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Design and Development of Herbal Based Hydrophilic Ointment and Gel to Control Zoonotic Bacterial Diseases Transmitted by Dog Bites

Thiruvengadam S[1*], Karthikeyan K[1], Ivo Romauld S[2], Shruthi ER[1], Ezhilarasu A[3]

[*] [1]Department of Biotechnology, Rajalakshmi Engineering College, Chennai-602105, TN, India.
[2]Department of Bio-Engineering, VISTAS, Chennai - 600117, TN, India.
[3] Department of Microbiology, Selvamm Arts and Science College(Autonomous), Namakkal-637003,TN, India.

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KEYWORDS

Canine, *Enterobacter hormaechei*, *Bacillus cereus*, *Aeromonas veronii*, Hypersensitivity.

ABSTRACT:

Introduction: Creature nibbles are critical reasons for dismalness and mortality around the world, because of the exchange of microorganisms through salivation that prompts a few deadly diseases.

Objectives: The current investigation revolves around the bacterial disease spread through canine chomps.

Methods: The bacterial strains were screened based on their morphology, biochemical characters, and 16s ribosomal RNA by single-pass sequencing.

Results: The new strains publicized were Enterobacter hormaechei, Bacillus cereus, and Aeromonas veronii. The antibiogram examination of MBC and MIC with various concentrates of Datura metel leaf and Calotropis gigantea blossom shows the most noteworthy action against each of the three microbial detaches with various extents. The hydrophilic ointment created with 0.1 percent and 0.5 percent drug extract was more stable than the gel formulations in the Physiochemical investigation.

Conclusions: The gel formulation performed better in the Swelling Index, Extrudability, and Spreadability Tests, indicating that gel formulations may transport drugs more effectively than hydrophilic ointments.

1. Introduction

Creature nibbles are a critical reason for grimness and mortality around the world. They represent a significant general medical issue in kids and grown-ups around the world. Dog bites represent a danger of bacterial and viral disease. The estimated number of dog bites cases in India per year is about 17.4 million, leading to 20,000 deaths per year and in that bacterial infection is unfamiliar[1]. If a dog nibble pierces a person's skin, bacteria from the dog's mouth including saliva can get into the body, which contains bacteria. If the bacteria stay in the body, they can cause an infection, such as Tetanus, Capnocytophaga sepsis, or other distinctive infection[2]. In certain cases, an infection can proliferate to other parts of the body.It is the famous actuality that the greater part of the important medications utilized in allopathic meds is gotten from plant assets. The ethno-pharmaco organic field concentrates in the rustic and ancestral zones assume a fundamental part in discovering new medication material, which is obscure or less thought about a significant number of the plant types of our rich verdure[3-4]. Regardless of advancement in allopathic medication, in a developing country like India, nearly 70% of the population still relies on herbs, which are explored by ethnic societies, exploiting them for the treatment of various diseases. Moreover, these herbal remedies are relatively cheaper and easily available to the local population at their doorsteps[4]. In many instances, the therapeutic benefits of crude plant preparations have shown to be either equal or superior to modern therapeutic approaches. Around 25% of

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allopathic medicines have been derived from plants[5]. Presently, the utilization of such plants has been expanding progressively overall due to different positive viewpoints like moderateness; openness; cost-adequacy; social conviction; treatment of different illnesses, and safe acknowledgment of new medications. The treatment methods using ethnomedicine and allopathic medicine are used in Nepal and Bangladesh[6]. The leaf extracts of *Datura metel* were antagonistic against the several bacteria species with inhibitory zones and *Staphylococcus aureus* was the most inhibited with the ethanol extract. The aqueous extract of the *D. metel* displayed an antioxidant activity of between 49.30-23.82% and can consider the plant as a natural source of antioxidants[7]. The aqueous extract of the latex of *Calotrophis gigantea* was reported to exhibit a significant inhibitory effect on *Staphylococcus aureus, Bacillus, Escherichia coli* and *Candida*[8]. The crude latex of *C. gigantea* was been evaluated for its wound healing activity in albino rats using excision and incision wound models. The treated wounds were found to epithelise faster as compared to controls[9]. Here, we use *D. metel* and *C. gigantea* as an ethnomedicine against dog bite and their infections.

Our novelty is to control the bacterial infection transmitted to humans by infected saliva through dog bites and to direct ethnopharmacobotanical field investigations in Tamil Nadu, India concerning the herbal-based topical drug preparations to figure out the effectiveness and application of medicinal plants towards pathogens.

2. Materials and methods

Collection and Plating of saliva sample

The dog's saliva sample was collected by using the cotton swab method. The sterile cotton swab was rubbed gently on the dog's cheek pouch and under the tongue for 30-60 seconds to passively absorb saliva. The collected sample was transferred immediately to a prepared peptone water medium and then, it was sealed tightly taken to the laboratory. A loop full of samples from the peptone water medium were streaked on Nutrient agar (NA), MacConkey agar (MA), and Blood agar (BA) plates by quadrant streaking method. The plates were incubated at 37℃ for a period of 24-48 hrs. The grown colonies were examined and the pure cultures were prepared by picking well-isolated colonies and re-streaking them on fresh agar slants. Also, Gram staining portrays microbes as Gram-positive (or) Gram-negative.

Identification of organism by biochemical test and 16s ribosomal RNA gene sequencing

Biochemical screening like catalase test, citrate usage test, urease test, indole test and H_2S gas production test were performed by standard protocol[10].

Then, 16s ribosomal RNA gene sequencing was done. DNA confinement from Microbial examples was finished utilizing the EXpure Microbial DNA Isolation Kit created by Bogar Bio Bee stores Pvt Ltd(Janda and Sharon, 2002). Polymerase Chain Reaction (PCR) was completed that utilizes groundwork to intensify genomic DNA from deoxyribonucleotide triphosphate substrates (dNTPs) and primer (27F and 1492R) on a single-stranded DNA template. The decontaminated PCR item was sequenced by single-pass sequencing technique utilizing ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (Applied Bio-frameworks). In this kind of sequencing, the cycle sequencing responses, color eliminator expulsion, and resulting examination were done together on a Genetic Analyzer.

Annotation - The 16s rRNA grouping was impacted utilizing the NCBI impact comparability search apparatus. The phylogeny examination of inquiry arrangements with the firmly related succession of impact results was performed trailed by different grouping arrangement MSA (Multiple Sequence Alignment). The program MUSCLE 3.7 was utilized for numerous arrangements of successions[11]. The subsequently adjusted successions were restored utilizing the program Gblocks 0.91b[12]. PhyML was demonstrated to be at any rate as exact as other existing phylogeny programs utilizing mimicked information while being one significant degree quickerand HKY85 as a substitution model. The program Tree Dyn 198.3 was utilized for tree delivery.

Extraction of active compounds from *D. metel and C. gigantea*

The new and illness-free leaves of *D. metel* and blossoms of *C. gigantea* were gathered from the nursery house and confirmed by a botanist. The gathered plant materials were washed and conceal dried at 29℃ ± 2℃ for 5 to 7 days and pulverized into fine powder.

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The watery concentrate of the plant sample was set up by the shake flask method. The finely powdered sample was weighted and taken in a fresh conical flask. Sterile refined water was added to it. At that point, the conical flask was stored in an incubator shaker at 80 rpm for 24-48 hrs. After extraction, the content was centrifuged and the pellet was disposed of. The unrefined compound present in the supernatant was accumulated by vanishing at 50℃. The natural concentrate of the plant sample was set up by the soxhlet extractor with organic solventacetone and hexane respectively[13].

Phytochemical analysis

The phytochemical mixtures like Tannins, Terpenoids, Saponins, Flavonoids, Glycosides, Anthocyanins, Alkaloids, Steroids, Phenols and Resins were screened by utilizing standard methods described by Yadav & Agarwala (2011)[14] and results were tabulated.

Antibiogram assay

The isolated strains of *E. hormaechei*, *B. cereus* and *A. veronii* were lawn cultured on Muller-Hinton agar plates. Four equidistant wells were made using the agar well cutter. 100 µl of different plant extracts (crude, aqueous, acetone and hexane extracts of *D. metel* and *C. gigantea*) were added to the well. Ciprofloxacin of 5 mcg was used as a positive control. The plates were then incubated at 37°C for 24 – 28 hrs and the zone of inhibition was determined by measuring the diameter of clear zones by using Vernier caliper.

Determination of MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration)

The MIC and MBC were constrained by inoculating 100 µl of culture using a sterile micropipette into a test tube containing 9 ml of cleaned supplement stock medium close by 900 µl of different centralizations of plant extracts. The test tubes were incubated at 37℃ for 24 hrs. The absorbance regard when bring forth was controlled by assessing OD (Optical Density) regard at recurrence 610 nm with supplement stock as control. The last OD regards were gotten by deducting the OD regard when incubating. The base obsession at which the last OD regard becomes zero was resolved as MIC.

The MBC value was determined by streaking the overnight cultures along with the extract from the test tubes on the MHA (Muller Hinton Agar) growth medium. The plating was done by the simple and quadrant streaking method. The concentration of plant extract at which there is no more growth on the MHA plates was calculated as MBC.

Anti-Inflammatory Activity

The counteracting inflammation of plant extracts was dictated by the protein denaturation method with 20 µl of plant extract [14]. The rate hindrance of protein denaturation was determined as follows,

% inhibition of denaturation = 100 × [1 – (A2/A1)]

Where A1 and A2 are the absorption of the control and test samples respectively.

Hydrophilic ointment preparation

The calculated amount of stearyl alcohol of 15 g was melted with white petrolatum of 30 g on a hot plate. The mixture (oleaginous phase) was heated to 70°C. The remaining ingredients Sodium lauryl sulfate (SLS) of 1 g, Propylene glycol of 14 g along with extract of 0.1 ml in A1, 0.5 ml in A2 and 0 ml in A3 were dissolved in water (made up to 100 ml) and the solution (aqueous phase) was heated to 70°C. The oleaginous phase was then slowly added to the aqueous phase with constant stirring. The mixture was then removed from heat and stirred until it congeals[15]. A1&A2 are drug-loaded formulations and A3 is a hydrophilic ointment with no drug formulation used as a control.

Formulation of carbopol gel

The water required for preparation was divided into two parts. In one part, the calculated quantity of propylene glycol of 4 ml and ethanol of 3 ml was added in 30% water. The other part contains 4g of carbopol 940, 0.1 ml in B1, 0.5 ml in B2 and 0 ml in B3 was dissolved in 70% water and to this solution methylparaben of 0.2 g, propylparaben of 0.02g, and Ethylenediaminetetraacetic acid (EDTA) of 0.03g were added with continuous stirring[16]. The above combinations were mixed in a beaker. Tri-ethanolamine (TEA) of 1.2ml was added to it and made up to 100ml. After stirring the ingredients, it becomes a gel. B1&B2 are drug-loaded formulations and B3 is gel with no drug formulation used as a control.

Physio-chemical screening hydrophilic ointment and gel- organoleptic characteristics

All combinations of ointment and gel (A1, A2, A3, B1, B2, and B3) were screened for actual appearance, shading, surface, smell, and homogeneity. These attributes were assessed by visual perception and the presence of coarse particles was utilized to assess the surface and homogeneity. Quick skin feels which

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incorporates solidness, coarseness, and oiliness were likewise assessed and recorded.

Spreadability test

The spreading capacity of all definitions was controlled by estimating the spreading breadth of 1 gram of test between two-level glass plates of size 10 cm × 20 cm. The weighted sample was put on the lower plate. From that point onward, the upper plate was put over it. The standard load of roughly 25 grams was applied to the upper plate. The distance across every definition was estimated after 1 min and it was done three-fold.

Extrudability test

The pre-arranged plans were filled in covered folding aluminum tubes and fixed by creasing as far as possible. Loads of the void and plan stacked cylinders were recorded and were placed between two glass slides and braced together. A strongarticlethat weighed around 500 g was set preposterous glass slide and the cap was taken out. The measure of the expelled gel and treatment was gathered and gauged.

The level of the expelled test was determined as follows,

% Extrudability = (W_2 / W_1) x 100

Where, W_1 and W_2 are Weight of prepared sample and extruded sample (g).

Swelling index

It was dictated by taking 1 g of the pre-arranged example on a petri-dish and set it in a container containing 10 ml of water. At that point, the examples were taken out from containers at various time stretches and put on a dry spot for quite a while, and re-gauged.

The swelling index was determined as follows,

Swelling Index (SI) % = $[(W_t - W_0) / W_0] \times 100$

Where,

SI % = Equilibrium percent swelling;

W_t= Weight of the swollen sample (g) after time t and W_0 = Original weight of the sample (g) at time t=0.

Washability test

The gel and ointment formulations were applied on the skin and afterward, the simplicity ease, extent and degree of washing with water for every one of the details were checked for each of the formulations physically.

Centrifugation test

This test was done to check for any phase separation in any of the prepared formulations by centrifuging the sample at 3000 rpm for 7 days, 14 days and 30 days at different temperatures 4℃ and 30℃ respectively.

pH determination

The pH of the formed ointment and gel was dictated by utilizing a computerized pH meter. Precisely 5 g of the test was gauged and scattered in 50 ml of refined water and put away for 2 hours. The estimation of pH for every plan was done three-fold.

Acid value

One gram of the pre-arranged example was taken and broken down in 10 ml of supreme ethanol (99%). At that point, it was warmed on a hot plate for 5 mins and 2 to 3 drops of phenolphthalein marker was added to it and titrated against 0.1 N KOH until a pale pink tone showed up.

The acid value was determined as follows,

Acid value = $[(56.1 \times T \times N) / W]$

Where, T = Titre value of KOH;

N = Normality of KOH (N); and

W = Weight of the sample (g).

Peroxide value

In 250 ml of the conical flask, 5 g of the pre-arranged example was taken and 30 ml of 1:1 acetic acid and chloroform solution were added and twirled tenderly to disintegrate. 0.5 ml of potassium iodide solution was added with consistent shaking and 30 ml of water was added. The preparation was titrated against a 0.1 M sodium thiosulfate arrangement with energetic shaking until the yellow tone vanished. At that point, 0.5 ml of 1% starch was added and the titration was preceded with incredible shaking to deliver all iodine from the chloroform until the blue tone vanished.

Peroxide value was determined as follows,

Peroxide value = $[(T \times M \times 1000) / W]$

Where,

T = Titre value of sodium thiosulfate,

M = Molarity of sodium thiosulfate solution (M) and W = Weight of the sample (g).

Total fatty matter

Two grams of arranged sample was taken in a measuring glass and 25 ml of 1:1 diluted HCl was added. It was then warmed on a water shower until the solution turns out to be clear. The sample was moved into a 250 ml separating funnel and permitted to cool at room temperature. 50 ml of petrol ether was then added to the funnel, shaken, and left for separation of organic phase from the aqueous layer. The organic phase was isolated from the watery layer. The above watery layer was apportioned twice with the same amount of petrol ether. The natural layers were dissipated to acquire the

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concentrate which was therefore washed with water. The concentrate gain sifted, dried and the substance was resolved which was then sifted and sodium sulfate was added to it. The concentrate combination was a

Total fatty matter (%) by mass = $100 \times (M_1 / M_2)$ (6)

Where, M_1 = Mass of residue (g) and

M_2 = Mass of the sample (g).

Stability Study

The stability of prepared formulations was tested at various storage conditions (4°C and 30°C) and checked for their actual qualities like color, appearance, odour, spreading capability, washing ability, and centrifugation test and chemical characteristics like pH, acid value, peroxide value, and total fatty matters for a period of 30 days.

Patch Test

Ten of our pear bunch volunteers were chosen and the test was performed on the lower arms of each volunteer to cover a span of 0.6 cm skin surface. 0.5g of arranged details was applied independently to decide any potential responses on the skin. The careful tape was utilized to fix them in a spot and the test locales were checked. Care was taken not to wash the applied region. After 48 hrs, the patches were taken out. After one hour, the skin was analyzed for any irregularities like redness or irritation.

3. Results and Discussion

Isolation of bacteria from dog's saliva

Dog's saliva was been collected and platted in NA, BA and MA agar medium from different subjects successfully by cotton swab method. Most of the strains isolated from dog bite wound infection were gram-positive cocci and gram-negative bacilli and some of the strains were gram-positive bacillus. The gram-positive coccus strains exhibited γ – hemolytic property. The gram-negative and gram-positive bacillus strains exhibited β - hemolytic properties. The results confirmed that the cultures from samples S_2, S_3, S_4, S_6, S_8, S_{10} and S_{11} have similar staining and hemolytic property to the strains.

Identification of organism by biochemical test and 16s ribosomal RNA gene sequencing

Biochemical tests (Table I) were performed only for the cultures from the samples S_2, S_3, S_4, S_6, S_8, S_{10} and S_{11},which are selected based upon their property on the previous examination.

The genomic DNA was isolated from the samples (S_6, S_8 and S_{10}). PCR was performed using 16s rRNA universal primers and multiple copies of 16s rRNA gene sequence were produced[17].

Annotation result - The 16s rRNA gene sequence was BLAST (Basic Alignment Local Search Tool) to identify similarities in sequences that have been already identified and submitted in the NCBI database by comparing and calculating the statistical significance of matches. The Phylogenetic tree was constructed by MSA with closely related sequences from blast results.The Phylogenetic tree and BLAST hits confirmed that the identified sequences were found to be *Enterobacter hormaechei* (S_6), *Bacillus cereus* (S_8) and *Aeromonas veronii* (S_{10}) (Figure 1). The results showed that the E value was found to zero for all three organisms and it confirmed that there were zero sequences in the database to match the query as well or better than the hit that was found. The percentage identifier of *A. veronii* was found to be around 98% and it could be a novel one. In 2008, Ofukwu *et al* (2018)[18] also carried out similar type work in different dogs and isolated *Enterobacter and Bacillus* species in different frequencies. Microbes recuperated from contaminated chomp wounds are frequently intelligent of the oral vegetation of the gnawing creature [19]. Viral diseases like rabies, norovirus and bacterial contaminations including *Brucella, Pasteurella, Salmonella, Campylobacter, Bordetella, Coxiella, Yersinia Leptospira,* and Methicillin-resistant *staphylococcus aureus* are the recognized viral and bacterial zoonotic contaminations communicated to people by canines[20].

Extraction of active compounds from *D. metel and C. Gigantean*

The measure of plant sample utilized per ml of water or natural dissolvable is 100 mg and the last convergence of the concentrate is 100 mg/ml. The extracted active compounds are kept ready for further analysis.

Phytochemical analysis

The study on *D.metel* leaf extracts and *C. gigantea* flower extracts revealed the presence of medicinally active compounds such as alkaloids and phenols. Flavonoids were present in all extracts of *D. metel* except hexane and present only in the crude extract of *C. gigantea*. Terpenoids were present in all extracts of both plants except the crude and hexane extracts of

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D.metel. But, tannins were present only in these two extracts (Table II). There is no presence of resins in any of the extracts and anthocyanins were present only in *C. gigantea* extracts.

Anti-biogram assay

In crude and acetone extract of *D. metel* and *C.gigantea*, shown clear zones against all three strains. The diameter of clear zones was measured and recorded in table III.

The results showed that the aqueous and hexane extracts of *D. metel* leaf and *C.gigantea* flower were ineffective against the isolated strains of *A. veronii, E. hormaechei* and *B. cereus*. It also showed that the acetone extract of *D. metel* was effective only against *E. hormaechei* and the acetone extract of *C. gigantea* was effective against *E. hormaechei* and *B. cereus* (Figure 2). The results confirmed that the crude extract of *C. gigantea* and *D. metel* were effective than all other types of extracts and showed antibacterial activity against all three strains at different levels. So, the MIC and MBC values were calculated only for the crude plant extracts.

Determination of MIC and MBC

The MIC values of plant crude extracts were screened for different concentrations of 5 to 100% with an interval of 5%. The MIC was then calculated by subtracting the absorbance value before and after incubation. The absorbance value for bacterial growth was found to be zero in *C. gigantea* concentration ranging from 85 to 100% for all three strains and in *D. metel* concentration ranging, from 65 to 100% for *A. veronii*, from 75 to 100% for *B. cereus*, and from 85 to 100% for *E. hormaechei* (Figure 3).

The MBC of plant crude extracts was determined by inoculating on Muller-Hinton Agar plates. The bacterial growth was observed until 90% concentration of *D.metel* and *C. gigantea* crude leaf extract for *E. hormaechei*, *B. cereus* and *A. veronii* strains.

The results showed that the MIC value of *C. gigantea* was 0.85 against all three strains. For *D. metel*, it was 0.65 against *E. hormaechei*, 0.75 against *B. cereus* and 0.85 against *A. veronii*. In addition to that, the MBC value of D. metel was 0.90 against all three strains. For *C. gigantea*, it was 0.95 against *E. hormaechei* and *B. cereus* and 0.90 against *A. veronii*. The value of MBC to MIC was found to be in the range of 1.06 – 1.39 for *D.metel* drug extract and 1.06 – 1.12 for *C. gigantea*. For a drug formulation to be effective, the ratio of MBC to MIC value of drug extract should be less than 4.

Anti-inflammatory activity

Crude extracts of *D. metel* and *C. gigantea* were able to inhibit protein denaturation in a concentration-dependent manner (Table IV). Percentage inhibition of plant extracts was found to be within the range from 18% - 63% at the concentration range of 25% to 100%. Flowers of *C. gigantea* exhibited a higher level of inhibition compared to leaves of *D. metel*.

Physio-chemical screening hydrophilic ointment and gel- organoleptic characteristics

The different types of hydrophilic ointment (A1, A2 &A3) and gel (B1, B2 & B3) were prepared according to tables II & III and analysed for their physical and chemical parameters. The drug-loaded formulations were prepared by adding 90% concentrated crude extract of *D. metel* and 95% concentrated *C. gigantea*. Because, the *E. hormaechei*, *B. cereus* and *A. veronii* strains were killed completely by the plant extracts at those concentrations.

Physical analysis

The prepared base (A3) and drug-loaded formulations (A1 and A2) of hydrophilic ointment were white and the prepared base (B3) and drug-loaded formulations (B1 and B2) of gel were translucent. There was no change in appearance, odour and colour up to the observation period of 30 days. However, there is a slight change in homogeneity (uniformity) of gel formulations B1, B2 and B3 at 30°C after 30 days. The result confirmed that the prepared formulations A were stable at different storage conditions. All the gel and hydrophilic ointment formulations were free of stiffness, grittiness and greasiness.

Centrifugation test for six different formulations, there is no phase separation after centrifugation in A1, A2 and A3 at 4°C but there is phase separation at 30°C after 30 days due to non-uniformity.

Determination of Swelling index, Extrudability and Spreadability Test

The calculated values of percentage swelling index for A1 to A3 formulations range from 77 to 85% and for B1 to B3 formulations range from 83 to 96% respectively. The swelling index of gel formulation was high comparatively.

The calculated values of percentage extrudability for A1 to A3 formulations range from 79 to 85% and for B1 to B3 formulations range from 86 to 90% respectively. The swelling index of gel formulation was high comparatively.

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The spreadability test was done three times and the average value (MEAN ± S.D) was calculated. The values of spreadability for A1 to A3 formulations range from 9 to 11 mm and for B1 to B3 formulations range from 25 to 26 mm respectively. The spreadability character of gel formulation was better than hydrophilic ointment.

pH determination

The pH was found to be in the range of, 6.09 to 6.65 for "A" hydrophilic ointment formulations and 6.52 to 6.91 for "B" gel formulations. The pH value of the drug-loaded formulations kept at 4℃ and 30℃ for 30 days did not show a large change and was ($p < 0.05$) significant over base formulations.

Peroxide value, total fatty and an acid value

Acid value, peroxide value and absolute greasy matter of gel and ointment kept at various capacity conditions were noticed for 30 days and examined by standard strategy. Peroxide esteem was discovered to be in the scope of 1.56 to 1.75, acid value discovered to be in the scope of 2.41 to 2.93 and add up to greasy issue were discovered to be in the scope of 14.08 to 14.19. The information of all plans was discovered to be noteworthy ($p < 0.05$).

Washability test

The drug-loaded gel and ointment formulations were analysed for washability and it was found that both hydrophilic ointment and carbopol gel were in the aqueous phase, they can be easily washed with water with no residuals remained on the skin.

Stability Study

The Stability study confirmed that the appearance of the formulation was clear and no significant changes were noticed in pH, spreadability, viscosity and drug content for an optimized time of 30 days and we found that hydrophilic ointment formulations were more stable than the gel formulations.

Patch test

It was done to check the safety of the formulations on human skin. The prepared formulations were applied on the forearms of the volunteers and left undisturbedfor 48 hrs. The volunteers reported that there was no irritation and redness after the application of prepared formulations.

The pH values of all prepared formulations were ranged from 6–7 which are considered acceptable to avoid the risk of skin irritation upon application to the skin because the human skin pH is around 5.5. The percentage-swelling index of B2 gel formulation was more than B1 gel formulation. It indicated that the drug release was better controlled in B2 formulation than B1.

In this study, we were able to isolateand identify *Enterobacter hormaechei*, *Bacillus cereus*, and *Aeromonas veronii* in different frequencies from the dog's saliva and submitted those sequences to NCBI. The antibiogram assay of different extracts showed that the crude extracts of *D. metel* leaf and *C. gigantea* flower show the highest activity against all three microbial isolates with different proportions. The results of the phytochemical test showed that the presence of flavonoids, alkaloids, and phenols was responsible for the plant extract's anti-microbial activity. The ratio of MBC to MIC of plant extracts concerning each microbial isolates was significant and within the range of effectiveness. In addition to that, the crude extract of the *C. gigantea* flower shows the highest anti-inflammatory activity. In the Physiochemical study, the hydrophilic ointment formulated with 0.1% and 0.5% drug extract was more stable than the gel formulations. But, the gel formulation showed better results in the Swelling index, Extrudability and Spreadability Test, this shows the drug delivery for gel formulation may be better than hydrophilic ointment. There is no sign of irritation and redness after applying the prepared formulations on the skin.

As indicated by the after-effects of antibacterial activity and physicochemical investigation of plant extricate from *D. metel* and *C. gigantea*, we can gather readily available local plant-based gel that can be prepared for canine wound contamination. Diverse exploration reports in natural-based skin drug definition uncovered equivalent antimicrobial and physicochemical boundaries. *Lantana Camara* removes gel concentrated against *Staphylococcus aureus* and *Staphylococcus epidermis*[20], antibacterial and antifungal movement of natural gel containing *Aloe vera*, *Azadirachta indica* and *Lycopersicon esculentum* seed separate[21]. *Tridax procumbens* and *Areca catachu extracts* gel shown respectable physiochemical and antimicrobial properties[22-23]. Kumar Avinash Bharati & Mukesh Kumar (2014)also found thirteen species of plants were identified and their medicinal properties compared with the Materia Medica from India.

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4. Conclusion

As indicated by the aftereffects of appraisal tests, for example, phytochemical screening, antibacterial measure and physicochemical investigation of plant extricate and drug definition from *D. metel and C. gigantea*, we can gather that local plant-based gel and analgesic can be prepared powerful for canine eat wound contamination. It tends to be used close by polymers give synergistic effect similarly as wound recovering effect on skin without antagonistic impact. The pack taken in the current examination can likewise be replaced with different medications having more prominent development.

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Conflict of Interest

All authors declare no conflict of interest.

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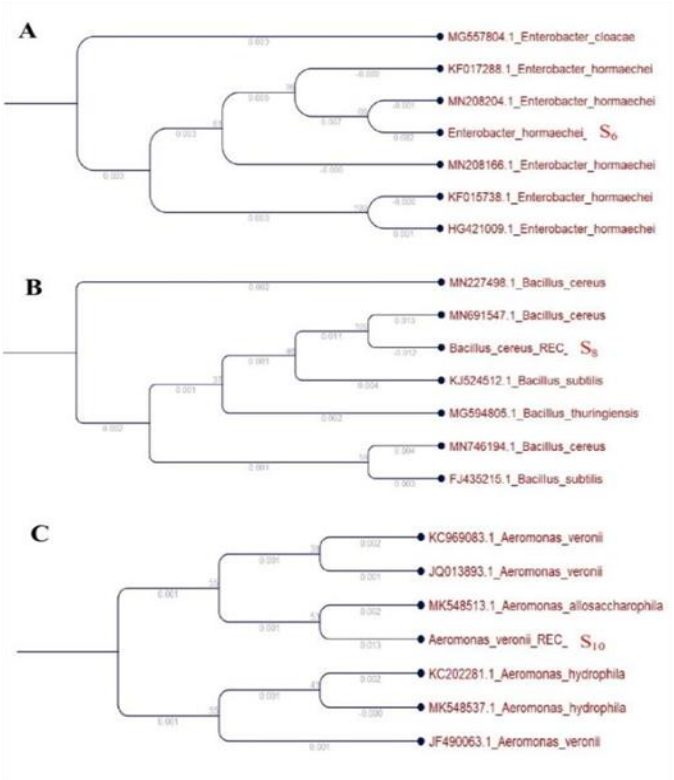

Figure 1. Phylogenetic tree reconstruction

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A: Phylogenetic tree of sample **S_6** (*Enterobacter hormaechei*) **B:** Phylogenetic tree of sample **S_8**(*Bacillus cereus*)
C: Phylogenetic tree of sample **S_{10}**(*Aeromonas veronii*)

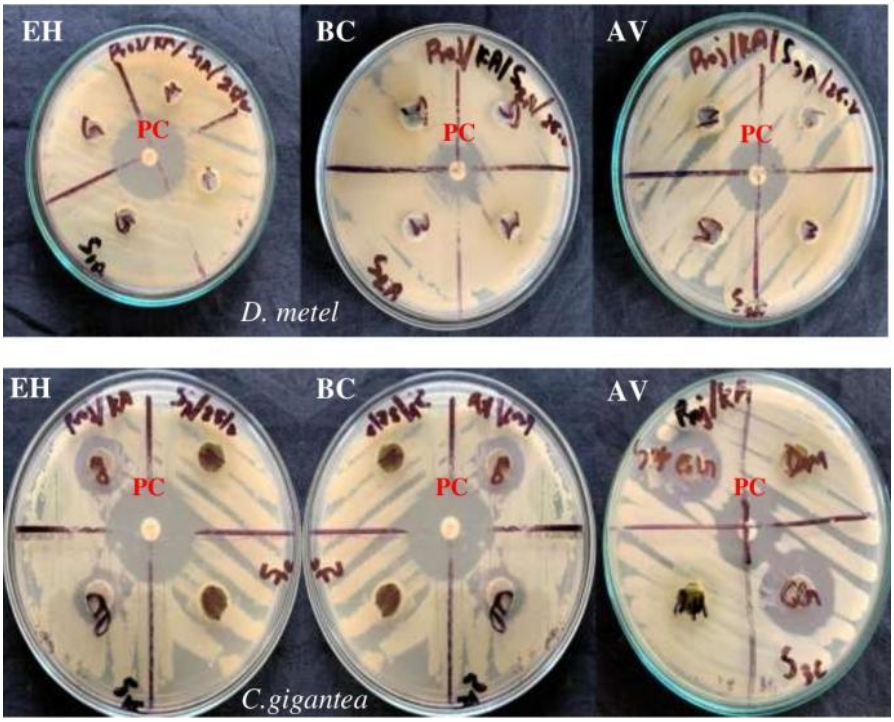

Figure 2. Antibacterial activity of crude extract of *D. metel* and *C.gigantea* against *E.hormaechei* (EH), *B. cereus* (BC) and *A. veronii* (AV) with Control

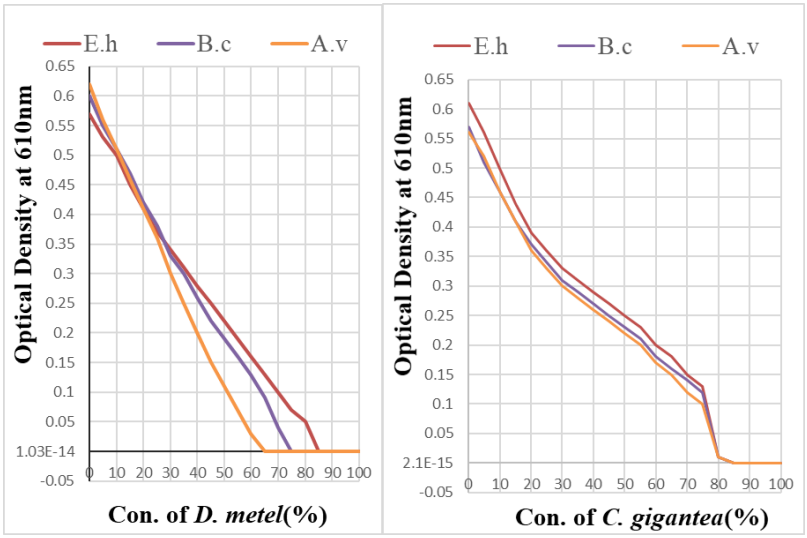

Figure 3. MIC value is determined by optical density vs Concentration of *D.metel* and *C.gigantea*

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Table I. Biochemical tests for unknown bacterial samples

Sample No.	Catalase	Citrate	Indole	Urease	H_2S gas
S_2	+	-	-	+	-
S_3	+	+	+	-	+
S_4	-	+	+	+	+
S_6	+	+	-	+	-
S_8	+	+	+	-	-
S_{10}	+	+	-	+	-
S_{11}	+	+	-	-	+

Table II. Phytochemical analysis of D. *metel* and *C. gigantea* extract

Phytochemical	*D. Metel* leaf extract				*C. gigantea* flower extract			
	CR	AQ	AC	HE	CR	AQ	AC	HE
Tannins	+	-	-	+	-	-	-	-
Terpenoids	-	+	+	-	+	+	+	+
Saponins	+	+	+	+	-	-	+	-
Flavonoids	+	+	+	-	+	-	-	-
Glycosides	+	+	+	-	+	+	+	-
Alkaloids	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+
Anthocyanins	-	-	-	-	+	+	+	+
Steroids	-	+	+	-	+	+	-	+
Resins	-	-	-	-	-	-	-	-

Note: CR (Crude), AQ (Aqueous), AC (Acetone), HE (Hexane)

Table III. Zone of inhibition of *D.metel* leaf extracts and *C.gigantea* flower extracts of *E. hormaechei* , *B. cereus* and *A. veronii*

Strains	Zone of inhibition (mm)								
	Control (5mcg) Ciprofloxain	*D. metel* extract				*C.gigantea* extract			
		AQ	AC	HE	CR	AQ	AC	HE	CR
E. hormaechei	29	Nil	Nil	Nil	10	Nil	15	Nil	18
B. cereus	27	Nil	18	Nil	13	Nil	14	Nil	15
A. veronii	22	Nil	Nil	Nil	16	Nil	Nil	Nil	21

Note: AQ (Aqueous), AC (Acetone), HE (Hexane), CR (Crude)

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Table IV. Absorbance value and percentage inhibition of protein denaturation (correlated with anti-inflammatory activity) of plant extracts with different concentration.

Concentration of Sample (%)	*D. metel*		*C. gigantea*	
	Absorbance (660 nm)	**% Inhibition**	**Absorbance (660 nm)**	**% Inhibition**
Control	0.3793	-	0.3521	-
25	0.3108	18.06	0.2753	21.81
50	0.2646	30.24	0.2189	37.83
75	0.2285	39.76	0.1794	49.05
100	0.1802	52.49	0.1316	62.62