Potential drug target from breast milk *Lactobacillus* against vaginal pathogens

Alvo potencial de drogas de *Lactobacillus* do leite materno contra patógenos vaginais

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ABSTRACT

The term “Probiotics” refers to the micro-organisms that confers health benefits to hosts when administered in adequate amounts. In this work, *Lactobacillus* was isolated from breast milk of a 26 yr old women and was treated against vaginal pathogens by varying in different concentration (50µl, 40µl and 30µl). Identification of *Lactobacillus* was carried out by motility, gram staining and biochemical test. The antibacterial effects of the *Lactobacillus* against vaginal pathogens were carried out by disc Agar diffusion method and Antibiotic sensitivity test was also analyzed for the pathogens. The antimicrobial activity of the sample revealed that the *Lactobacillus* isolated from breast milk showed significant effectively against vaginal pathogens especially higher for *Klebsiella pneumonia.* GC-MS was carried out to identify bioactive compounds, followed by the identification of novel bioactive compounds in the corresponding fraction. The main aim is to assess the probiotic nature of *Lactobacillus* in preventing cervical pathogens by studying the effectiveness of antimicrobial activity against vaginal pathogens by identifying the effective compounds by GC-MS and they may widened up

the panorama in research and may act as a promising natural human source based drug in medical field without taking any chemical drugs which cause side effects.

Keywords: probiotic, vaginal pathogens, *Lactobacillus,* LMW compounds, Gc-Ms, antimicrobial compounds.

RESUMO
O termo "probióticos" refere-se aos microrganismos que conferem benefícios à saúde dos hospedeiros quando administrados em quantidades adequadas. Neste trabalho, o Lactobacillus foi isolado do leite materno de uma mulher de 26 anos e foi tratado contra patógenos vaginais variando em diferentes concentrações (50µl, 40µl e 30µl). A identificação do Lactobacillus foi realizada por motilidade, coloração de Gram e teste bioquímico. Os efeitos antibacterianos do Lactobacillus contra patógenos vaginais foram realizados pelo método de difusão em disco de Agar e o teste de sensibilidade aos antibióticos também foi analisado para os patógenos. A atividade antimicrobiana da amostra revelou que o Lactobacillus isolado do leite materno apresentou eficácia significativa contra patógenos vaginais, especialmente maior para Klebsiella pneumonia. O GC-MS foi realizado para identificar compostos bioativos, seguido pela identificação de novos compostos bioativos na fração correspondente. O principal objetivo é avaliar a natureza probiótica do Lactobacillus na prevenção de patógenos cervicais, estudando a eficácia da atividade antimicrobiana contra patógenos vaginais por meio da identificação dos compostos eficazes por GC-MS, o que pode ampliar o panorama da pesquisa e atuar como um promissor medicamento natural de origem humana no campo da medicina, sem a necessidade de tomar nenhum medicamento químico que cause efeitos colaterais.

Palavras-chave: probiótico, patógenos vaginais, *Lactobacillus*, compostos LMW, Gc-Ms, compostos antimicrobianos.

1 INTRODUCTION

A literal probiotic should preferably be of human origin, safe, and free of vectors that are able to transfer resistance to antibiotics and of pathogenicity or toxicity factors. In addition, a probiotic should have great range to survive under intestinal conditions (acidic pH, enzymes, biliary salts, etc.). Moreover, a probiotic should exhibit antagonism against pathogens and stimulation of the immune system and, ultimately, must have demonstrable beneficial effects on the host [1-3]. Finally, maintenance of the activity, viability, and growth potency of the probiotic upon technologic treatment should be demonstrated[4-5]. The effects of probiotics on host health have been reported in many articles, reviews, and systematic reviews (6-7). These studies have documented the role of probiotics in the prevention of health problems, including digestive disorders such as diarrhea caused by infections (4), antibiotic-associated diarrhea (8), irritable bowel syndrome (IBS) (9), *Clostridium difficile*–associated diarrhea in adults and children (10), inflammatory bowel disease (IBD), only in ulcerative colitis (11), and allergic disorders

such as atopic dermatitis (eczema) (12) and allergic rhinitis (13).Probiotics are made of good live bacteria and/or yeasts that naturally live in our body. Though there are many types of bacteria that can be considered to be probiotics, there are two specific types of bacteria that are common probiotics found in stores. These include *Lactobacillus* and *Bifidobacteriu* [14].

Antibiotic treatments can upset the gut microbiome and its normal balance of "good" and "bad" bacteria, leading to diarrhoea. Probiotics taken before, during, and after antibiotic treatment can reduce the chances of diarrhoea, according to several studies. But, researchers have had mixed results regarding the benefits of probiotics in preventing traveler's diarrhoea [15].

Many other claims are made for probiotics—that they lower cholesterol, alleviate allergic skin conditions (like eczema), treat ulcers and urinary tract infections, improve vaginal health, reduce the risk of colon cancer, ease anxiety and ward off traveler's diarrhea. Good evidence to support these claims is lacking. Research on probiotics for weight loss has yielded conflicting results, and even studies with positive results have mostly found very small benefits, as was seen in an analysis of 15 clinical trials in Obesity Reviews in 2018 [16]. There is remarkable prevalence of RTIs in pregnant females (68%). These infections are known to produce inevitable conclusion in pregnancy. This alarms for a needful action in this group of females because of the complications associated with these infections in pregnancy[17]. Increase number of cases were seen in third trimester of gestation. These infections are more seen in third trimester of pregnancy because as pregnancy advances, various hormonal changes take place and thus occurrence of endogenous RTIs increases.16 Overall, candidiasis was the most prevalent infection seen in pregnant women (36.36%) followed by BV (25%), TV (4.5%) [18].

To apprehend the emerging needs of supplements, probiotics used in health food industry moreover many investigations are aiming on probiotics potential LAB isolation from different resources of fermented milk, foods, Taiwanese pickled cabbage and faeces of breast-fed infants [19-21]. In addition, it also contains bioactive compounds responsible for a wide range of beneficial effects such as the promotion of immune system maturation and the protection against infections. Amidst these bioactive agents, probiotic bacteria have been recently isolated from human milk. Among these bioactive agents, probiotic bacteria have been recently isolated from human milk [22].

Several studies have reported that human breast milk contains complex microbial community. This community impacts both, the shape of the infant gut microbiota as well

as consequently impacts host health. *Lactobacillus* is an important probiotic and has many applications in the functional food industry [23]. The role of *Lactobacillus* species in the female urogenital tract act as a barrier to infection is of considerable interest. These organisms are believed to contribute to the control of vaginal microbiota by competing with other microorganisms for adherence to epithelial cells and by producing antimicrobial compounds [24]. The maternal gut is the vital source of commensal bacteria in the infant gut during the lactation stage, where breast milk acts as an intermediary for the transfer of potential probiotic bacteria consortia, including *Lactobacillu* [25]. The isolation of probiotic bacteria with beneficial effects for the host provides scientific support for the supplementation of infant formula with these bacteria, in order to advance the pursuit of the main goal of formula: to mimic breast milk and its functional effects as firmly as possible.

2 MATERIALS AND METHODS

2.1 SAMPLE COLLECTION

Breast milk was collected from a breast feeding mother of 26 yr. old healthy women. The sample was maintained in a sterile container and pure culture was done. Bacterial colonies were stored in 0.8% MRS agar overlaid with 50% glycerol at -20^{o}C.

2.2 ISOLATION OF LACTOBACILLUS SPECIES FROM BREAST MILK

Lactobacillus sp. was isolated from breast milk and the sample was taken in sterilized flask, and experiment was carried out. The milk was serially diluted to get different dilutions. From that dilution the sample was spreaded on MRS medium and incubated at 37^{0}C which is optimum temperature for *Lactobacillus* growth. Incubation is carried out for 24 hours. After the period of incubation, the isolated colonies were grown and colony characterization was done for this colonies which are found to be *Lactobacillus* species. The isolated colony formed on the MRS agar plates was identified using gram staining and biochemical test. The

identification was done according to Bergey's manual of determinative of bacteriology.

2.3 MORPHOLOGICAL EXAMINATION OF CULTURE

Cultural and Morphological examination was carried out by using Gram's staining method described by Hans Christian Gram (1884).

2.4 IDENTIFICATION OF THE PURE CULTURE

Pure culture isolated from MRS agar slant was identified with the help of biochemical tests like motility test, catalase test, urease test, TSI test, citrate utilization test, oxidase test, MR-VP, indole and sugar fermentation test.

2.5 MOTILITY TEST

Soft agar test was used for the testing if the bacteria were motile or non-motile through stab inoculation.

2.6 BACTERIAL STRAIN AND CULTURE CONDITIONS

One-gram negative and two gram positive bacteria were used for antibacterial assay respectively. *Klebsiella pneumoniae* , *Staphylococcus aureus* and *Enterococcus faecalis* were purchased from Magnum Diagnostic Centre, Trichy. These gram positive and gram negative test organisms were maintained in brain hear infusion agar butt slants in screw capped tubes and kept at $4^{O}C$.

2.7 BIOCHEMICAL CHARACTERIZATION OF THE ISOLATED BACTERIAL STRAIN

Identification of the isolated bacteria as *Lactobacillus sp.* and vaginal pathogens were performed according to their morphological, cultural, physiological and biochemical characteristics by the procedures as described in bergey's manual of systematic bacteriology. The test carried out were gram staining, motility test, production of catalase, indole, methyl red, voges-proskauer, citrate utilization , urease, TSI , oxidase, indole and carbohydrate fermentative test.

2.8 ANTIBACTERIAL/ ANTIMICROBIAL ACTIVITY TESTING

(a) Microorganism

Staphylococcus aureus, *Enterococcus faecalis*, *Klebsiella pneumoniae* were purchased from Magnum Diagnostic Centre, Trichy.

(b) Antimicrobial activity screening using *Lactobacillus* strain

The Mueller Hinton agar (MHA) medium was used for agar well diffusion approach to examine antibacterial test. The Mueller Hinton agar (MHA) medium was poured in petri plate and allowed to solidify. Bacterial suspension (*Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*) was enriched in saline for 4

hours. 100µl of bacterial culture was spread aseptically over the Mueller Hinton agar plates using a sterile cotton swab. *Lactobacillus* species isolated from three different sample breast milk was centrifuged at 5000 rpm for 15 minutes and supernatant was taken without disturbing the pellet. Using sterile 100mµl tip (micropipette tips) agar gel was punctured to create well. Supernatant of three different sample were loaded into those wells and saline water was used as control. All plates were incubated at 37^0C for 24 hours. The antimicrobial properties were determined by measuring of the zone of inhibition (diameter).

(c) Antibiotic sensitivity test

Three bacterial colonies (*Klebsiella pneumoniae, Staphylococcus aureus* and *Enterococcus faecalis*) were touched by cold heat sterilized nichrome wire inoculation loop and the same was inoculated into sterile 2µl of normal saline water. The saline water was incubated at 37^0C for 4 hours to get light to moderate turbidity, which were yield young colonies. The freshly grown organisms were uniformly inoculated aseptically into Mueller Hinton agar by using sterile swab. The required antibiotic disc Ampicillin, Oflaxacin, Gentamycin, Amphotericin B, Vancomycin, Nitrofurantoin were placed over the lawn culture by using sterile forceps. The petriplate was incubated at 370C for 18-24 hours. After the completion of this period the inhibited zones by the antibiotics were measured in a well light sourced safety hood.

2.9 GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GC-MS)

GC-MS analysis of extracellular compounds were performed as previously described. Briefly, the compounds were extracted with methanol and chloroform. 1µl of both the methanol and chloroform extracts was separated on a nonpolar HP-5MS capillary column (30 m × 0.18 mm) in a Agilent GC fitted to an Agilent MS detector. The injector temperature was 250°C and the oven temperature was programmed at an initial temperature of 50° C for 1 min, rising at 25°C per minute to 160° C and maintained at that temperature for 1 min. The temperature was subsequently increased by 10° C per min to 230° C and maintained at that temperature for a further 4.6 min. The carrier gas helium was kept at a constant pressure of 5 kPa. The GC was directly interfaced with an Agilent mass spectrometer with an interface temperature of 250°C. Sample ionization was done by 70 eV electron impact and was analysed in positive mode. Structural determination was by comparison of mass spectral patterns to NIST data bases.

3 RESULTS AND DISCUSSION

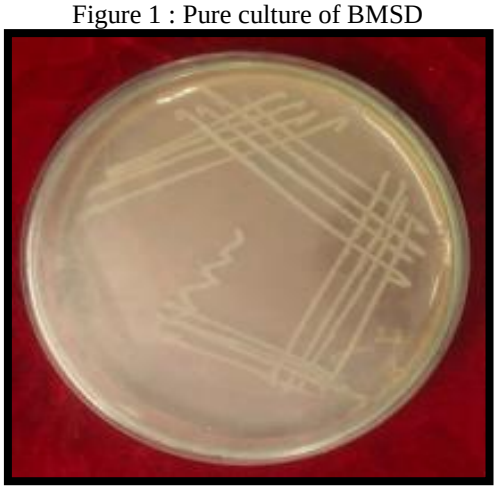
3.1 ISOLATION OF PURE COLONIES FROM BREAST MILK SAMPLE

In the present investigation, *Lactobacillus* was isolated from breast milk and named as BMSD.

3.2 SUB CULTURING DIFFERENT COLONIES (SUBSEQUENT DAYS)

Subculture the different colonies of sample (BMSD) for subsequent hours as following 34 hours, 48 hours, 72 hours, 96 hours, 120 hours. On MRS agar plates, pure white colonies were observed and this was identified as *Lactobacillus* sp. (Fig 1)

Figure 1 : Pure culture of BMSD

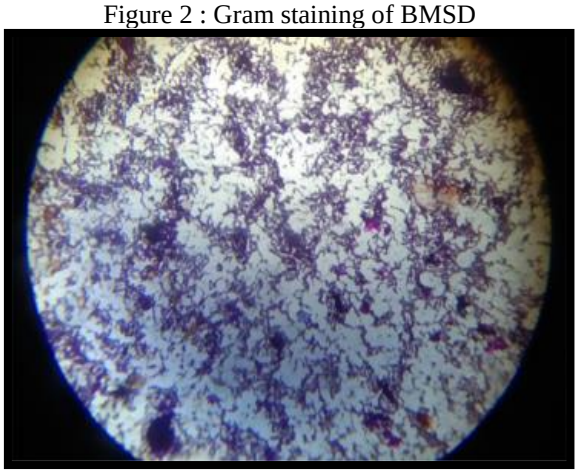


Source: Breast Milk

3.3 GRAM'S STAINING

Gram's staining was performed for all colonies for the study of different morphologies. Luxuriantly grown bright white colonies from MRS agar were selected for grams staining. When specimen was observed under 100X (oil immersion) gram positive purple colour rod shaped bacilli were observed. (Fig 2)

Figure 2 : Gram staining of BMSD



Source: Breast Milk

3.4 PREPARATION OF STOCK CULTURE

In 80µl of MRS broth, culture of *Lactobacillus* sp. was inoculated and incubated the standing culture at 37^0C in an incubator. This obtained broth is the stock culture which is used for future studies.

3.5 BIOCHEMICAL CHARACTERIZATION

The biochemical characterization of the sample BMSD was as follows: (Table:1)

Table:1 Biochemical characterization of BMSD

BIOCHEMICAL TEST	**SAMPLE**
	BMSD
Triple sugar iron agar	+
Urease test	-
Citrate utilization test	-
Catalase test	-
Oxidase test	-
Indole test	-
Methyl red test	-
Voges Prauskauer test	-
Sucrose	+
Glucose	+
Lactose	+

Source: Breast Milk

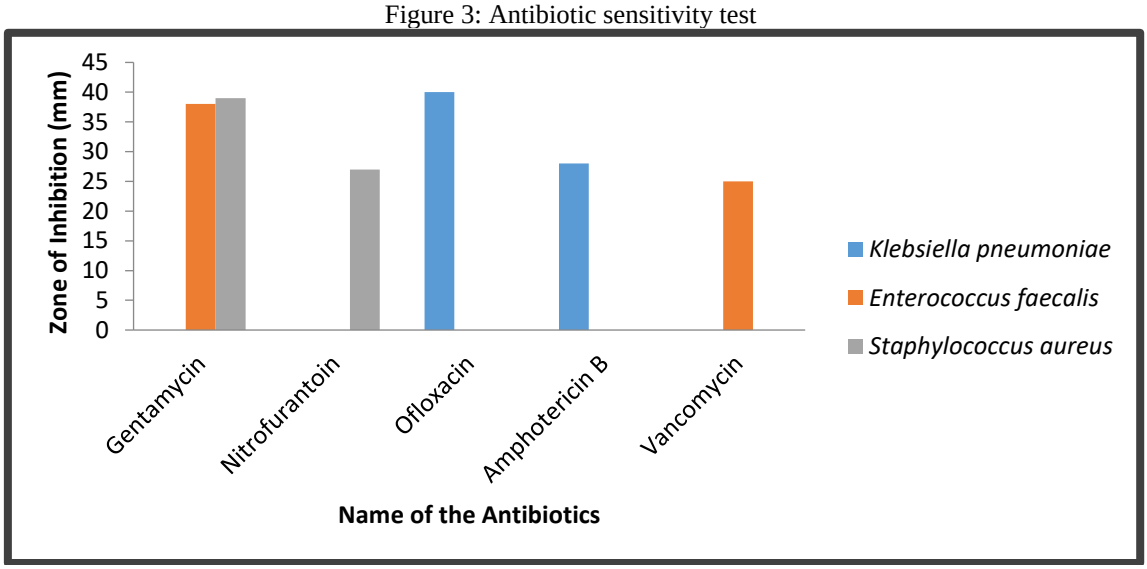
3.6 Antimicrobial screening using *Lactobacillus* spp.

The zone of inhibition was measured for both antimicrobial and antibiotic sensitivity test against the pathogenic organisms:

3.6.1 Antibiotic Sensitivity Test:

The zone of inhibition was measured for antibiotics against the pathogenic organisms which were shown in (Fig 3)

Figure 3: Antibiotic sensitivity test



Source: Different antibiotics

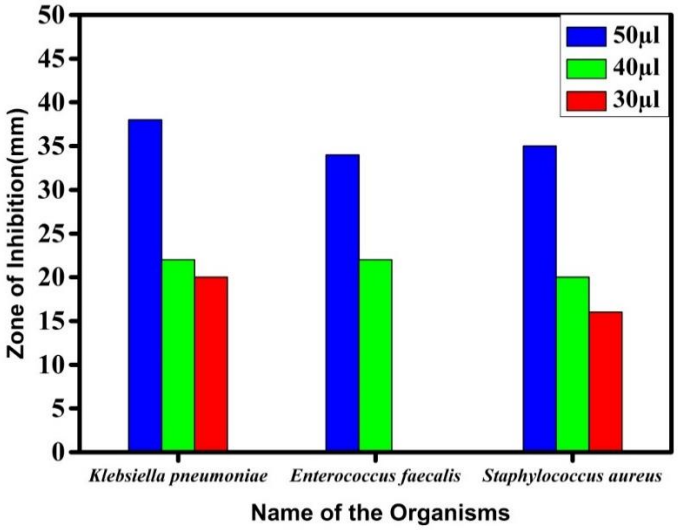
The antibiotic sensitive test of antibiotics was investigated against pathogenic organisms *Klebsiella pneumoniaee*, *Staphylococcus aureus* and *Enterococcus faecalis*.

For *Klebsiella pneumoniaee*, oflaxcin showed maximum zone of inhibition than Amphotericin B,followed by *Enterococcus faecalis*, Nitofurantoin showed maximum zone of inhibition than vancomycin and for *Staphylococcus aureus*, gentamycin showed maximum zone of inhibition than vancomycin.

3.6.2 Antimicrobial Screening

The zone of inhibition was measured for BMSD against the pathogenic organisms were shown in (Fig 4)

Figure 4: Antimicrobial screening

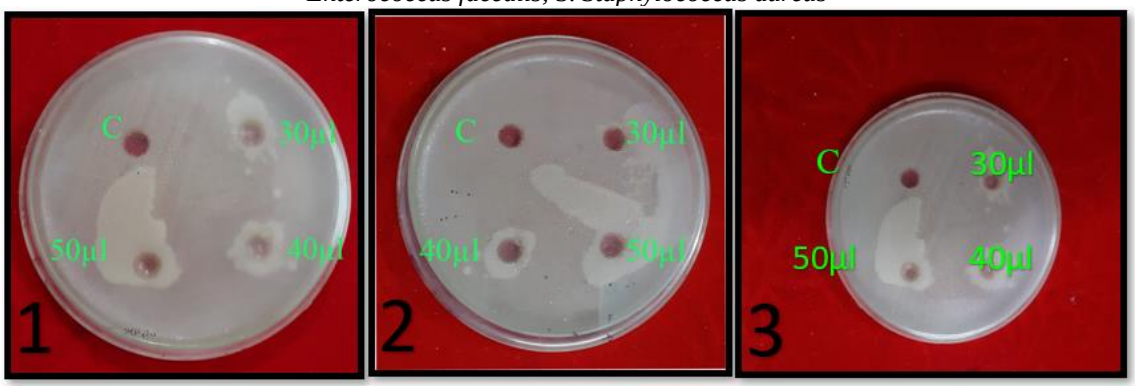


Source: Micro-organisms

The anitimicrobial activity of BMSD was investigated against pathogenic organisms *Klebsiella pneumoniaee*, *Staphylococcus aureus* and *Enterococcus faecalis*. The highest antimicrobial activity was observed for BMSD against *Klebsiella pneumoniae* (38mm), *Enterococcus faecalis* (34mm), *Staphylococcus aureus* (35mm) in 50 µl concentration respectively.

The antimicrobial activity of BMSD-1 was investigated against pathogenic organisms *Klebsiella pneumoniaee*, *Staphylococcus aureus* and *Enterococcus faecalis* in different concentrations. The highest antimicrobial activity was observed against *Klebsiella pneumoniae* (38mm in 50 µl conc.), (22mm in 40µl conc.), and (20mm in 30µl conc.) respectively, followed by *Enterococcus faecalis* (34mm in 50 µl conc.), (22mm in 40µl conc.), and *Staphylococcus aureus* (35mm in 50 µl conc.), (20mm in 40µl conc.), and (16mm in 30µl conc.) (Fig 5)

Figure 5: Maximum zone of inhibition shown by BMSD-1 against 1. *Klebsiella pneumoniae*, 2. *Enterococcus faecalis*, 3. *Staphylococcus aureus*

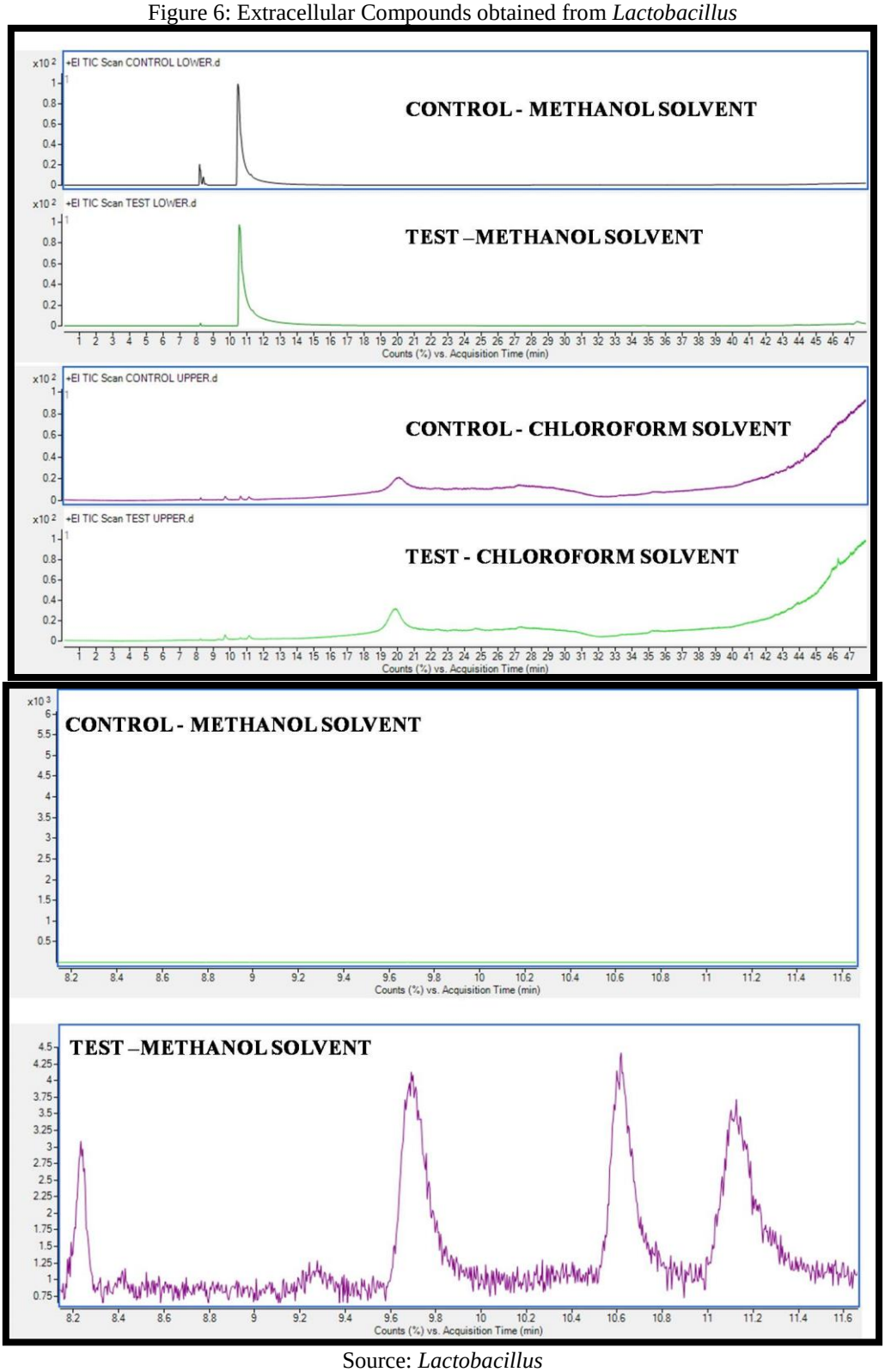


Source: Different micro-organisms

3.7 GC-MS ANALYSIS OF THE EXTRACELLULAR COMPONENTS

Fig 6 shows the extracellular compounds found from *Lactobacillus*. The main component was found to be lactic acid.

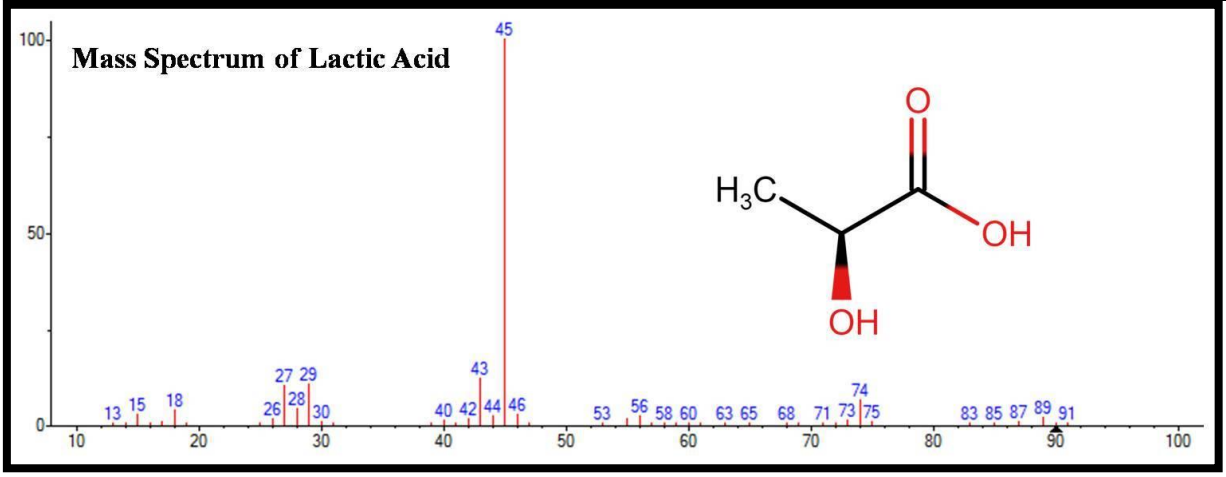
Figure 6: Extracellular Compounds obtained from *Lactobacillus*



Source: *Lactobacillus*

Table 2: Prediction of Target for Lactic Acid: Probability scores for lactic acid - higher probability with indicated protein class is assumed as bioactive and to have this protein as target.

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*
Tyrosine-protein kinase LCK	LCK	P06239	CHEMBL258	Kinase	0
Tyrosine-protein kinase FYN	FYN	P06241	CHEMBL1841	Kinase	0
Matrix metalloproteinase 9	MMP9	P14780	CHEMBL321	Protease	0
Matrix metalloproteinase 2	MMP2	P08253	CHEMBL333	Protease	0
HMG-CoA reductase	HMGCR	P04035	CHEMBL402	Oxidoreductase	0
Histone deacetylase 3	HDAC3	O15379	CHEMBL1829	Eraser	0
Estrogen receptor beta	ESR2	Q92731	CHEMBL242	Nuclear receptor	0
Egl nine homolog 1	EGLN1	Q9GZT9	CHEMBL5697	Oxidoreductase	0
Aldose reductase	AKR1B1	P15121	CHEMBL1900	Enzyme	0.085
Adenylosuccinate synthetase 2	ADSS	P30520	CHEMBL4875	Enzyme	0
DNA Adenine Methylase	DAM	P0AEE9	CHEMBL1075075	Enzyme	0.11



Source: *Lactobacillus*

4 CONCLUSION

A study was initiated to know the antimicrobial activity of *Lactobacillus* isolated from breast milk against vaginal pathogens (*Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecalis)*.In present investigation, *Lactobacillus* were isolated from breast milk sample from a healthy mother and was identified by grams staining, biochemical and motility test. The *Lactobacillus* was tested against vaginal pathogens for its antimicrobial screening in three different concentration (30µl, 40µl and 50µl). Antibiotic sensitivity test for the vaginal pathogens were tested by using various antibiotic disc, by this test the sensitivity and resistivity to different antibiotics of vaginal pathogens were examined. In this work, *Lactobacillus* isolated from breast milk of a healthy mother showed maximum zone of inhibition in all three concentration against *Klebsiella pneumonia* (38mm in 50 µl conc.), (22mm in 40µl conc.), and (20mm in 30µl conc.) respectively, followed by *Enterococcus faecalis* (34mm in 50 µl conc.), (22mm in 40µl conc.), and *Staphylococcus aureus* (35mm in 50 µl conc.), (20mm in 40µl conc.), and (16mm in 30µl conc.) The bioactive compounds present in *Lactobacillus* were determined

by GC-MS method and molecular docking of these compound will be done in further studies. Hence, *Lactobacillus* a probiotic isolated from breast milk showed great effect against vaginal infection and these steps toward establishing "good science" may result in the approval of health claims in the near future.

REFERENCES

1. AO/WHO (2001) Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Acid Bacteria. Report of a Joint FAO/WHO Expert Consultation,Córdoba,Argentina.http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf?ua=1

2. Henrik Andersson, Nils-Georg Asp, Åke Bruce, Stefan Roos, Torkel Wadström, Health effects of probiotics and prebiotics A literature review on human studies, Näringsforskning, 45:1, (2001) 58-75, DOI: 10.3402/fnr.v45i0.1790

3. Aceti, A., Gori, D., Barone, G. et al. Probiotics for prevention of necrotizing enterocolitis in preterm infants: systematic review and meta-analysis. Ital J Pediatr 41, 89 (2015). https://doi.org/10.1186/s13052-015-0199-2

4. Plaza-Díaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. Immune-mediated mechanisms of action of probiotics and synbiotics in treating pediatric intestinal diseases. Nutrients 10 1:42(2018) doi: 10.3390/nu10010042.

5. Fontana, L., Bermudez-Brito, M., Plaza-Diaz, J., Muñoz-Quezada, S. & Gil, A. Sources, isolation, characterisation and evaluation of probiotics. Br. J. Nutr. 109 (2013) S35–S50 doi: 10.1017/S0007114512004011

6. Naseri A, Seyedi-Sahebari S, Mahmoodpoor A, Sanaie S. Probiotics in Critically Ill Patients: An Umbrella Review. Indian J Crit Care Med. 26(3) 2022 339-360. doi: 10.5005/jp-journals-10071-24129..

7. Martinelli M, Banderali G, Bobbio M, Civardi E, Chiara A, D'Elios S, Lo Vecchio A, Olivero M, Probiotics' efficacy in paediatric diseases: which is the evidence? A critical review on behalf of the Italian Society of Pediatrics. Ital J Pediatr.46(1): 2020 104. doi: 10.1186/s13052-020-00862-z.

8. Cremonini F, Videlock EJ. Probiotics are associated with a decreased risk of antibiotic-associated diarrhoea. Evid Based Med. 18(2): 2013 71-2. doi: 10.1136/eb-2012-100863.

9. Quartero AO, Meineche-Schmidt V, Muris J, Rubin G, de Wit N. Bulking agents, antispasmodic and antidepressant medication for the treatment of irritable bowel syndrome. Cochrane Database Syst Rev.18;(2):2005 doi: 10.1002/14651858.CD003460.

10. Goldenberg JZ, Yap C, Lytvyn L, Lo CK, Beardsley J, Mertz D, Johnston BC. Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. Cochrane Database Syst Rev 12(12) 2017 doi: 10.1002/14651858.CD006095.pub4.

11. Ghouri YA, Richards DM, Rahimi EF, Krill JT, Jelinek KA, DuPont AW. Systematic review of randomized controlled trials of probiotics, prebiotics, and synbiotics in inflammatory bowel disease. Clin Exp Gastroenterol. 7 2014 473-87. doi: 10.2147/CEG.S27530.

12. Anania C, Brindisi G, Martinelli I, Bonucci E, D'Orsi M, Ialongo S, Nyffenegger A, Raso T, Spatuzzo M, De Castro G, Zicari AM, Carraro C, Piccioni MG, Olivero F. Probiotics Function in Preventing Atopic Dermatitis in Children. Int J Mol Sci. 23(10): 2022 5409. doi: 10.3390/ijms23105409..

13. Matsuoka T, Shamji MH, Durham SR. Allergen immunotherapy and tolerance. Allergol Int. 62(4) 2013 03-13. doi: 10.2332/allergolint.13-RAI-0650

14. Su GL, Ko CW, Bercik P, Falck-Ytter Y, Sultan S, Weizman AV, Morgan RL. AGA Clinical Practice Guidelines on the Role of Probiotics in the Management of Gastrointestinal Disorders. Gastroenterology. 159(2) 2020 697-705. doi: 10.1053/j.gastro.2020.05.059.

15. 15.Guo Q, Goldenberg JZ, Humphrey C, El Dib R, Johnston BC. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Cochrane Database Syst Rev. 2019 4(4): 2019 CD004827. doi: 10.1002/14651858.CD004827.pub5.

16. Nazir Y, Hussain SA, Abdul Hamid A, Song Y. Probiotics and Their Potential Preventive and Therapeutic Role for Cancer, High Serum Cholesterol, and Allergic and HIV Diseases. Biomed Res Int. Aug 29;2018:3428437. doi: 10.1155/2018/3428437.

17. Amiri M, Lavasani Z, Norouzirad R, Najibpour R, Mohamadpour M, Nikpoor AR, Raeisi M, Zare Marzouni H. Prevalence of Urinary Tract Infection Among Pregnant Women and its Complications in Their Newborns During the Birth in the Hospitals of Dezful City, Iran, 2012 - 2013. Iran Red Crescent Med J. 17(8): 2015 e26946. doi: 10.5812/ircmj.26946..

18. Soong D, Einarson A. Vaginal yeast infections during pregnancy. Can Fam Physician. Mar; 55 (3): 2009 255-6.

19. Chang S, Tsai C, Wee C, Yan T Isolation and functional study of potentially probiotic Lactobacilli from Taiwan traditional paocai. Afri J. Micro.Resr. 7: 683-691.

20. Kaboosi H Antibacterial effects of probiotics isolated from yoghurts against some common bacterial pathogens. Afri J. Micro.Resr. 5: 2011 4363-4367.

21. Wang CY, Lin PR, Ng CC, Shyu YT (2010) Probiotic properties of strains isolated from the feces of breast-fed infants and Taiwanese pickled cabbage. Elsevier Anaerobe 16: 578-585.

22. Federico Lara-Villoslada1, Mo´nica Olivares1, Saleta Sierra1, Juan Miguel Rodrı´guez2 , Julio Boza1 and Jordi Xaus1 Beneficial effects of probiotic bacteria isolated from breast milk Brit J. Nutri 98(1), 2007 S96–S100 doi: 10.1017/S0007114507832

23. Jiang M, Zhang F, Wan C, Xiong Y, Shah NP, Wei H, Tao X. Evaluation of probiotic properties of Lactobacillus plantarum WLPL04 isolated from human breast milk. J Dairy Sci. 99(3): 2016 1736-1746. doi: 10.3168/jds.2015-10434

24. McGroarty JA. Probiotic use of lactobacilli in the human female urogenital tract. FEMS Immunol Med Microbiol. 6(4): 1993 251-64. doi: 10.1111/j.1574-695X.1993.tb00337.x.

25. Jost T, Lacroix C, Braegger C, Chassard C. Impact of human milk bacteria and oligosaccharides on neonatal gut microbiota establishment and gut health. Nutr Rev. 73(7): 2015 426-37. doi: 10.1093/nutrit/nuu016.