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Formulation and Evaluation of Antibacterial and Anti-Inflammatory Emulgel Containing Eugenia caryophyllus Buds Extract

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Eugenia caryophyllus is a valuable aromatic spice used in household cooking. Traditional healers frequently employ the plant, which is said to have therapeutic qualities, in herbal concoctions to treat a variety of illnesses and conditions. Eugenia caryophyllus clove buds have antibacterial, analgesic, and anti-inflammatory properties. The formulation of clove emulgel and its assessment using a variety of evaluation criteria were the main objectives of the current study. Clove emulgel was evaluated for its anti-inflammatory and anti-microbial properties. We extracted and assessed the oils from Eugenia caryophyllus buds for phytochemical testing using solvents such as methanol, ethanol, petroleum ether, and N-hexane. We prepare emulgel and test it for spreadability, pH, and other organoleptic characteristics. We used egg albumin denaturation as a protein to measure the in vitro anti-inflammatory efficacy. We tested the antibacterial susceptibility using the agar-well diffusion method. Alkaloids, saponins, tannins, steroids, carbohydrates, and glycosides were the phytochemical components found in clove bud extract. Physical factors such as color, consistency, and state—such as semi-solid, smooth, and brownish-gummy—are included in this category of evaluation parameters. The emulgel's pH was 5.35, its spreadability ranged from 1.6 to 2.5 cm, and no phase separation was noticed while the emulgel was stored. An in vitro anti-inflammatory test (spectroscopy method) and an antimicrobial test (zone of inhibition) were used to evaluate the anti-inflammatory activity of emulgel. Comparing clove oil emulgel to a typical medication, these evaluations found that it demonstrates strong anti-inflammatory and antibacterial resistance. In conclusion, Clove Buds Emulgel has demonstrated antibacterial activity against both gram-positive and gram-negative bacterial strains. Emulgel demonstrated the inhibitory response percentage in relation to the standard. This validates the roles that E. caryophyllus has been shown to play in protecting human health. This innovative emulgel mixture was applied to arthritis to lessen microbial infection and joint discomfort. In addition, more preclinical and clinical trials will be needed for this research in order to successfully commercialize the emulgel formulation for the treatment of inflammation and antimicrobial activity.

Keywords: Anti-inflammatory; Anti-microbial; Clove; Evaluations; Emulgel; Spectroscopy.

Emulgels are the newest and most popular drug delivery technique for hydrophobic drugs. This formulation is thought to be a novel sort of drug delivery method since it combines gel and emulsion[1, 2]. The properties of emulgels include transparency, emollience, persistence, thixotropic

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properties, spreadability, and aesthetic appeal. These days, emulgels are used to administer a variety of drugs, such as antifungal, anti-inflammatory, analgesic, and anti-acne[3, 4]. As a result, it has significant pharmacological usefulness and is virtually side-effect-free. Historically, a variety of medicinal plants have treated and managed a wide range of illnesses and disorders. The combined action of all the physiologically active ingredients in these therapeutically significant medicinal plants strengthens antioxidant defense mechanisms and reduces lipid peroxidation, thereby providing favorable benefits[5]. In addition to being a common household spice used in cooking, clove, or Eugenia caryophyllus, is a significant medicinal plant in the Myrtaceae family [6, 7]. Cloves can be smoked in cigars or consumed as a tea[8]. Eugenia caryophyllus has also been used topically to cure toothaches[9]. Clove is thought to be useful in preventing a variety of degenerative diseases because it contains significant amounts of several chemical elements that have antioxidant activity[10].

Numerous health benefits, including antiherpetic, antioxidant, anticandidal, anticarcinogenic, antipyretic, antiplatelet inhibitory, and aphrodisiac acyivity, have been linked to Eugenia caryophyllus in recent studies. The main chemical components of Eugenia caryophyllus are ethylene, caryophyllene, and tannins[11]. Eugenol, sesquiterpenes (á-and â-caryophyllenes), acetyl-eugenol, and trace amounts of ketones, alcohols, and esters make up the 14%–20% volatile oils found in cloves. Moreover, cloves have stigmasterol, tannins, and sitosterol[12]. 72% to 90% of the essential oil recovered from cloves is eugenol, which is primarily responsible for the aroma that cloves release. This is truly quite incredible. Acetyl eugenol, â-caryophyllene, and vanillin; crategolic acid; tannins; gallotannic acid; methyl salicylate (an anesthetic); flavonoids (kaempferol, eugenin, eugenitin and rhamnetin); triterpenoids (oleanolic acid, stigmasterol, and campesterol); and several sesquiterpenes are additional significant components of clove seed essential oil. Vanillin, gallotannic acid, crategolic acid, methyl salicylate, kaempferol, eugenin, rhamnetin, eugenitin and triterpenoids, such as oleanolic acid[13], are additional essential oil components of clove oil. Furthermore, methyl salicylate, methyl amyl ketone, benzaldehyde, á and â-humulene, chavicol and â-ylangene are present in the oil[14]. Syzygiumaromaticum (L.) is categorized taxonomically from the order Plantae all the way down to Species[15].

Pharmacologically, cloves and their constituents possess analgesic, antioxidant, antibacterial, anticancer, and anesthetic qualities. They also showed evidence of having insecticidal, antipyretic, aphrodisiac, and repellent qualities for mosquitoes[16]. Because clove essential oil has a high percentage of eugenol, it has good biological and bactericidal properties[17, 18]. In many pathophysiological situations, including diabetes, hypertension, and neurological and cardiovascular illnesses, oxidative stress and inflammation are closely connected processes. CEO (Clove Essential Oil) and eugenol have anti-inflammatory qualities that, like diclofenac gel, can reduce inflammation from 60% to 20% in just three hours. Similarly, mice treated with CEO for wounds shown an impressive 95% reduction in size within the first 15 days. These findings demonstrated that wound healing in mice given CEO was comparable to that of animals given neomycin, an anti-inflammatory medication frequently used to speed up wound healing. Thus, it is possible to prevent the adverse effects of synthetic antibiotics, both short-term and long-term, particularly if they are taken frequently[19].Eugenol was given intravenously and intragastrically to rabbits in order to evaluate its analgesic properties[20]. Anacetamol was a common medication. More fever-reducing potential was demonstrated by eugenol than by paracetamol[21]. The US Food and Drug Administration (USFDA) has approved clove oil, oleoresins, and clove buds as generally recognized safe food additives[22]. Clove oil and its main active ingredient, eugenol, have been shown in numerous studies to have antibacterial properties against common food sources, including Salmonella, Pseudomonas aeruginosa, Bacillus substiles, and Escherichia coli, as well as Gram-positive bacteria like Streptococcus, Listeria, and Bacillus substiles. according to how it prevents adhesion, migration, the formation of biofilms, virulence factors, and other processes [23, 24].

Based on the facts above, the goal of this research study is to ascertain how clove essential oil affects its anti-inflammatory qualities. the creation of a novel drug delivery system for clove

oil emulgel's anti-inflammatory qualities. Clove oil's antimicrobial properties are also determined. To overcome the drawbacks of parenteral and oral drug delivery systems in order to improve patient compliance. To create and assess a topical drug delivery system with clove oil emulgel. The drawbacks of both emulsion and gel can be avoided by formulating in an emulgel[25, 26].

MATERIALS AND METHODS

Cultivation and Collection

The clove tree is an evergreen that can grow up to fifteen meters tall. The best soil for seeding is rich, loamy soil with a high humus content that drains effectively. It thrives near the ocean and likes a warm, humid environment. August to October is when plants are planted. The spacing between seeds in nursery beds is 10 cm. They take 4-5 weeks to germinate. Clove seedlings are tiny and vulnerable. For the first two to three years, the clove is allowed to grow in the shadow between banana plants. Clove is harvested from plants that range in age from six to seventy years[27]. The buds are gathered and gently dried in the sun when they turn crimson from green. Cloves are sorted by plucking the buds that still have a stalk or by beating them with bamboo sticks[28].

Preparation of plant extract

Solvent extraction is one of the most widely used and common techniques for removing essential oils from plants. Several solvents, including ethanol, methanol, N-hexane, and petroleum ether, have been used to extract clove essential oil. The 50gm clove bud samples were weighed before being placed in a 500ml reflux flask (or RBF) with an extraction thimble for filter paper. After that, the samples were extracted using a Soxhlet apparatus for almost six hours using 250ml of 100% ethanol. Using the evaporation process in conjunction with the Soxhlet extraction procedure, concentrate was produced[29, 30].

Evaluations tests[31]

Test for Proteins

a) Xanthoproteic test: 1ml of plant extract was treated with 0.25ml of nitric acid. The presence of proteins was revealed by the white precipitate's appearance.

b) Biuret test: Added 1 ml of plant extract, 4% NaOH, and 1% CuSO4 to a test tube. The violet-pink tint indicated that proteins were present.

Test for alkaloids

a) Mayer's test: Two drops of chloroform, two drops of Mayer's reagent, and one milliliter of clove flower bud extract were added. White deposits were produced as a result of a positive alkaloid response.

b) Dragendorff's test: Mixed 1 ml of plant extract with 2 ml of Dragendorff's reagent. Alkaloids were present when an orange-white precipitate formed.

c) Wagner's test: Wagner's reagent treatment of 1 ml of extract indicates the presence of alkaloids by producing a brown, reddish precipitate.

Test for Carbohydrates

a) Fehling's test: Boil two milliliters of pure water, then filter one milliliter of plant extract. Next, boil 2 ml of Fehling's reagent and add it to 2 ml of the filtrate. A precipitate that is reddish brown suggests that glucose, or carbohydrates, are present.

b) Molisch's test: Added 0.4 ml of Molisch's reagent with 1 ml of plant extract. Next, we placed one milliliter of concentrated sulfuric acid along the edge of the test tube. When there are carbs (starch) present, the hue becomes purple.

c) Benedict's test: For five minutes, one milliliter each of plant extract and Benedict's reagent were cooked. An orange precipitate was produced, indicating the presence of carbs.

Test for Tannins

a) Gelatine test: 500 µl of the filtrate was mixed with 1% gelatine solution. Tannin was present because a curdy white precipitate formed.

b) Ferric chloride test: The filtrate was mixed with five drops of a 5% ferric chloride solution. The development of a blue-green tint suggested the presence of tannin.

c) Lead acetate test: Five milliliters of a 10% lead acetate solution were added to the filtrate. The presence of tannin is shown by the formation of white precipitate.

Test for Saponin

Foam test

A test tube containing one milliliter of plant extract and a modest amount of water was used. After adding sodium bicarbonate, give it a good five minutes of vigorous shaking. The presence of saponins was detected by foam formation.

Salkowski test for sterols

0.5 ml of the plant extract was exposed

to 2 ml of concentrated sulfuric acid and 2 ml of chloroform, one after the other, from the test tube's side. We gently shook the test tube for a few minutes. The presence of sterols was shown by the chloroform layer turning red.

To test for flavonoids

To 0.5 ml of plant extract, add 1 ml of concentrated sulfuric acid and 5 ml of diluted ammonia. A yellow hue, which disappeared upon standing, indicated the presence of flavonoids.

Cardiac Glycoside Test (Keller Killiani test)

We evaporated each plant extract at 40 °C, yielding around 5 ml of residue, which we then collected. In 5 milliliters of water, there was some residue suspended. We mixed it with two milliliters of glacial acetic acid and one drop of ferric chloride solution. We underplayed this solution with 1 milliliter of concentrated sulfuric acid. A brown ring at the contact revealed one of the characteristics of cardiac glycosides, deoxy sugar.

Test for Resins

Two drops of concentrated sulfuric acid and 0.5 milliliter of acetic acid were put to a dry test tube. The presence of resins was indicated by a purple tint that turned violet in around ten minutes.

Test for anthraquinones

Shake 2 milliliters of each plant extract with 10 milliliters of benzene, then add 5 milliliters of 10% ammonia solution. To achieve the anthraquinone color, the mixture was agitated. The presence of anthraquinones was revealed by the ammoniacal layer turning pink.

Triterpene test

5 ml of chloroform was combined with a few milligrams of plant extract residue, and the mixture was heated for 30 minutes at 40 °C. Add a few drops of concentrated sulfuric acid and thoroughly stir. Because of the red color's emergence, triterpenes were present.

The Formulation of Emulgel in Essential Oils of Clove

The emulgel formula made reference to the findings of the earlier investigation, which are shown in Table 1. The process of preparing emulgel began by immersing the gelling ingredient, Carbopol 940, in 30 milliliters of hot distilled water for a full day. The water phase and oil phase were then melted at 60°C on the water bath. There was a mix during the two sessions. The liquid cooled before the essential oil of clove was added. Ultimately, the emulsion mixture was mixed with the Carbopol 940 solution to create a homogenous mixture[32].

Manufacturing Process

Step 1: Preparation of gel

Separately, we dissolved carbopol 940 in distilled water and stirred continuously to create the gel bases. We brought the formulation's pH to 6-6.5 using triethanolamine (TEA).

Step 2: Preparation of Emulsion

A). Preparation of Aqueous Phase

To create the aqueous component of the emulsion, pure water was used to dissolve sorbitol, span 80, tween 80, and triethanolamine.

B). Preparation of Oil Phase

We dissolved oleic acid, propyl paraben, methyl paraben, and paraffin liquid in propylene glycol. The oily and aqueous phases were then separately heated to 60 °C. Next, we combined the aqueous phase with the oil phase and continuously mixed it until it reached room temperature.

Step 3: Addition of clove oil

After the mixture cooled, clove essential oil was added.

Step 4: Preparation of Emulgel

Finally**,** the emulgel was created by gently combining the emulsion that had been obtained with the gel[33].

Evaluation of formulation organoleptic properties[34]

a) Physical appearance: The color, consistency, homogeneity, and phase separation of the prepared Emulgel are examined visually.

b) pH Evaluation: This is a crucial factor, particularly in topical formulations. Emulgel's pH should be between 5.8 and 6 to replicate the skin's pH range. Patient discomfort may result from an acidic or basic pH in the manufactured emulgel. Using a digital pH meter, the prepared emulgel's PH was determined by dipping a glass electrode into it.

c) Spreadability: The spreadability of emulgel is measured by measuring the diameter of the emulgel circle that forms when the substance is sandwiched between two glass plates of a particular weight. Place 350 mg of weighted emulgel on one glass plate, then drop another glass plate from a height of 5 cm. We measure the circumference of the spread emulgel circle.

d) Grittiness: Using a light microscope, the formulation was examined microscopically to see if any noticeable particle matter was present. Therefore, it is evident that the emulgel preparation satisfies the necessary condition of being free of specific material and grittiness, which is desirable for any topical preparation.

In-vitro Anti-Inflammatory Test

Inhibition of albumin denaturation

Measurement of the in vitro anti-inflammatory effect was done using the suppression of protein denaturation method (egg albumin).

Control Solution (50 ml): Freshly made egg albumin (2 ml) was combined with 28 ml of pH 6.4 phosphate buffered saline and 20 ml of distilled water to create the control solution.

Standard Solution (50 ml): After transferring 28 ml of pH 6.4 phosphate buffer saline to 2 ml of freshly made egg albumin, 20 ml of diclofenac sodium solution (varying concentrations from 10 to 2000 µg/ml) was added to the mixture to create the standard solution.

Test Solution (50 ml): 28 ml of pH 6.4 phosphate buffer saline was added to 2 ml of newly made egg albumin. 20 ml of varying concentrations of clove oil, ranging from 10 to 2000 µg/ml, was then added to the mixture to create the test solution.

Following a 15-minute incubation period at 37 ± 2 °C, each solution was heated for five minutes at 70 °C in a water bath. We allowed the solutions to cool at room temperature. Next, we measured the absorbance at 660 nm using a UV-visible spectrophotometer, using the vehicle as a blank [35]. We determined the percentage inhibition of protein denaturation from the control using the following formula:

$$\text{Percentage inhibition} = (Vt/Vc - 1) \times 100$$

Where,

Vt = Absorbance of the test sample

Vc = Absorbance of control

Antimicrobial Assay

The antibacterial susceptibility was tested using the agar well diffusion method. In accordance with standard protocol28, inoculums were generated and calibrated to 0.5 McFarland turbidity. The gram-negative E. Coli and gram-positive Bacillus subtilis inoculum suspension from the nutrient broth were uniformly streaked on nutrient agar media. The grass cultures were punctured with wells that were filled with pathogen-inoculated medium using a sterile borer that had a 6 mm diameter. Aseptically, the well was filled with 10 µl, 20 µl, 50 µl, 100 µl, and 200 µl of each extract. The plates were kept at room temperature for an hour in order to give the extract time to permeate into the agar. It was then incubated for 24 hours at 37°C. The zone of inhibition's diameter was measured to document the outcomes after a period of 24 to 48 hours. As a positive control, antibiotic amikacin was used at concentrations of 10 µl, 20 µl, 50 µl, 100 µl, and 200 µl. Conversely, without the herbal extracts, ethanol medium was utilized as a negative control at concentrations of 10 µl, 20 µl, 50 µl, 100 µl, and 200 µl. After the wells were incubated, the clear zone around them was measured in millimeters to determine the zone of inhibition. The experiment was run at several concentrations, and the findings were recorded[36].

Statistical analysis

Analytical statistics GraphPad Prism 9 was used to perform statistical analysis on all the data. Using an independent sample t-test, the percentage of inhibition at various sample concentrations was examined. When $P < 0.05$, the differences were deemed statistically significant [37].

RESULT AND DISCUSSION

Phytochemical Evaluations

The following outcomes were obtained and documented from a phytochemical examination of the different ethanolic and chloroform Eugenia caryophyllus bud extracts. As seen in Table 2, the plant extract contained flavonoids, sugars, tannins, alkaloids, saponin, sterols, and glycosides.

Evaluation Parameters of Formulation

The clove buds extract emulgel was found to have an aromatic scent, a consistent consistency and homogeneity, and to be well-preserved with a brownish-gummy color. Table 3 illustrates that the emulgel formulation's pH values fell between 5.2 ± 0.17 and 5.5 ± 0.20, which is a close range of neutral pH and suitable for topical application and medical purposes.

Fig. 1. Preparation of gel

Fig. 2. Preparation of Oil Phase

Percentage Inhibitory Response

The anti-inflammatory effects of clove oil are determined using the egg albumin denaturation assay method, which is an in-vitro test. Comparing the usual medicine, diclofenac sodium, with an emulgel containing clove buds extract, Figure 6 illustrates the drug's anti-inflammatory susceptibility. A formula provided in the anti-inflammatory test technique was used to compute the % inhibition. Higher concentrations were shown to have stronger inhibition of the denaturation of egg albumin protein. The % inhibition of Diclofenac Sodium and clove buds extract emulgel was determined and compared using the unpaired "t" test. Analysis using GraphPad Prism 9.0 software demonstrated a statistically significant

Fig. 3. Clove Emulgel

Table 1. Formulation of *Eugenia caryophyllus* Emulgel

Sr. No.	Ingredients	Quantity Taken (gm)	Uses
1	Essential Oil of Clove	3.3	Anti-inflammatory, Antimicrobial, Pain Reliever for Tooth Condition and Muscle pain
2	Carbopol 940	1.2	Gelling Agent
3	Paraffin Liquid	0.37	An Emollient
4	Triethanolamine	2.4	Surfactants, Emulsifier
5	Propylene Glycol	1.5	Moisturizer
6	Oleic Acid	1.5	Increase Fluidity of Gel
7	Span 80	0.75	Surfactant (for o/w emulsion)
8	Tween 80	5.25	Emulsifier, Wetting Agent, Penetrating Agent
9	Propyl Paraben	0.01	Preservative
10	Methyl Paraben	0.05	Preservative
11	Sorbitol	0.6	Plasticizers
12	Distilled Water	30	Vehicle

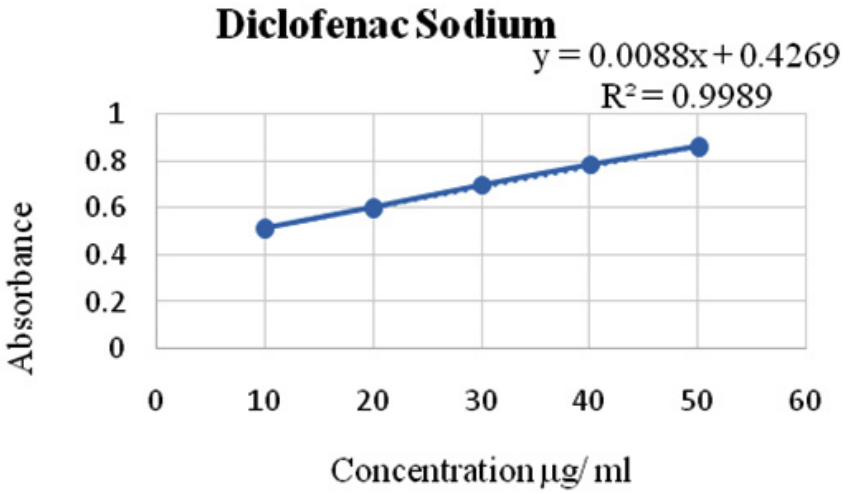

Fig. 4. Calibration curve of standard using UV spectrophotometer

Table 2. Phytochemical Evaluations of Extract

Sr. No.		Tests	Ethanol	Chloroform
1	Proteins	Xanthoproteic	-	-
		Biuret	-	-
2	Alkaloids	Mayer's	+	+
		Dragendorff's	+	+
		Wagner's	-	-
3	Carbohydrates	Fehling's	-	-
		Molisch	+	-
		Benedict's	+	+
4	Tannins	Gelatine	+	+
		Ferric Chloride	+	+
		Lead Acetate	+	+
5	Saponin	Foam Test	+	+
6	Sterols	Salkowski	+	+
7	Flavonoids	-	-	
8	Cardiac Glycosides	Keller Killiani	+	+
9	Resin's	-	-	
10	Anthraquinones	-	-	
11	Triterpene's	-	-	

(+ Detected, - Not Detected)

Table 3. Formulation Evaluations

Sr. No.		Evaluation Parameter's	Result
1	Physical Appearance	Colour	Brownish Gummy
		Homogeneity	+++
		Consistency	+++
		Phase Separation	None
2	pH Evaluation	5.35	
3	Spreadability	1.6–2.5 cm	

distinction ($P<0.05$) between the test and standard samples.

Antimicrobial Assay

Diameter of Inhibition Zones of antibacterial activity of emulgel of clove buds extract against E. coli using the Agar Well Diffusion Method:

The selected organisms were tested for susceptibility to the emulgel of clove buds extract, and the results were observed and recorded. The plant extracts exhibited strong antimicrobial activity against E. coli as shown in Table 4 and Figure 7.

Diameter of Inhibition Zones of antibacterial activity of emulgel of clove buds extract against B.subtilis using the Agar Well Diffusion Method:

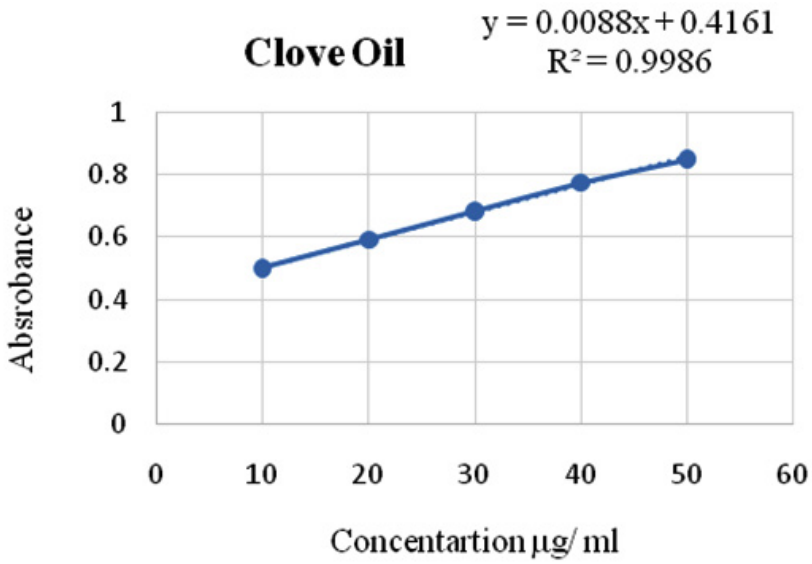

Fig. 5. Calibration curve of Test Sample (Clove oil) using UV spectrophotometer

Table 4. Effect of Extract on *E. coli* micro-organism zone of inhibition obtained are shown in below Table

Sr. No.	Particular	Concentration in µg/ml	Diameter of Zone of Inhibition in mm
1	Emulgel of Clove Buds Extract (Test)	10	6.5
		20	7.5
		50	8.5
		100	10
		200	10.5
2	Positive Control (Amikacin)	10	7.5
		20	8.5
		50	10.5
		100	12
		200	13.5
3	Negative Control (Ethanol)	10	6
		20	6.5
		50	6.5
		100	7
		200	8

The chosen organisms were subjected to an emulgel containing plant extracts extracted from clove buds, and the outcomes were noted and documented. Table 5 and Figure 7 demonstrate the plant extracts' significant antibacterial action against B. subtilis.

An anti-microbial assay for clove buds extract emulgel was conducted in the current investigation. through the measurement of the zones of microbial inhibition. Via the agar well diffusion method, the antimicrobial susceptibility was evaluated. As the zone of inhibition observed is at high concentrations, it is demonstrated that

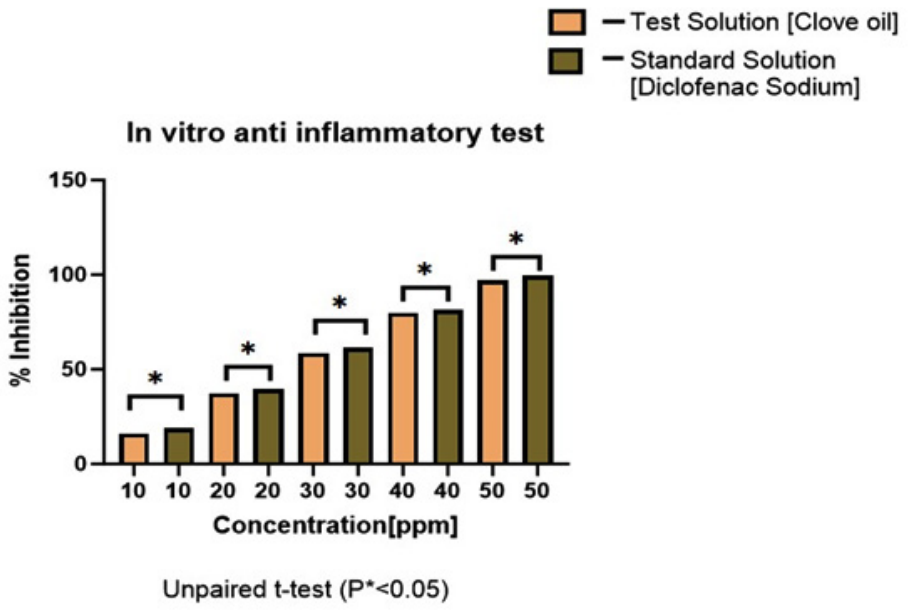

Fig. 6. % Inhibition of protein (Anti-inflammatory assay)

Table 5. Effect of Extract on *B. subtilis Bacillus* micro-organism, zone of inhibition obtained are shown in below Table 5

Sr. No.	Particular	Concentration in µg/ml	Diameter of Zone of Inhibition in mm
1	Emulgel of Clove Extract (Test)	10	8
		20	9
		50	11
		100	13
		200	14
2	Positive Control (Amikacin)	10	8.5
		20	10
		50	11
		100	13.5
		200	15
3	Negative Control (Ethanol)	10	6.5
		20	6.5
		50	7
		100	8
		200	8.5

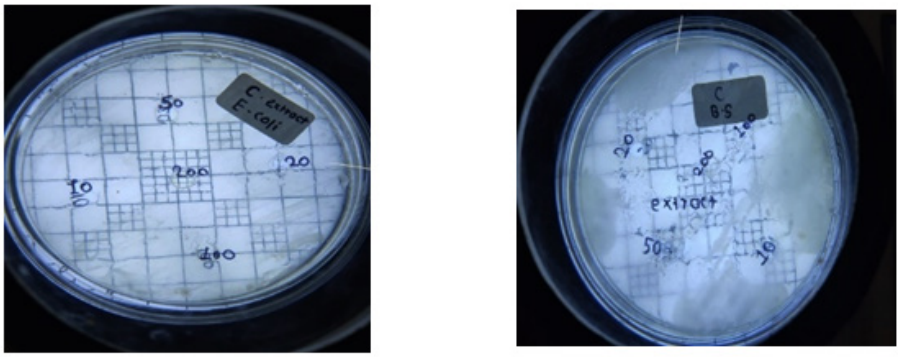

Fig. 7. Zone Inhibition by Extract

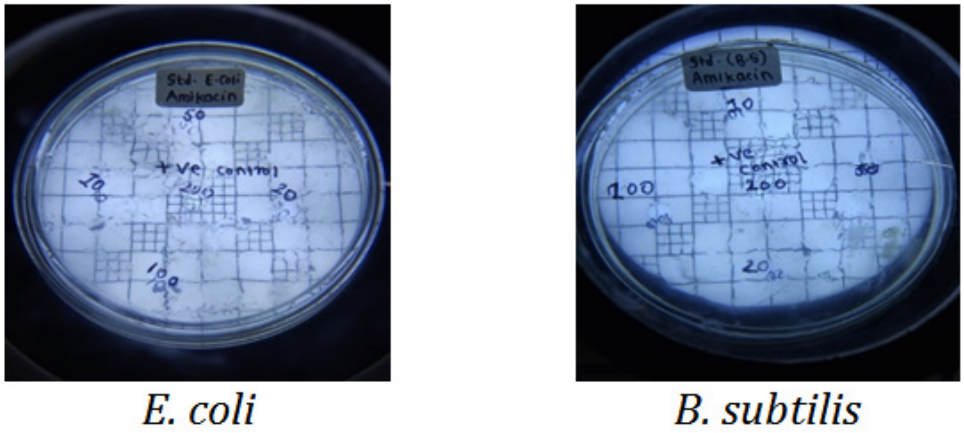

Fig. 8. Zone Inhibition by Amikacin

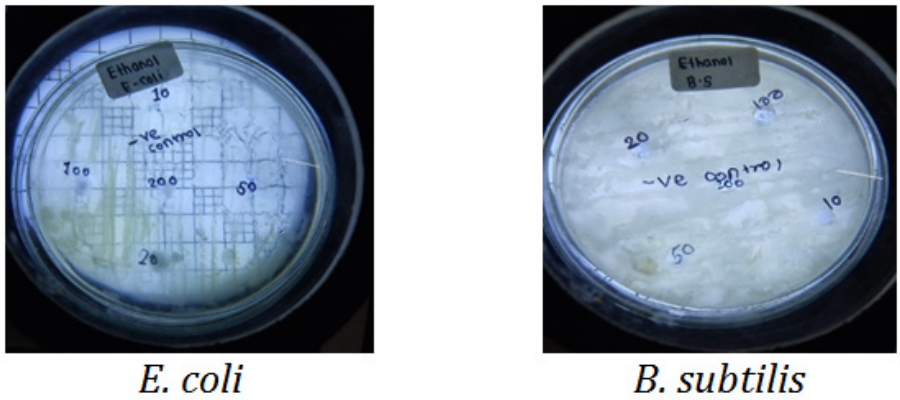

Fig. 9. Zone Inhibition by solvent (Ethanol)

the emulgel's activity is concentration dependant. Clove extract emulgel was utilized as the test sample, and amikacin served as the positive and negative controls, respectively. A comparison between the standard medicine amikacin and test results indicates that the former has stronger zones of inhibition. As illustrated in Figure 10, the contrast. inaddition to figure 11. The anti-microbial susceptibility of the clove buds extract emulgel is quite good at varying concentrations.

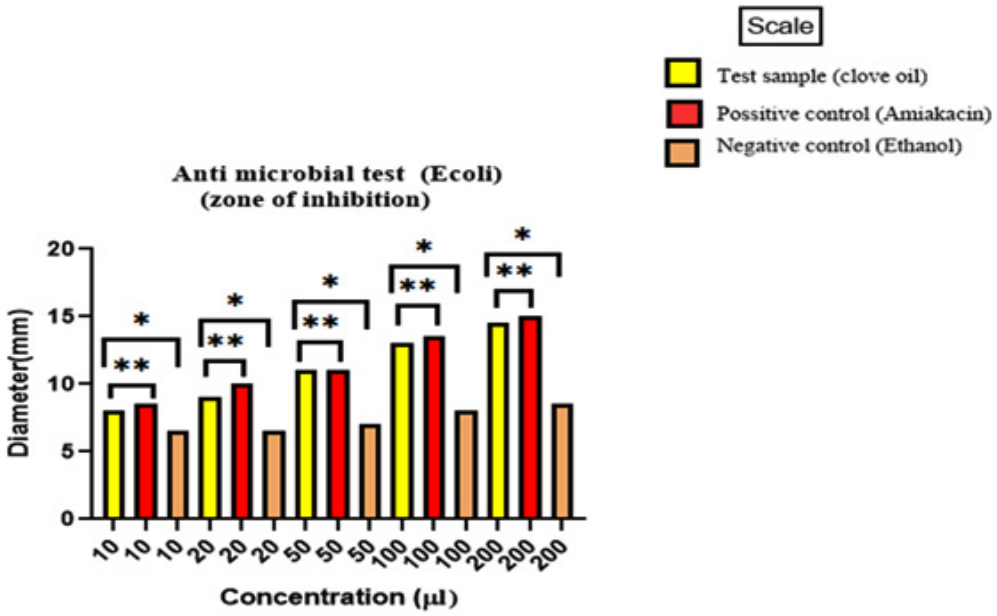

Fig. 10. Zone of inhibition using different Concentration of Test, Positive Control and Negative Control for *E. Coli.*

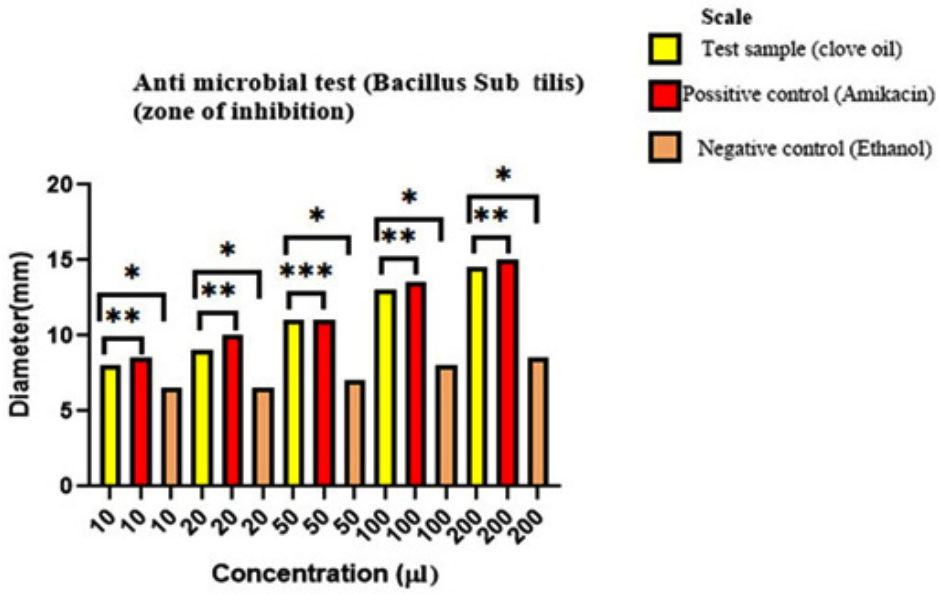

Fig. 11. Zone of inhibition using different Concentration of Test, Positive Control and Negative Control for *B. subtilis*

A student "T" test and statistical analysis will be used for comparison. A noteworthy distinction was discovered between the test, positive, and negative samples by the use of E. Coli and B. subtilis. Where $P<0.001$, $P<0.01$, and $P<0.05$ are found.

CONCLUSION

The current study uses Eugenia caryophyllusemugel's herbal formulation to determine its therapeutic efficacy for human health care. The research investigated the anti-inflammatory and anti-microbial activity of the clove emulgel. Researchers have previously discovered numerous therapeutic or medicinal properties in the crude drug clove. Clove oil possesses a multitude of pharmacological properties, including antimicrobial, anticancer, anti-inflammatory, wound-healing, analgesic, and anesthetic properties. Evaluation tests are used to identify the various primary and secondary metabolites that Eugenia caryophyllus clove possesses, which are important in a variety of diseases and disorders. Anticipated phytoconstituents are also present in this study on emulgel's anti-inflammatory and anti-microbial activity. The egg albumin method is used to measure the anti-inflammatory action of cloves in vitro, while the agar-well diffusion method is used to measure the zone of bacterial inhibition. There are several different medical uses for clove oil. The current investigation found that the clove bud extract in the emulgel has anti-inflammatory properties. The investigations conducted to determine clove oil's anti-inflammatory and antimicrobial properties were successful in every way. To increase patient compliance, the topical medication delivery system will be widely utilized. This simplifies and enhances the efficiency of applications and uses for pharmaceutical and cosmetic products. The combination of plant extracts and antibiotics against resistant bacteria produces new therapy possibilities for infectious disorders. When the proper antibiotic loses its efficacy on its own during therapeutic therapy, this effect allows for its continued usage.

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Conflict of Interest

The author declare that I have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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All required data is available.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Authors' Contribution

Research work carried out: Mr. Juber shaha S. Fakir, Mr. Chandrakant M. Ahire, Dr. Khemchand R. Surana; Data collection, analysis and Interpretation of Results and Manuscript Draft Preparation: Mr. Juber shaha S. Fakir Dr. Khemchand R. Surana, Ayaz A. Ahamad, Mr. Abdul Kalam, Madhuri D. Davanage Dr. Sunil K. Mahajan; Reviewed the results and approved the final version of the manuscript: Mr. Juber shaha S. Fakir, Dr. Khemchand R. Surana, Dr. Sunil K. Mahajan

REFERENCES

1. Likitha B, F R Sheeba, Yeshavantha Kumar, Shivanand K Mutta, H S Keerthy. Emulgel: A Novel Topical Drug Delivery. *Research Journal of Pharmaceutical Dosage Forms and Technology*. 2023; 15(2):123-130. http://dx.doi.org/10.52711/0975-4377.2023.00021
2. Parijat Pandey, NehaMinocha, NeelamVashist, Rashmi Shah, Sapna Saini, Manish Makhija, DeepikaPurohit and DhirenderKaushik. Emulgel: An Emerging Approach towards Effective Topical Drug Delivery. *Drug Delivery Letters*. 2022; 12(4):227-242. http://dx.doi.org/10.2174/2210303112666220818115231
3. Maria Talat, Muhammad Zaman,Rahima, Khan,MuhammadJamshaid,Muneeba Akhtar, Agha ZeeeshanMirza. Emulgel: an effective drug delivery system. *Drug Development and Industrial Pharmacy*. 2021; 47(8):1193-1199. https://doi.org/10.1080/03639045.2021.1993889

4. Milutinov J, Krstonošiæ V, Æirin D, Pavloviæ N. Emulgels: Promising Carrier Systems for Food Ingredients and Drugs. *Polymers (Basel)*.2023;15(10):2302. https://doi.org/10.3390/polym15102302
5. Wang CZ, Mehendale SR, Yuan CS. Commonly used antioxidant botanicals: active constituents and their potential role in cardiovascular illness. Am J Chin Med. 2007;35(4):543–58. https://doi.org/10.1142/s0192415x07005053
6. Singh AK, Dhamanigi SS, Asad M. Anti-stress activity of hydroalcoholic extract of Eugenia caryophyllus buds (clove). *Indian J Pharmacol*. 2009;41(1):28–31. https://doi.org/10.4103%2F0253-7613.48889
7. Cortés-Rojas DF, de Souza CR, Oliveira WP. Clove (Syzygiumaromaticum): a precious spice. Asian Pac J Trop Biomed. 2014;4(2):90-6. Milind P, Deepa K. Clove: a champion spice. *Int J Res Ayurveda Pharm*. 2011;2:47–54. https://doi.org/10.1016%2FS2221-1691(14)60215-X
8. Malson JL, Lee EM, Murty R, Moolchan ET, Pickworth WB. Clove cigarette smoking: biochemical, physiological, and subjective effects. *PharmacolBiochemBehav*. 2003;74(3):739–45. https://doi.org/10.1016/s0091-3057(02)01076-6
9. Alqareer A, Alyahya A, Andersson L. The effect of clove and benzocaine versus placebo as topical anesthetics. *J Dent*.2006;34(10):747–50. https://doi.org/10.1016/j.jdent.2006.01.009
10. Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. Syzygiumaromaticum L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. *Biomolecules*. 2020;10(2):202. https://doi.org/10.3390%2Fbiom10020202
11. Li Bao, EerdunbayaerEerdunbayaer, A. Nozaki, E. Takahashi, K. Okamoto, Hideyuki Ito, T. Hatano. Hydrolysable Tannins Isolated from Syzygiumaromaticum: Structure of a New C-GlucosidicEllagitannin and Spectral Features of Tannins with a Tergalloyl Group.*Heterocycles*. 2012;85(2):365. https://doi.org/10.3987/COM-11-12392
12. P K Lai and J RoyLai. Antimicrobial and chemopreventive properties of herbs and spices. *Current medicinal chemistry*.2004;11(11), 1451-1460. https://doi.org/10.2174/0929867043365107
13. Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K, Bakhrouf A. The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzigiumaromaticum L. Myrtaceae): a short review. *Phytother Res*. 2007;21(6):501-516. https://doi.org/10.1002/ptr.2124
14. Temesgen S, Sasikumar JM, Egigu MC. Effect of Extraction Solvents on Total Polyphenolic Content and Antioxidant Capacity of SyzygiumAromaticum L. Flower Bud from Ethiopia. *Biomed Res Int*.2022:4568944. https://doi.org/10.1155%2F2022%2F4568944
15. Kiki, ManalJameel.In Vitro Antiviral Potential, Antioxidant, and Chemical Composition of Clove (Syzygiumaromaticum) Essential Oil. *Molecules*.2023; 28(6):2421. https://doi.org/10.3390/molecules28062421
16. Surana KR, Ahire ED, Mahajan SK, Patil DM, Jadhav KR. Antimicrobial And Antiinflammatory Action of Flavonoids. InThe Flavonoids 2024 Feb 6 (pp. 263-276). Apple Academic Press. https://doi.org/10.1201/9781003399964
17. Khairnar SJ, Ahire ED, Jagtap MR, Surana KR, Kshirsagar SJ, Keservani RK. Nutritional Properties of Polyphenols. InAdvances in Flavonoids for Human Health and Prevention of Diseases 2024 (pp. 1-22). Apple Academic Press. https://doi.org/10.1201/9781003369813
18. Ahire ED, Surana KR, Sonawane VN, Talele SG, Talele GS, Kshirsagar SJ, Khairnar SJ, Thombre NA. The Metabolic Syndrome: A Concerning Area for Future Research. InThe Metabolic Syndrome 2023 Aug 4 (pp. 231-249). Apple Academic Press. https://doi.org/10.1201/9781003329732
19. Mounika AM, Sushma MS, Sidde LS, Malathi SM, Rajani KR. Invitro evaluation of antimicrobial activity of Clove buds (Euginea aromatica). *Int. j. indig. herbs drugs*. 2020 Aug 27:25-33. DOI: https://doi.org/10.46956/ijihd.vi.95
20. Chavan PR, Kalshetti MA, Navindgikar NI. Formulation and evaluation of polyherbal cream. *Int. J. Curr. Pharm. Res.* 2020;12(4):75-7. DOI: https://doi.org/10.22159/ijcpr.2020v12i4.39086
21. Aher S. N, Sonawane S. N, Sonawane P. R, Surana K. R, Mahajan S. K, Patil D. M, Katkade P. N. Insilico Drug Design, Synthesis and Evaluation of Anti-inflammatory Activity Pyrimidine Analogue. Biotech Res Asia 2024;21(2). Available from: https://bit.ly/3XcHvg9
22. Haro-González JN, Castillo-Herrera GA, Martínez-Velázquez M, Espinosa-Andrews H. Clove Essential Oil (Syzygiumaromaticum L. Myrtaceae): Extraction, Chemical Composition, Food Applications, and Essential Bioactivity for Human Health. *Molecules*. 2021;26(21):6387. https://doi.org/10.3390%2Fmolecules26216387
23. Chaieb, K., Hailaoui, H., Zmantar, T., Kahla-Nakbi, A. B., Rouabhia, M., Mahdouani, K. and Bakhrouf, A. The chemical composition

and biological activity of clove essential oil, Eugenia caryophyllata (Syzygiumaromaticum L.). *Phytother. Res*. 2007; 21(6):501-506.https://doi.org/10.1002/ptr.2124

24. Nuñez L, Aquino MD. Microbicide activity of clove essential oil (Eugenia caryophyllata). *Braz J Microbio*l. 2012;43(4):1255-60.https://doi.org/10.1590/s1517-83822012000400003
25. Banerjee K, Madhyastha H, Sandur VR, Manikandanath NT, Thiagarajan N, Thiagarajan P. Anti-inflammatory and wound healing potential of a clove oil emulsion. Colloids Surf B Biointerfaces.2020;193:111102. https://doi.org/10.1016/j.colsurfb.2020.111102
26. MohammadiNejad S, Özgüneº H, Baºaran N. Pharmacological and Toxicological Properties of Eugenol. *Turk J Pharm Sci*. 2017;14(2):201-206. https://doi.org/10.4274%2Ftjps.62207
27. J. Feng, J. Lipton. Eugenol: antipyretic activity in rabbits. Neuropharmacology. 1987;26(12): 1775-1778.https://doi.org/10.1016/0028-3908(87)90131-6
28. Issac A., Gopakumar G., Kuttan R., Maliakel B., Krishnakumar I.M. Safety and anti-ulcerogenic activity of a novel polyphenol-rich extract of clove buds (Syzygiumaromaticum L.) Food Funct. 2015;6(3):842–852. https://doi.org/10.1039/c4fo00711e
29. Hu Q, Zhou M, Wei S. Progress on the Antimicrobial Activity Research of Clove Oil and Eugenol in the Food Antisepsis Field. *J Food Sci*. 2018;83(6):1476-1483.https://doi.org/10.1111/1750-3841.14180
30. JingwenBai, Jianqiang Li, Zhiyuan Chen, Xuedong Bai , Zhenyuan Yang , Zitong Wang ,Yu Yang. Antibacterial activity and mechanism of clove essential oil against food borne pathogens. *LWT-food and science technology*. 2022; 173:114219.https://doi.org/10.1016/j.lwt.2022.114249
31. Lijo Thomas, K Anees and V A MuhammedNissar. The economic landscape of clove production in India: challenges and opportunities. *Journal of Spices and Aromatic Crops*.2023; 32 (2): 161-169.https://doi.org/10.25081/josac.2023.v32.i2.8880
32. Humbal BR, Sadariya KA, Prajapati JA, Bhavsar SK, Thaker AM. Anti-inflammatory activity of Syzygium aromaticum (L.) Merrill & Perry oil in carrageenan-induced paw edema in female rats. *Annals of Phytomedicine*. 2019;8(2):167-71. DOI: http://dx.doi.org/10.21276/ap.2019.8.2.21
33. Patil SJ, Surana KR, Mahajan SK. Quantification of active phytoconstituents in ethanolic extract of Menthapiperita by modern analytical tools. *Res. Jr. Agril. Sci.* 2024; 15(3): 615-21. DOI: NA
34. Patil SJ, Surana KR, Mahajan SK. In vitro antimicrobial and antifungal activity of Menthapiperita active phytoconstituents. *Res. Jr. Agril. Sci.* 2023; 14(6): 1875-7. DOI: NA
35. Anees Ahmed Khalil, Ubaidur Rahman, MoazzamRafiq Khan, AmnaSahar , Tariq Mehmood and Muneeb Khan. Essential oil eugenol: sources, extraction techniques and nutraceutical perspectives. *RSC Adv*. 2017; 7:32669-32681.https://doi.org/10.1039/C7RA04803C
36. Dharmadeva S, Galgamuwa LS, Prasadinie C, Kumarasinghe N. In vitro anti-inflammatory activity of Ficusracemosa L. bark using albumin denaturation method. *Ayu*. 2018;39(4):239-242. https://doi.org/10.4103%2Fayu.AYU_27_18
37. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal*. 2016;6(2):71-79.https://doi.org/10.1016/j.jpha.2015.11.005