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EVALUATION OF EFFECTIVENESS AND SAFETY ASSESSMENT OF ELECTROHOMOEOPATHY REMEDIES WITH NOVEL BACILLUS SUBTILIS

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ABSTRACT

Spore-based probiotics have a significant advantage over other probiotics in that theycan withstand the harsh gastric conditions of the stomach as well as bile salts in the smallintestine, germinating in the digestive tract. Protection of natural flora is very much important for greater bioavailability and effectiveness of oral medication.In this study group of Electrohomoeopathy remedies are used to check the safety and efficacy with help of natural flora Bacillussubtilis.Through isolation and culture of Electrohomeopathy sample, the culture characteristics, morphology observation and biochemical test were performed.It was discovered that the bacteria are Grampositive spore chain Bacillus subtilis. The sample Acidome,Bioticome and BPlome morphological test for Bacillus subtilis was positive, while BP Home and Gangreome tests were negative.Various group of Electrohomoeopathy remedies haveshown excellent result during this microbial study.

INTRODUCTION

Antibacterial medications are frequently used in the prevention and treatment of bacterial illnesses in humans and animals, perpetuating the widespread phenomena of irrational antibiotic usage. This eventually leads to diseases becoming more resistant to antibacterial treatments and the emergence of pathogens. [1] There are currently no non-resistant bacteria or bacteria that are responsive to antimicrobial medications, and the rise in resistant pathogenic strains is a major impediment to illness prevention and treatment, making treatment more difficult. [2] Bacillus subtilis strains are known to be safe and effective probiotics that are non-pathogenic to people and animals.Bacteriocin is a protein or polypeptide produced by this bacterium during its growth and reproduction. This chemical has a high antibacterial activity as Well as a broad antibacterial range and good thermal stability. Furthermore, pH has minimal bearing on stability or action. As a result, it has a lot of potential as an antibacterial medication substitute .Bacillus subtilis is a Gram-positive germ with a rod-shaped morphology. This bacteria'smorphologicalcircularcolonyisrough ,opaque,fuzzywhiteorslightlyyellowwithjagge dedgeswhenculturedonconventionalnutritiona gar.[3]Therefore,inthisstudywearelookinginto anantibacterialactivematerialthatdoesnotcause drugresistanceinthehopesofreplacingantibacte rialmedicationsinthetreatmentofbacterialinfect ionswithElectrohomoeopathyremedies.Inthetr eatmentorprevention of intestinal diseases B. subtilis is frequently utilised as a probiotic preparation. It'salso used to make antibiotics, as a fungicide, and in complementary and alternative medicine.Bacillus anthracis

belongs to the same family as this bacteria (anthrax). Bacillus subtilis is a well-studied bacterium that is frequently used as a model organism for Gram-positive bacteria. B.subtilis is a rod-shaped bacterium that generates endospores, which enable it to survive in harsh environments such as heat and desiccation.

Electrohomoeopathy and Spagyric process during this study

Plants extraction process involves the separation of medicinally active ingredient from plants cell so inactive or inert components can be separated by using selective solvents in standard extraction procedures. During this study we have taken group of Electrohomoeopathy remedies and extraction done via Cohobation process. In modern day, Spagyric process a plant to retain its botanical properties and nutrients before separating the three parts and extracting nutrients andenergies therein before reuniting them. The careful separation, extraction, and reunification process ensures that as much of the plant's nutrition that can be drawn into the liquid herbal extract. Equally important is the ratio of the said nutrients which in synchrony allow the plant to live and thrive in nature-that is retained in the spagyric liquid extract. Spagyric Medicine may contain numerous medicinal Plants/ herbs, minerals in a dynamized form (Medicinal plant).Spagyric most commonly refers to a plant tincture to which has also been added to ash of thecalcinedplantation.[4]Spagyricistorestoreth eimbalanceofthethreephilosophicallyAlchemy elementswhichinmodernscienceknownasAro maticCompoundslowmolecularweightorganic molecules and micro andamp macroelements found in our human body and its cell in a balanced proportion.

Material and Method: This study conducted in Feb-2021. NAM (Nutrient agar media) has been used in this study.Electrohomoeopathy remedies Acidome, Bioticome, BPlome, BPHome and Gangreome usedduringthisstudy.

Composition of Media: Preparation of Culture Media: Nutrient's Agar media were prepared forculturegrowth.Nutrient'sAgar:Beefextract-1.5gm,Peptone-2.5gm,NaCl-2.5gm,Agar-7.5gm,D.W.-500mlandpH-6.0-7.0usedforthepreparation.Autoclavethemediaf orplateproductionandthenpour into sterile Petriplates.After solidify, samples were added on Petriplates.

Methodsofspreading:2-3drops of samples were spread on the surface of petriplates and then incubate at24- 48hours for NAMand3-5daysfor PDAfor isolate and identify the colonies.

Microbiological Testing for Bacteria:

Gram staining Techniques- Gram staining is one of the most important microbiology staining procedures. Gram-positive organism is an organism that preserve their primary colour and appear purple-brown under a microscope. Gram-negative organisms are those that do not takeup primary stain and look red under a microscope. Gram staining is a differential method of staining used to recognize the type of bacterial species (grampositive and gramnegative).Gram staining involves the staining bacteria, fixing the color with crystal violet with a mordant (gramiodine), decolorizing the cells with alcohol or acetone, and applying a counter stain (safranine solution).

Procedure of Gramstaining during:

Applying a primary stain (crystal violet) to a heat-fixed smear, followed by the addition of amordant(Gram'sIodine),quickdecolorization withalcohol,acetone,oracombinationofalcohol and acetone, and finally counterstaining with safranin are the four basic processes of the GramStain. [5]

Preparation of slide: Preparation of slide included during this study prepares a smear and heat fixes the bacteria to the slide by passing through the flame of Bunsen burner. Slide stained with crystal violet and allows it to sit for 1 min and rinse the slide for 5 sec with distilled water. Few drops of Gram iodine were added on slide for 1mins and then rinse it. Rinse the slide with alcohol for 30 sec- 1 mins to decolorize the cells and rinse with D.W. After the decolorizing step, a counter stain (safran in solution) was added to the slide and allows sitting for 1min and then gently rinse with D.W. Air Dry the slide at room temperature. After the prescribed procedure result was closely monitored on bacteria. The result of the gramstained is

viewed under the microscope. The gram-positive bacteria should be stained purplecolor, while gram-negative bacteria will appear pink or colorless ,the bacteria should be identified through their size, shape and arrangement.

Biochemical test- Biochemical tests are used to determine or identify the bacterial species based on their differences in the biochemical activities of the bacteria.

Biochemical testing: IMVIC tests**:** The IMViC tests are a collection of separate assays used in microbiology labs to identify coliform organisms. Acoliform is a gram-negative,aerobic or facultatively an aerobic rod that generates gas from lactose in less than 48 hours. Fecal contamination is indicated by the presence of certain coliforms. [6] Indole test: The indole test is used to detect an organism's capacity to divide the aminoacid tryptophan into the chemical indole.[7]The methylred test,or MR test, is used to assess an organism's capacity to develop and maintain stable acid end products from glucose fermentation.[8]The Voges-Proskauer(VP)test is to see if an organism makes acetylmethyl carbinol from glucose fermentation, do this test. [9] Indole test: identifies the bacteria which is capable of converting tryptophan to indole or pyruvic acid by using the enzyme tryptophanase. During this study for the optimal result 1gm peptone in 100ml distilled water and pH- 6.0-7.0 maintained during the study. All the preliminary precaution and aseptic condition has been closely monitored during this period here 1gm peptone added in 100ml D.Wand sterilize by autoclave as per prescribed temperature. 5ml of media is added in eachtube and bacterial sample is inoculated into tubes and incubated for 24hrs. Few drops of kovac's reagent are added to the tubes. The following observation predicated after the completion of procedure if indole is produced, "cherry red"color ring forms on the surface of tubes.

Effect of Electrohomoeopathy on bacterial Flora Bacillus subtilis

Bacillus subtilis is a Gram-positive germ with a rod-shaped morphology. This bacteria's morphological circular colony is rough, opaque, fuzzy white or slightly yellow with jagged edges when cultured on conventional nutrition agar.[10]

Microbiological testing for group of Electro homoeopathy medicine: The use of biological, biochemical, molecular, or chemical procedures for the detection, identification, or enumeration of microorganisms in a sample is known as microbiological analysis of group of Electrohomoeopathy sample. Study done to understand and monitoring of non-pathogenic bacteria.

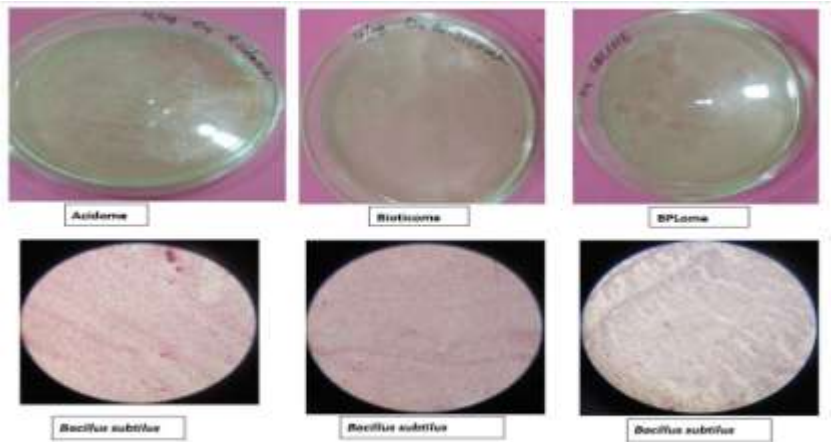
Various techniques for Microbiological analysis: During this study colony morphology technique has been used to explain the traits of an individual colony of microorganism developing on agar in a Petri dish. It helpful to identify microorganism and its effect with the group of Electrohomoeopathy medicine. [11] When the appropriate concentration of microorganisms is plated, this technique is often used to separate microorganisms contained with in a decent samplevolume that is dispersed across the surface of an agar plate, resulting in the formation of discrete colonies distributed uniformly across the agar surface. Spread-plating is commonly used in enrichment, selection, and screening procedures, in addition to viable plate counts, in which the total number of colonies forming units on a singleplate is counted and used to quantify the concentration of cells in the tube from which the sample was plated. If the end goal of an enumeration experiment is to isolate colonies for further analysis, the spread plate technique may be preferred over the pour plate technique because colonies grow accessible on the agar surface with the spread plate procedure, whereas they become embedded in the agar with the pour plate procedure.[12]

Bacterial colony morphology: During this study 5 sample of Electrohomoeopathy medicine Acidome- 6.3 (hyperacidity, heartburn) Bioticome-4.6(Antibacterial,Antiviral),BPLome6.7(Autonomicnervoussystem)BPHome7.7(Hypertension,peripheralresistance)Gangreome6.5(Diabetic foot,antiseptic, wounds and ulcer healing property) sample used in dilution 4. Number of colonies noted along with characteristic and causal organism.

S.no.	Samples	No.of colonies	Characters	Causal organism
1.	Acidome	1	Pale Irregular	Bacillussubtilis
2.	Bioticome	1	Pale Irregular	Bacillussubtilis
3.	BPlome	1	Pale Irregular	Bacillussubtilis
4.	BPHome	0	Nogrowth	Nogrowth
5.	Gangreome	0	Nogrowth	Nogrowth

Table for Gram staining:

S.no.	Samples	Stain/type of bacteria	Causal organism
1.	Acidome	+bacilli	Bacillus subtilis
2.	Bioticome	+bacilli	Bacillus subtilis
3.	BPlome	+bacilli	Bacillus subtilis
4.	BPHome	Nogrowth	No growth
5.	Gangreome	Nogrowth	No growth



Observation: During the colony morphology **p**ale irregular colony of Bacillussubtilis was observed in the Acidome,Bioticome and BP lome whileabsence of colony noted in th eBaphometandGangreome sample.

Table for Gramstaining: During this study a Gram stain is done to find out Bacillus subtilise in the group of Electro homoeopathy sample. Bacteria colony grow on NAM plate

Observation: During the Gram staining +bacilli stain of Bacillus subtilis was observed in the Acidome, Bioticome and BPlome while absence of stain noted in the Baphomet and Gangreome sample.

Biochemical testing

Biochemical tests are one of the most used ways for identifying microorganisms, and they arefrequently used in association with phenotypic identification. Various biochemical tests arebased on microbial' ability to use specific biomolecules to produce valuable organic compounds for themselves. [13] IMVIC tests: This test is done to find out Bacillus subtilis in the group ofElectrohomoeopathysample. Methyl red and VogesProskauer-lucoseoxidationandProductionofneutralendpro ducts.

Glucose test and Sucrose test: Observation during this study pink colour showed positive test while no colour indicate negative test.

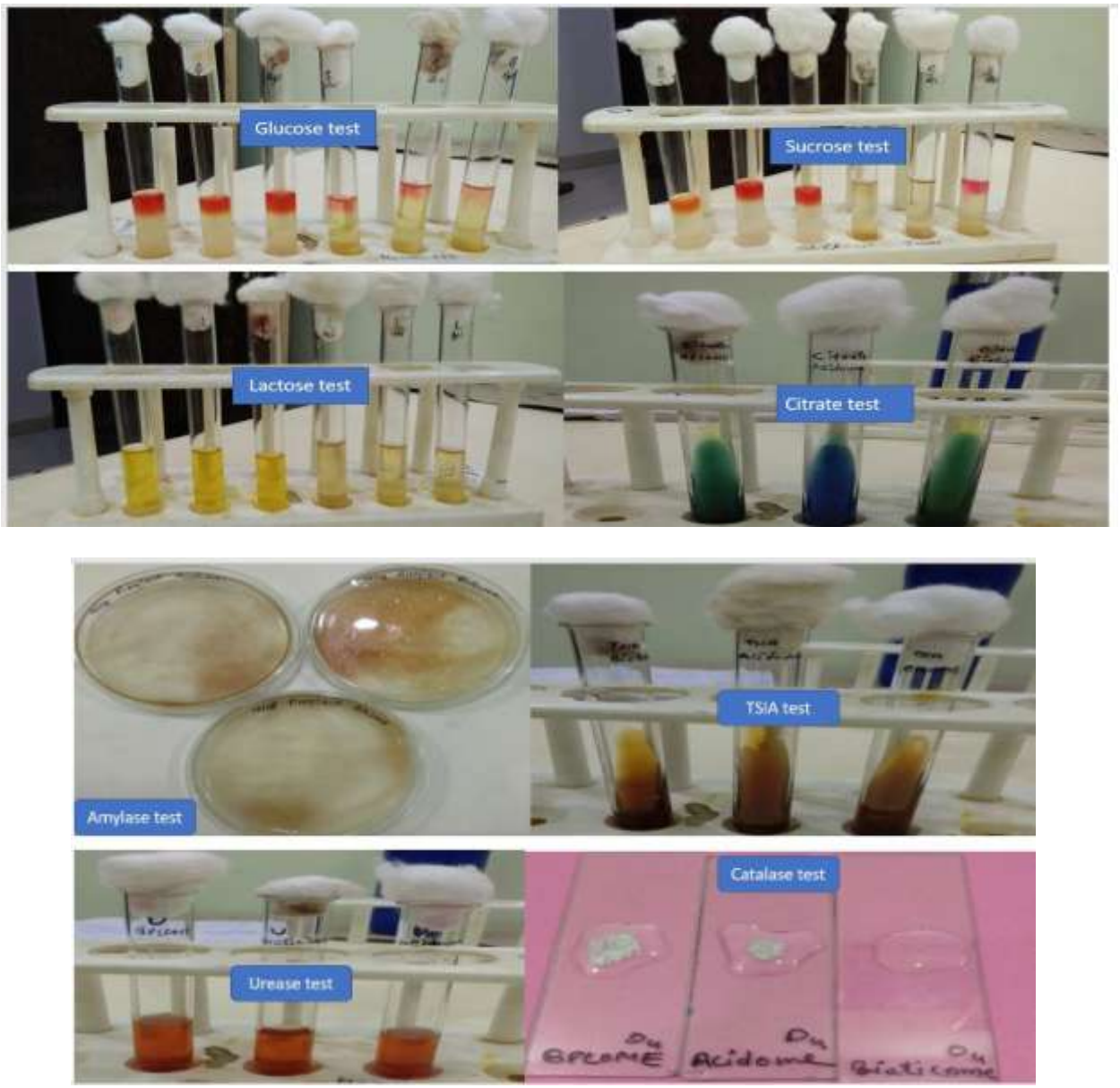
Lactose test: Presence of yellow colour showed positive test while no colour indicate negativetest.

Citrate test- Citrate fermentation observation during this study blue color showed positive test while no colour indicate negative test.

Amylase test-Amylase is an enzyme (atype of protein) that contributes in the digestion of food.The pancreas and salivary glands produce the majority of your amylase.

Presence of blue colour showed positive test while no colour indicate negative test.

The Triple Sugar Iron Agar (TSIA): test is used to discriminate between distinct Enterobacteriaceae families or genera, which are all gramme negative bacilli capable of fermenting glucose with the generation of acid, and other gramme negative intestinal bacilli.



Presence of color Change in Slant and Butt, Blackening or cracks in medium colour showed positive test while no colour indicate negative test.

The urease test: To determines whether organisms can hydrolyze urea and create ammonia and carbon dioxide. It's mostly utilised to distinguish between urease-positive Protease and other Enterobacteriaceae. **Presence of r**ed color colour showed positive test while no colour indicate negative test.

The catalase test: looks for catalase, an enzyme that converts hydrogen peroxide into water and oxygen. When hydrogen peroxide is introduced to an organism that can make catalase, it produces oxygen bubbles. **Presence of** Active bubbling occurs showed positive test while no colour indicate negative test

Result: During this study bacterial fermentation of Bacillus subtilis was observed in the sample of Electrohomoeopathy remedies with various biochemical analysis. Acidome,and BPLome showed positive indicator in the Citrate, Lactose, Glucose and Catalase test while Bioticome shows positive indicator in Lactose, Glucose, Dextrose, Sucrose and Catalase analysis. BPLome shown in positive indicator in Citrate, Lactose, Glucose, Dextrose, Sucrose and Catalase analysis. Broth is inferior to crude bacteriocins inhibitory effect, indicating that the content of the bacterial factors influences the size of the inhibition zone.

Sample	Indole	Citrate	Amylase	Lactose		Glucose		Dextrose		Sucrose		TSIA		Urease	Catalase
				MR	VP	MR	VP	MR	VP	MR	VP	Gas	H2S		
Acidome	-	+	-	+	-	+	+	-	-	-	-	-		-	+
Bioticome	-	-	-	+	-	+	+	+	+	+	-	-		-	-
BPLome	-	+	-	+	-	+	+	-	+	+	+	-		-	+
BPHome	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG		NG	NG
Gangreome	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG		NG	NG

CONCLUSION:

From given sample, the growth of organisms is benign during this study. This bacterium is naturally found in our environment and non-pathogenic in nature. So, this Electrohomoeopathy remedies is beneficial for human health with great possibility of pharmacological activity. It was closely monitored that good bacteria are necessary for our bodies to battle bad bacteria and restore equilibrium inside the body, allowing us to feel healthier. Bacillus subtilis which is identify in this study keeps us healthy by supporting our immune function and controlling inflammatory pathway. Some harmless bacterium that residue in our body and helps you to digest food and destroys some disease causing and provide nutrients. Found bacteria are harmless and gave lesser effect to human body. Importantly, the crude bacteriocin of Bacillus subtilis has been shown to suppress the growth of Staphylococcus aureus, Escherichia coli, Enterococcus, and Salmonella, implying that it could be useful in the future. Since the ancient period, natural plants have aided in the discovery of novel pharmaceuticals. There are many distinct systems of medicine in the world, and while they all use the same treatments, they differ due to a variety of factors such as the principal, philosophy, creator, and changes in pharmacological activity. The Electrohomoeopathy system of medicine, which has a positive impact on humanity, should been courage to innovate, investigate, and develop further.

Limitation: Currently, bacteria are identified mostly through traditional approaches such as studying bacterial colony features and shape, as well as biochemical testing for more complete assessments. Although these approaches can identify the most common clinical bacteria, the current type and form of infection-causing clinical pathogenesis more complex, and the identification results are frequently unsatisfactory. With the improvement of the nucleic acid sequence analysis techniques, the conserved bacterial genomic regions are sequenced compared with that from the GenBank sequences which is common limitation observed in this study. Toxicological study with the help of animal model is the further scope in this kind of study.

Ethics approval

This study was approved by the EHF scientific committee and conducted at Biome spagyric Pvt Ltd Bhilai, Durg, Chhattisgarh.

Study Centre: Biome spagyric Pvt Ltd, Bhilai Durg, Chhatishgarh.

Microbiologist: Dr. Nidhi Shukla, Director-operations in Biome spagyric Pvt Ltd.

Data Availability

The data is available with the authors for further information and details you can communicate to the corresponding author via email as mentioned in the article.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Authors'contributions

All the authors are equally contributed in this article through the study.

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