Koyambedu farm house	Cow milk	Gram +ve rods	Creamish white, circular
2		Goat milk	Gram +ve rods	Creamish white, circular

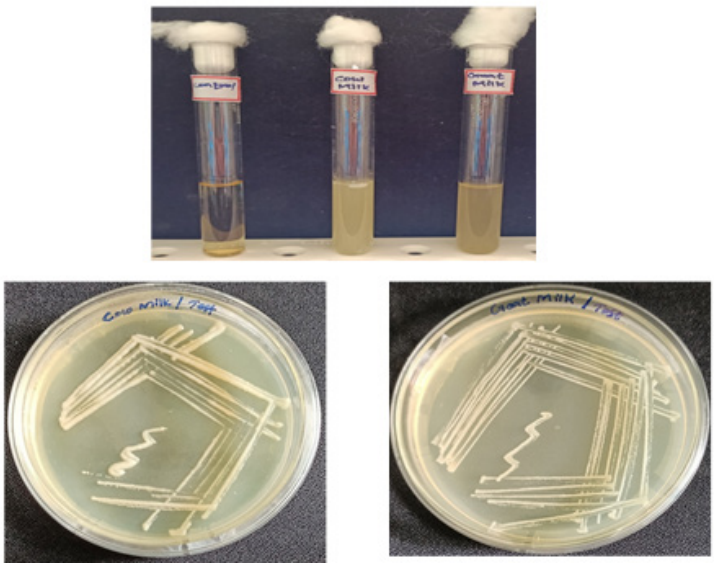

Fig. 1. Colony morphology of isolates Mother culture from Cow and Goat milk

reference and it is kept for incubation at 37 °C for 15 min. Then, 100 µL of the starch solution was added to start the reaction and incubated at 37 °C for 60 min. After that 10 µL of 1 M HCl and 100 µL of the iodine reagent were added to the test tube. The absorbance of these mixtures were measured at 565 nm and the activity of á-amylase inhibition was measured using the following formula,

% Inhibition = [(OD of test - OD of control)/OD of test] x 100

RESULTS

In this study the sample of cow and goat raw milk were collected from Koyambedu farm house in Chennai. These samples were undergone to isolation, purification of *Lactobacillus* sp., extraction of secondary metabolites, antibacterial activity and alpha amylase inhibition activities.

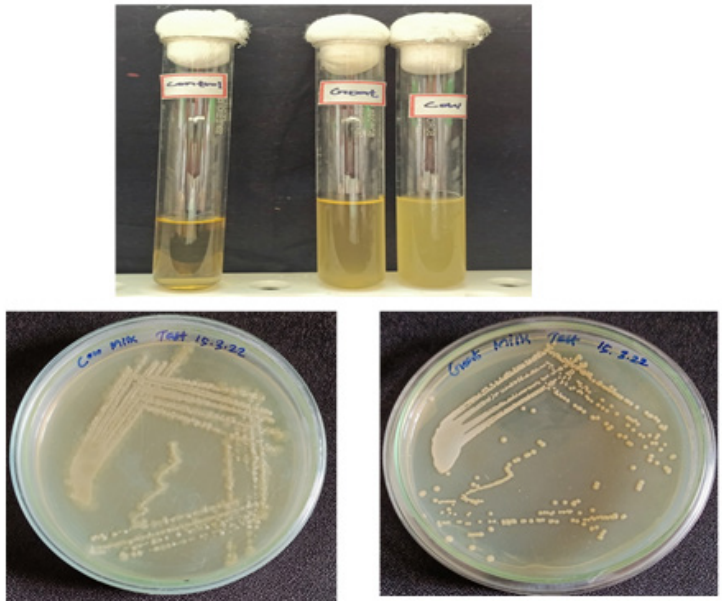

Fig. 2. Colony morphology of isolates pure culture from Cow and Goat milk

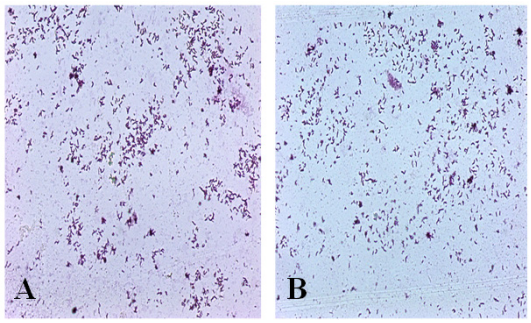

Fig. 3. A: Gram staining shows the gram positive rods in Cow milk and B: Gram staining shows the gram positive rods in Goat milk

Isolation and purification of *Lactobacillus* sp. from cow and goat milks

A total of two purified *Lactobacillus* sp. cultures were isolated from cow and goat milk using deMan Rogosa Sharpe medium. On the basis of colony morphology (viz. colour, shapes of the colony) the gram staining test were analyzed.

Table 2. Extraction of secondary metabolites from *lactobacillus* sp.

S.No.	Sample	Culture volume (mL)	Extract (mg)
1	Cow milk isolate	1000	138
2	Goat milk isolate	1000	96

Morphological and cultural characterization of isolates

Cultural characterization

The isolated bacterial cultures were observed for colony morphology on MRS agar plates and the isolated both colonies were found Gram +ve rods, creamish white in colour and circular in shape (Table 1 and Figure: 1 and 2). The cultures were subjected to further confirmation using the analysis of Gram staining.

Gram staining

The Gram staining characteristics and cellular morphology of isolated bacterial cultures were examined using standard staining procedures. The Gram-positive bacteria retained the crystal violet colour after gram staining. The isolated bacterial culture of cow milk was found purple coloured and rod shaped of varying sizes, under 40x are shown in Figure 3 (A) , and the isolated bacterial culture of goat milk was also found purple coloured and rod shaped, under 40x (Figure 3 B) which confirms the presence of *Lactobacillus* sp.

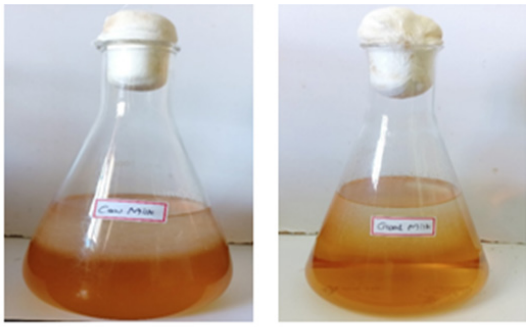

Fig. 4. Extraction of secondary metabolites from the isolated *Lactobacillus* sp. from Cow and Goat milks using Ethyl acetate

Extraction of secondary metabolites from *Lactobacillus* sp.

The two isolated *Lactobacillus* sp. from goat and cow milk samples were subjected to the extraction of secondary metabolites using the solvent ethyl acetate. The yield of the secondary metabolites from cow milk *Lactobacillus* sp was found138 mg and the goat milk *Lactobacillus* sp was found 96 mg respectively (Table 2 and Figure 4). The high yield of secondary metabolites was found from cow milk *Lactobacillus* sp. when compared to the goat milk *Lactobacillus* sp.

Antibacterial activity

Three different dosages i.e., 500, 1000 and 2000 µg of each extract of the four species were tested for the antibacterial activity. Two Gram-positive and two Gram-negative strains were selected for this study (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*). The results were measured zone of inhibition in mm by each extract at each dose. Streptomycin was used as the antibacterial standard drug. DimethylSulphoxide (DMSO) used as the control of antibacterial activity. The data are summarized and presented in Table

Table 3. Antibacterial activity of the cow milk *Lactobacillus* sp. extract

Species	The Zone of inhibition (mm)				
	500 µg	1000 µg	2000 µg	Standard 20 µg	DMSO
Bacillus subtilis	0	8	10	19	0
Staphylococcus aureus	0	0	0	23	0
Escherichia coli	0	0	0	22	0
Pseudomonas aeruginosa	0	10	16	19	0

Table 4. Antibacterial activity of the goat milk *Lactobacillus* sp. extract sp.

Species	Zone of inhibition (mm)				
	500 µg	1000 µg	2000 µg	Standard 20 µg	DMSO
Bacillus subtilis	0	0	11	19	0
Staphylococcus aureus	0	0	10	23	0
Escherichia coli	0	0	8	22	0
Pseudomonas aeruginosa	0	0	9	19	0

3 and 4 and Figure 5 and 6. In this investigation, the antibacterial activity were observed at 1000 and 2000 µg in cow milks extractions against only the *Bacillus subtilis* and *Pseudomonas aeruginosa* (Figure 5) but in goat milk extractions was observed at 2000 µg against all the four species viz, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*

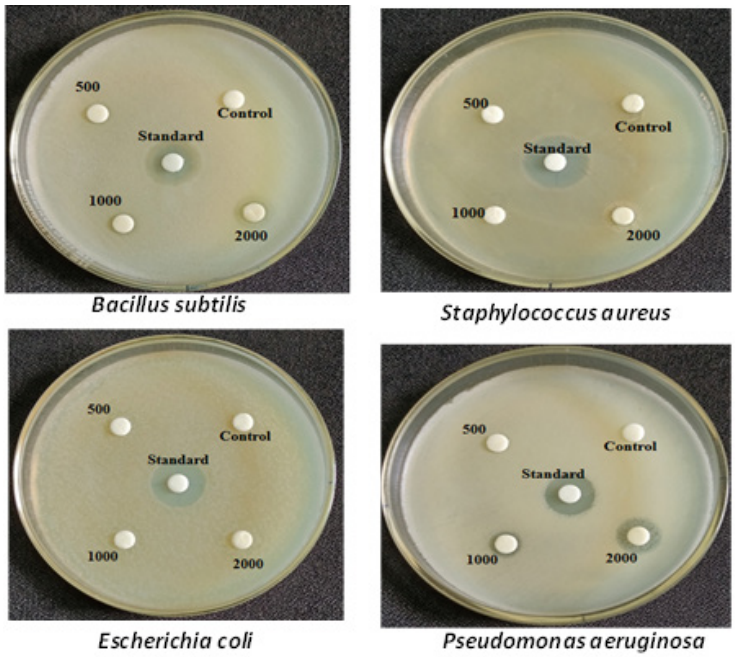

Fig. 5. Image shows the Zone of inhibition of ethyl acetate extract of cow milk's isolate.

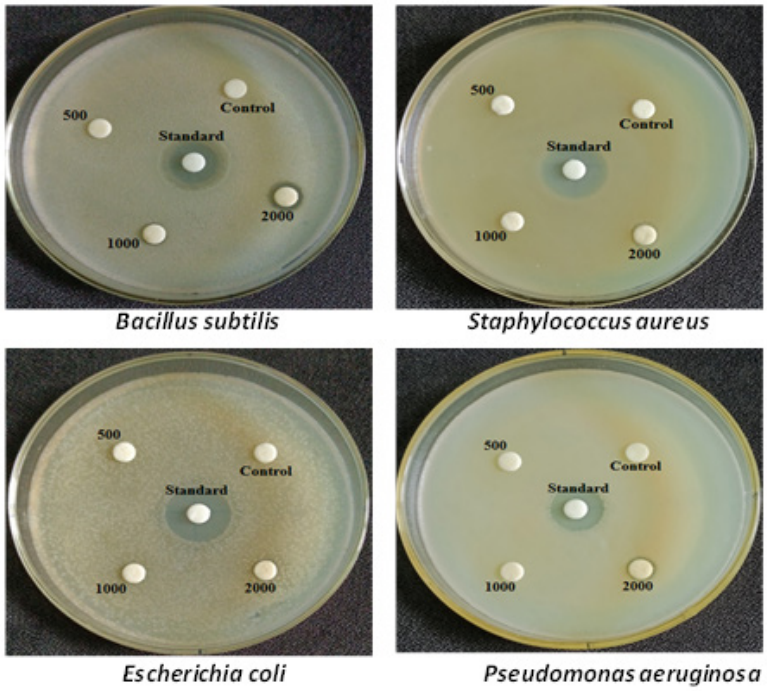

Fig. 6. Picture shows Zone of inhibition of ethyl acetate extract of goat milk's isolates

(Figure 6*)* but no activities were found in 500 µg in cow milk extractions and 500 and 1000 µg in goat milk extractions.

Antidiabetic activity

The alpha amylase inhibition assay was performed using Unuofin *et al.*, 2018. The result showed the percentage inhibition of ethyl acetate extract of cow milk *Lactobacillus* sp. was found 19.89% in 10 µg/mL concentration and 97.51% in 320 µg/mL concentration and the IC_{50} value was found 36.85 µg/mL (Table 5). Similarly, the percentage inhibition of ethyl acetate extract of goat milk *Lactobacillus* sp. was found 19.77% in 10 µg/mL and 97.45% in 320 µg/mL and the IC_{50} was found 33.07µg/mL (Table 6). The percentage inhibition of standard acarbose was found 60.32% in 10 µg/mL and 95.82% in 320 µg/mL and the IC_{50} was found 1.35µg/mL (Table 7).

The overall results of both cow milk and goat milk *Lactobacillus* sp. were showed good results of antidiabetic activity (Figure 7). Although the IC_{50} value were showed moderate activity in the two extractions when compared to acarbose standard.

DISCUSSION

Khedid *et al*.[33]in 2009 reported that the

Table 5. Antidiabetic activity of the ethyl acetate extract of cow milk's *Lactobacillus* sp

Sample	Different Concentrations (µg/mL)	% of inhibition	IC_{50} value (µg/mL)
Ethyl acetate extract of cow milk isolate	10	19.89	36.85
	20	25.62	
	40	44.36	
	80	84.05	
	160	96.34	
	320	97.51	

Table 6. Antidiabetic activity of ethyl acetate extract of goat milk's *Lactobacillus* sp.

Sample	Different Concentrations (µg/mL)	% of inhibition	IC_{50} value (µg/mL)
Ethyl acetate extract of goat milk's isolate	10	19.77	33.07
	20	30.77	
	40	53.06	
	80	80.73	
	160	96.92	
	320	97.45	

Table 7. Antidiabetic activity of the standard acarbose

Sample	Different Concentration (µg/mL)	% of inhibition	IC_{50} value (µg/mL)
Standard acarbose	10	60.32	1.35
	20	80.54	
	40	85.21	
	80	90.63	
	160	94.30	
	320	95.82	

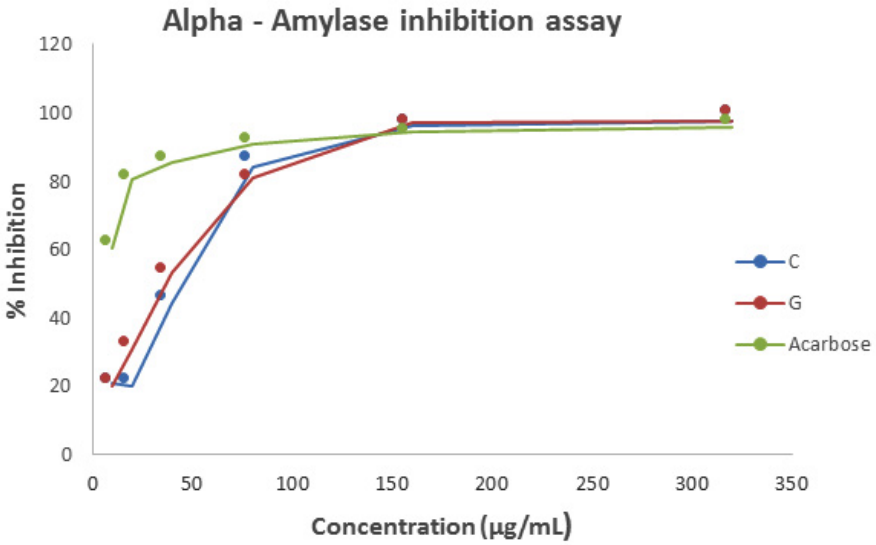

Fig. 7. Percentage analysis of Alpha amylase Inhibition activity of cow (C) extract, goat (G) extract and acarbose

presence of high amounts of LAB in cow and goat milks has useful microbiota which indicates that the source for explorations of biological materials of significant public health importance and vast applications in dairy industries. Therefore, this research cow and goat milk samples were collected from Koyambedu farm house, Chennai, Tamil Nadu, India. Based on previous reports, bacteria were grouped based on their gram stain, cell shape, cell organization, acid production from glucose and lactose, gas production from glucose, catalase activity and classify LAB strains[34, 35]. This research the gram stain was used for morphological identification of *Lactobacillus* sp. from cow and goat milk and which results confirmed the presence of *Lactobacillus* sp. The metabolites produced from lactic acid bacteria such as bacteriocin, hydrogen peroxide, acetic acid, lactic acid and low molecular weight proteins which results antimicrobial activities[36, 37, 38, 39]. The yield of metabolites from cow milk's *Lactobacillus* sp. was found to be high when compared to the metabolites from goat milk's *Lactobacillus* sp. These metabolites were used for the antibacterial activity. The Microbial peptides with pronounced antimicrobial activity are isolated from microbes, plants, animals, and in fermented food. Among these antimicrobial metabolites, the bacteriocins are proteins or peptides with bacteriostatic action which especially against to the closely-related species[40, 41]. Elegado *et al.*[42] in 2004 reported that the metabolite bacteriocin produced from *Lactobacillus plantarum* which is against to *Staphylococcus aureus.* In this study the antibacterial activity was carried out using the two extracted metabolites. The good results of zones were observed at 1000 and 2000 µg in cow milk *Lactobacillus* sp. extraction but goat milk *Lactobacillus* sp. extractions were observed only at 2000 µg. Therefore, in this investigation the *Lactobacillus* sp. producing secondary metabolites was good source for the activity of antibacterial. Various researchers have been reported that some of the health-associated benefits of consuming probiotics include immune stimulatory,[43, 44] and antidiabetic effects[45]. According to Zhang *et al.,* [46] in 2016 found that probiotics also have the ability to exert antidiabetic effects on insulin resistance in patients with type 2 diabetes. Based on the previous report in this study also proved that the extracted secondary metabolites from the *Lactobacillus* sp. were showed good antidiabetic activity.

CONCLUSION

The natural raw cow and goat milk were contains the lactic acid producing bacteria such as *Lactobacillus* spp. These bacteria contain various beneficial metabolites compounds and which is used in the field of pharmaceuticals. In this study the secondary metabolites from the *Lactobacillus*

sp. isolated from cow and goat milk were showed good results to treat microbial disease and diabetics. Moreover the *Lactobacillus* sp. is also use in many food industries. These are eco-friendly and cost effective at present and future.

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Conflict of Interest

There is no conflict of interest.

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REFERENCES

1. Lowy, F.D. *Staphylococcus aureus* infections. *New England Journal of Medicine*. 1998; 339: 520–532.
2. Savadogo, A., Ouattara Cheik, A.T., Bassole Imael, H.N., Alfred, T. Antimicrobial activities of lactic acid bacteria strain from Burkina Faso fermented milk. *Pakistan Journal of Nutrition*. 2004; 3: 174-179.
3. Gálvez, A., Abriouel, H., López, R.L., Omar, B.N. Bacteriocin-Based Strategies for Food Biopreservation. *International Journal of Food Microbiology*. 2007; 12: 51-70.
4. Lipsitch, M., Bergstrom, C.T., Levin, B.R. The epidemiology of antibiotic resistance in hospitals: paradoxes and prescriptions. *Proc. National Academy of Science, USA*. 2000; 97: 1938-1943.
5. WHO. Classification of diabetes mellitus 2019. https://www.who.int/health-topics/diabetes
6. Cheng, A.Y.Y., Fantus, I.G. Oral antihyperglycemic therapy for type 2 diabetes mellitus. *Canadian Medicinal Association Journal.* 2005, 172(2):213-226.
7. Tarling, C.A., Woods, K., Zhang, R., Brastianos, H.C., Brayer, G.D., Andersen, R.J., Withers, S.G. The Search for Novel Human Pancreatic á-Amylase Inhibitors: High-Throughput Screening of Terrestrial and Marine Natural Product Extracts. *ChemBioChem* 2008, 9:433-438.
8. FAO/WHO. "WHO working group report on drafting guidelines for the evaluation of probiotics in food," in *Guidelines for the Evaluation of probiotics in food* WHO, *Ontario, Canada.* 2002. Vol. 30.
9. Fesseha, H. "Probiotics and its potential role in poultry production: a review," *Veterinary Medicine - Open Journal*. 2019. vol. 4(2), pp. 69–76.
10. Gareau, M.G., Sherman, P.M., Walker, W.A. "Probiotics and the gut microbiota in intestinal health and disease," *Nature Reviews Gastroenterology & Hepatology*, 2010. vol. 7(9), pp. 503–514.
11. V´elez, M.P., Hermans, K., Verhoeven, T.L.A., Lebeer, S.E., Vanderleyden, J., De Keersmaecker, S.C.J. "Identification and characterization of starter lactic acid bacteria and probiotics from Columbian dairy products," *Journal of Applied Microbiology*, 2007. vol. 103(3), pp. 666–674,
12. Wegh, C.A., Geerlings, S.Y., Knol, J., Roeselers, G., Belzer, C. Postbiotics and their potential applications in early life nutrition and beyond. *Int J Mol Sci*, 2019. 20: 4673.
13. Tserovska, L., Stefanova, S., Yordanovu, T. Identification of lactic acid bacteria isolated from katyk, goat Milk and cheese. *J. Culture Collections*, 2002: 3: 48-52.
14. Chen, Y.S., Yangida, F., Shinahara, T. Isolation and identification of lactic acid bacteria, from soil using enrichment procedure. *Lett. Applied Microbiol*., 2005:40: 195-200.
15. Axelsson, L. Lactic Acid Bacteria: Classification and physiology. In: Lactic Acid Bacteria, Microbiological and Functional Aspects. Salminen, A.V. and A.O. Wright (Eds.) ouwehand. *Marcel Dekker*, *New York*, 2004: pp: 1-66.
16. Hayek, S.A., Ibrahim, S.A. Current Limitations and Challenges with Lactic Acid Bacteria: A Review. *Food and Nutrition Sciences*, 2013:4, 73-87.
17. Aslim, B., Onal, D., Beyatli, Y. Factors influencing auto aggregation and aggregation of *Lactobacillus delbrueckii* subsp. bulgaricus isolated from handmade yogurt. *Journal of Food Protection*. 2007; 70: 223-227
18. Morea, M., Baruzzi, F., Cocconcelli, P.S. Molecular and physiological characterization of dominant bacterial populations in traditional Mozzarella cheese processing. *Journal of Applied Microbiology*. 1999; 87: 574-582

19. Sieladie, D.V., Zambou, N.F., Kaktcham, P.M., Cresci, A., Fonteh, F. Probiotic properties of Lactobacilli strains isolated from raw cow milk in the western highlands of Cameroon. *Innovat. Roman. Food Biotechnol.* 2011. 9(12): 12- 28.
20. Georgieva, R., Yocheva, L., Tserovska, L., Zhelezova, G., Stefanova, N., Atanasova, A. Antimicrobial activity and antibiotic susceptibility of *Lactobacillus* and *Bifidobacterium* spp. intended for use as starter and probiotic cultures. *Biotechnol. Biotechnol. Equip* 2015. 29, 84–91.
21. Inglin, R.C., Stevens, M.J., Meile, L., Lacroix, C., Meile, L. High throughput screening assays for antibacterial and antifungal activities of *Lactobacillus* species. *J. Microbiol. Methods*, 2015. 114, 26–29.
22. Kolida, S., Saulnier, D.M., Gibson, G.R. Gastrointestinal microflora: Probiotics. *Adv. Appl. Microbiol.,* 2006. 59: 187-219.
23. Kumar, M., Dhaka, P., Vijay, D., Vergis, J., Mohan, V., Kumar, A. Antimicrobial effects of *Lactobacillus* plantarum and Lactobacillus acidophilus against multidrug-resistant enter aggregative *Escherichia coli*. *Int. J. Antimicrob. Agents*, 2016. 48, 265–270.
24. Ahn, K.B., Baik, J.E., Park, O.J., Yun, C.H., Han, S.H. *Lactobacillus* plantarum lipoteichoic acid inhibits biofilm formation of Streptococcus mutans. *PLoS One*, 2018. 13:e0192694.
25. Jamalifar, H., Rahimi, H., Samadi, N., Shahverdi, A., Sharifian, Z., Hosseini, F. Antimicrobial activity of different Lactobacillus species against multidrug resistant clinical isolates of Pseudomonas aeruginosa. *Iran J. Microbiol*., 2011. 3, 21–25.
26. Kang, M. S., Lim, H.S., Oh, J.S., Lim, Y.J., Wuertz-Kozak, K., Harro, J.M., *et al*. Antimicrobial activity of Lactobacillus salivarius and Lactobacillus fermentum against Staphylococcus aureus. *Pathog. Dis*., 2017. 75:ftx009.
27. Mirnejad, R., Vahdati, A.R., Rashidiani, J., Erfani, M., Piranfar, V. The antimicrobial effect of *Lactobacillus casei* culture supernatant against multiple drug resistant clinical isolates of Shigella sonnei and Shigella flexneri in vitro. *Iran Red Crescent Med. J* 2013. 15, 122–126.
28. McFarland, L.V. Probiotics for the primary and secondary prevention of *C. difficile* infections: a meta-analysis and systematic review. *Antibiotics* 2015. 4, 160–178.
29. Tabrizi, R., Moosazadeh, M., Lankarani, K.B., Akbari, M., Heydari, S.T., Kolahdooz, F., Asemi, Z. The effects of synbiotic supplementation on glucose metabolism and lipid profiles in patients with diabetes: a systematic review and meta-analysis of randomized controlled trials. *Probiotics Antimicro*, 2018. 10:329–342.
30. Gérard, C., Vidal, H. Impact of gut microbiota on host glycemic control. *Front Endocrinol*, 2019. 10:29.
31. Elumalai, E.K., Ramachandran, M., Thirumalai, T., Vinothkumar, P. Antibacterial activity of various leaf extracts of Merremia emarginata. *Asian Pac J Trop Biomed*. 2011. (5):406-8.
32. Unuofin, J.O., Otunola, G.A., Afolayan, A.J. In vitro á-amylase, á-glucosidase, lipase inhibitory and cytotoxic activities of tuber extracts of Kedrostis africana (L.) Cogn, *Heliyon*, 2018. 4(9), p.e00810.
33. Khedid, K., Faid, M., Mokhtari, A., Soulaymani, A., Zinedine, A. Characterization of lactic acid bacteria isolated from the one humped camel milk produced in Morocco. *Microbiol. Res*., 2009. 164:81–91.
34. Abd Gawad, E.l., Abd El Fatah A., Al Rubayyi, K. "Identification and characterization of dominant lactic acid bacteria isolated from traditional rayeb milk in Egypt," *Journal of American Science*, 2010. vol. 6, pp. 728–735.
35. El-Shenawy, M., Dawoud, E.I., Amin, G.A., El-S.K., Fouad, M.T., Soriano, J.M. "Antimicrobial activity of some lactic acid bacteria isolated from local environment in Egypt," *African Journal of Microbiology Research*, 2017. vol. 11, no. 8, pp. 327–334.
36. Barnby –Smith, F.M., Roller, S.D., Woods, L.F.J., Barker, M., Nightingale, M., Gibbs, P.A. Production of antimicrobial compounds by lactic acid bacteria. The British Food Manufacturing Industries Research Association. Research Reports. No.662.*Leatherhead Food RA*, 1989.
37. Larsen, A. G., Vogensen, F.K., Josephsen, J. Antimicrobial activity of lactic acid bacteria isolated from sour doughs: Purification and characterization of bavaricin A, a bacteriocin produced by *Lactobacillus bavaricus* MI401.*J.Appl. Bacterio,* 1993. 75:113-122.
38. Daeschel, M.A. Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technol*, 1989. 43: 164-167.
39. *Suchita Kholia.* Isolation of Lactic Acid Bacteria and Detection of their Antimicrobial. *Inter. J. on Emerging Technologies,* 2017. 8(1): 260-266.
40. Klaenhammer, T.R. Bacteriocins of lactic acid bacteria, *Biochimie*, 1988. 70: 337-349.
41. Garneau, S., Martin, N.I., Vederas, J.C. Two peptide bacteriocin produced by lactic acid bacteria. *Biochimie,* 2002. 84; 577-592.
42. Elegado, F.B., Guerra, M.A.R.V., Macayan, R.A., Mendozab, H.A., Lirazan, M.B. Spectrum of bacteriocin activity of Lactobacillus plantarum

BS and fingerprinting by RAPD-PCR. *Int.J.Food Microbiol*., 2004. 95:11-18.

43. Nagao, F., Nakayama, M., Muto, T., Okumura, K. "Effects of a fermented milk drink containing Lactobacillus casei strain shirota on the immune system in healthy human subjects", *Biosci Biotechnol Biochem*, 2000. Vol. 64 No. 12, pp. 2706-2708.
44. Gill, H.S. "Stimulation of the immune system by lactic cultures", *International Dairy Journal*, 1998. Vol. 8 Nos 5/6, pp. 535-544.
45. Zhang, Y., Zhang, H. "Microbiota associated with type 2 diabetes and its related complications", *Food Science and Human Wellness*, 2013.Vol. 2 Nos 3/4, pp. 167-172.
46. Zhang, Q., Wu, Y., Fei, X. "Effect of probiotics on glucose metabolism in patient with type 2 diabetes mellitus: a meta-analysis of randomized controlled trial", *Medicina*, 2016. Vol. 52 No. 1, pp. 28-34.