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A RESEARCH ON: FORMULATION AND EVALUATION OF HERBAL GEL PREPARATION OF ANTIBACTERIAL AND WOUND HEALING ACTIVITY CONTAINING *RHYNCHOSIA ROTHII* LEAVES EXTRACT.

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ABSTRACT

This study focuses on the formulation and evaluation of a herbal gel containing Rhynchosia rothii leaves extract, known for its antibacterial and wound healing properties. Rhynchosia rothii is a plant belonging to the Fabaceae family, traditionally used for its medicinal benefits. The objective was to develop a topical gel that can deliver the extract effectively for wound healing and antibacterial applications. Various concentrations of Carbopol and other excipients were used to formulate the gel. The prepared gels were evaluated for their physical properties, including homogeneity, pH, viscosity, and spreadability. Additionally, the antibacterial activity was assessed against common pathogens, and the wound healing efficacy was tested using an animal model. The results indicated that the herbal gel formulation exhibited significant antibacterial activity and enhanced wound healing, suggesting its potential as a natural alternative for topical wound care.

KEYWORDS

Rhynchosia rothii, Herbal Gel, Antibacterial Activity, Wound Healing, Natural Extracts, Phytomedicine, Fabaceae Family

INTRODUCTION

Gel as a Topical Delivery System.

On, Targeting, and Carriers (2013) state that gels are categorized as semisolids by the United States Pharmacopeia (USP). They can either be a mixture of big organic molecules combined with liquid or a mixture of small inorganic particles. This system can be classified as a genuine two-phase system since the inorganic particles scatter evenly throughout the continuous phase rather than dissolving[1]. Typically, complex organic compounds exist in a dissolved state as pliable chains that are randomly coiled. Regardless of whether they are manufactured or natural, polymers entangle with one other as a result of their random mobility. Systems like this can be seen as single phases, where the organic molecule dissolves in a continuous phase[2,3]. At the micro level, this system can be understood as consisting of two phases: the solvent and the colloidal polymer molecule. This phenomenon occurs due to the peculiar behavior of long molecules in solutions, resulting in a relatively elevated viscosity and the creation of a gel. Gels are utilized as delivery systems for sustained-release medications either via intramuscular injection or implanted into the body. They are also used for topical administration of pharmaceuticals directly to the skin, mucous membranes, or eyes. Gels can also serve as gelatinous capsule shells or simply as gels. Gels are widely recognized for their application on the surface of the body in the personal hygiene and cosmetics sectors. Hydrocortisone-17-valerate and sulconazole nitrate have been effectively included in hydroxypropyl cellulose gels for the treatment of fungal infections, even though they are not stable in conventional emulsion or cream systems[4–6].

Gels are categorized primarily using two techniques based on:

a) Based on nature of colloid phase

i) Inorganic gels ii) Organic gels

b) Based on nature of solvent

i) Aqueous gels ii) Nonaqueous gels[7,8].

Rhynchosia rothii

Rhynchosia is a diverse genus of flowering plants in the *Fabaceae* family, which is also known as the legume or pea family. This genus has multiple species, each possessing distinct traits, habitats, and roles. *Rhynchosia* is a member of the *Fabaceae* family, which is a broad group of flowering plants commonly known as legumes or peas[9]. This genus encompasses a diverse array of species exhibiting distinct traits, distribution patterns, and functions. *Rhynchosia* species have a global distribution, with a specific presence in Africa, Asia, the Americas, and other regions. The range of different species might vary depending on their specific characteristics and level of adaptation to their habitat[10]. *Rhynchosia* plants exhibit a diverse array of sizes and shapes, depending on the species. Their distinctive blooms, often with keel, banner, and wing petals, are a notable attribute shared by many plants in the *fabaceae* family. Typically, a compound leaf consists of three leaflets. Indigenous populations in different regions have employed specific species of *rynchosia* as sustenance and in

traditional medicinal practices. The specific usage may vary depending on local knowledge and cultural practices[11].

CLASSIFICATION:

Kingdom: Plantae

Phylum: Tracheophyta

Order: Fabales

Family: Fabaceae

Genus: Rhynchosia

Species: Rhynchosiarothii Aitch

Common name: Silky Snoutbean

Botanical name: Rhynchosiarothii

Synonyms: Rhynchosia sericea, Rhynchosiamollissima

Figure No 1 *Rhynchosiarothii*

PARTS OF HERB:

1. **Leaves:** Some species of *Rhynchosia* have long been used in herbal medicine.
2. **Roots:** Some legume species, such as *Rhynchosia* species, may have bioactive chemicals in their roots that have therapeutic potential.
3. **Seeds:** Leguminous plants are known to provide edible seeds, some of which may also have therapeutic or cultural value.

4. **Aerial parts:** Depending on the species, different parts of the plant, such as stems, may have uses in traditional medicine or other applications.

Rhynchosia Pharmacological Effects:

- **Antioxidant:** Flavonoids and rosmarinic acid are two substances found in *rhynchosia* that have antioxidant attributes.
- **Wound Healing:** Apigenin compound found in *rhychosia rothii* that have wound healing qualities. Body repairs and regenerates damaged or injured tissue to restore its structure and function
- **Antimicrobial:** *Rhynchosia* has been utilized historically for food preservation due to its antibacterial attributes. It could potentially help hinder the proliferation of fungus and bacteria.
- **Antibacterial:** The antibacterial efficacy of *Rhynchosia* a veloens whole plant ethanol extract was investigated against the pathogens Bacillus subtilis and Staphylococcus aureus.

Application of *Rhynchosia rothii*:

- **Antibacterial Properties:** Bacillus subtilis and Staphylococcus aureus were the pathogens against which the antibacterial activity of *Rhynchosiasu* a veloens whole plant ethanol extract was studied.
- **Anti-Inflammatory:** *Rhynchosia's* constituents have anti-inflammatory qualities. These compounds may have an anti-inflammatory effect on the body, which could be beneficial for ailments including back pain and arthritis.
- **Wound Healing:** Rhynchosia rothii has chemicals with wound-healing qualities that could help prevent further skin infections.
- **Effects of Antioxidants:** Rhynchosia is rich in antioxidants, such as rosemarinic acid. These substances enhance overall health and decrease the likelihood of chronic illnesses by assisting the body in combating free radicals. Rhynchosia contains bioactive compounds that have antibacterial properties, which can be beneficial in treating targeted infections and enhancing the immune system[9,10,12].

MATERIAL AND EQUIPMENTS

Materials:

The medication and substances currently in use research work are given in below table.

Drug: Extract of *Rhynchosia rothii*

Chemicals: All are excipients used of their analytical grade.

Table: Composition of the Batches of Gels

Make-Up	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Rhynchosia Rothi (g)	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**
Carbopol(g)	**1.01**	**1.01**	**1.01**	**1.01**	**1.15**	**1.15**	**1.15**	**1.15**	**1.6**	**1.6**	**1.6**	**1.6**
DMF% V/W	**-**	**13**	**-**	**-**	**-**	**13**	**-**	**-**	**-**	**13**	**-**	**-**
DMSO% V/W	**-**	**-**	**13**	**-**	**-**	**-**	**13**	**-**	**-**	**-**	**13**	**-**
PEG 400% V/W	**-**	**-**	**-**	**13**	**-**	**-**	**-**	**13**	**-**	**-**	**-**	**13**
Glycerine %V/W	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Triethanolamine e(ml)	**2**	**2**	**2**	**2**	**2.5**	**2.5**	**2.5**	**2.5**	**3**	**3**	**3**	**3**
Methyl paraben (W/V) ml	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Propyl Paraben (W/V) ml	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
Deionized water to produce(gms)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

PHYSICOCHEMICALEVALUATION Determination of Ash Values

Total ash: Approximately 2-4 grams of the air-dried material, which had been pulverized, were precisely measured using a silica crucible that had been previously heated and weighed. The substance was evenly distributed and set on fire by progressively raising the temperature to 500-600°C in a muffle furnace until the residue turns white, indicating the lack of carbon. The crucible was cooled in a desiccator and then measured for weight.

Ash insoluble in acid: To the crucible containing the entire ash, 25 ml of hydrochloric acid TS (70 g/l) was added. The crucible was heated at a low temperature for five minutes while it was under a watch glass. The watch glass was rinsed with 5 cc of hot water, and the resultant liquid was added to the crucible. Paper that was free of ash was used to filter the contents of the crucible. After rinsing the insoluble residue on ash-free filter paper with hot water, the liquid that passed through the filter was neither basic nor acidic. The first crucible held the filter paper containing the insoluble particles. After that, it was cooked to a constant weight and dried on a hot plate. The residual material was quickly weighed after cooling in the proper desiccator for thirty minutes. The amount of ash that was insoluble in acid was calculated as the milligrams per gram of air-dried material[13].

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Microbial Contamination Preparation of Plant Material for the Test

Each sample, weighing ten grams, was mechanically homogenized in 100ml of buffered lactone broth to assess the presence of E.coli, Salmonella spp., and the total aerobic count. For the assessment of Pseudomonas aeruginosa and Staphylococcus aureus, each sample was mechanically homogenized with 10 grams in 100ml of buffered sodium chloride and peptone solution.

Evaluation of Microbial Contamination

The sample was introduced into MacConkey broth and placed in an incubator at a temperature range of 43-45°C for a period of 18 to 24 hours in order to assess the presence of E.coli. Salmonella spp was introduced into MacConkey broth and placed in an incubator at a temperature range of 35-37°C for a period of 5-24 hours. Pseudomonas aeruginosa and Staphylococcus aureus were introduced into 100ml of soyabean-casein digest medium and incubated at a temperature range of 35-37°C for a period of 24-48 hours. The plates were positioned on a colony counter to ascertain the quantity of colony forming units.

Total viable Aerobic Count

10 milliliters of the sample solution were promptly placed onto two membrane filters and filtered without delay. The membrane underwent three rounds of washing using a buffered sodium chloride-peptone solution. Each membrane filter was placed onto a plate containing casein soybean digest agar. The plates were then incubated for five days at a temperature range of 30350°C to detect the presence of bacteria. The count of colonies was tallied and the density of microorganisms per gram of the tested substance was computed. The microbiological content was calculated as the average of the duplicate measurements[14,15].

Toxic Heavy Metal Analysis (Cadmium, Mercury, and Lead Determination)

The procedure employed to identify the metals present in the plant material is an adaptation of the technique suggested by Chow et al. 107. Concisely, 2 grams of the sample was transferred to a 100 milliliter Nessler tube. Then, 15 milliliters of a 10% nitric acid solution (volume/volume) was added. The mixture was subsequently placed in a water bath at a temperature of 100 degrees Celsius for duration of 3 hours. The examination of mercury involves subjecting the digested solution to cold vapor atomic absorption spectroscopy (AAS) after reducing it with sodium borohydride (NaBH4). The digested sample solutions for cadmium and lead were subjected to two reflux treatments with concentrated HNO3 before the metals were determined using flame AAS. Every sample was tested twice. The quantification thresholds for lead and cadmium will be 2 mg/g and 0.2 mg/g, respectively[16,17].

Preparation of plant extract

By using the Soxhlet extraction method, specific plant samples were extracted. The solvent was then distilled under low pressure in a rotating evaporator until the extract was entirely dry. For every extract, the yield percentage was calculated[18].

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Determination of Antimicrobial Action (Microorganisms used):

The test organisms that were employed were Candida albicans (ATCC10231), Group A.

Streptococcus, Pseudomonas aeruginosa (ATCC9027), Escherichia coli (ATCC2068), and

Staphylococcus aureus (ATCC29737).

Inoculum

In Soybean Casein Broth (SBCB), the bacteria were injected and then incubated for four hours at 35 ± 2°C. To achieve the desired turbidity standard of 1 McFarland, the resulting turbid suspension was diluted with SBCB. Approximately 3.0 × 108 CFU/ml corresponded to this degree of turbidity.

Agar diffusion assay

Modified versions of the agar well diffusion method was applied. The swab was evenly distributed across the whole sterile agar surface to inoculate the Mueller-Hinton agar plates. To ensure uniform dispersion of the inoculum, the process was repeated twice more using streaking, rotating the plate by about 60° each time. Finally, a swab was also performed on the agar's perimeter. The inoculum was allowed to evaporate at room temperature before 6 mm diameter wells were made in the agar. To evaluate the antibacterial activity of each extract, three different

wells were filled with 100 µL of a 4000µg/ml concentration At the same time, nystatin was employed as a positive control for Candida albicans and gentamicin sulfate as a positive control for S. aureus, P. aeruginosa, and E. coli. The positive controls had a value of 1.0 µg/ml. Petroleum ether, ethanol, and sterile distilled water made up the dilution medium for the positive controls. To enable the extract to permeate the agar, the plates were kept at room temperature for one hour. Following that, all of them—aside from C. albicans, which was incubated at a temperature of 29 ± 2°C—were kept in an incubator for a whole day at a temperature of 35 ± 2°C[19].

The potential antimicrobial compound from a selected extract was separated and purified using column fractional separation.

That in the primary comparative antibacterial research, the methanol extract shown substantial efficacy. As a result, a silica gel column was loaded with the methanol extract for chromatography. An effective stepwise gradient of methanol and chloroform at 2:1 and 1:1 ratio was used to separate the column. The antibacterial activity was assessed using Perez et al.'s modified agar well diffusion method, while thin layer chromatography was employed to track the elution process. The test organisms utilized were Pseudomonas aeruginosa (ATCC9027), Group A Streptococcus, Escherichia coli (ATCC2068), and Staphylococcus aureus (ATCC29737). Using a vacuum, equivalent fractions were mixed, condensed, and dehydrated. The most effective antibacterial properties were found in the fourth and fifth fractions, which were isolated using a 1:1 ratio of methanol to chloroform.

TLC

Aluminum sheets coated with silica gel 60 F254 (E.M.Merck, 0.20 mm thickness) were subjected to analytical thin-layer chromatography (TLC). The chromatograms were analyzed using UV light in a UV-viewing cabinet and made visible by applying a 4% solution of phosphomolybdic acid with a little amount of ceric sulfate in 5% H2SO4.

FTIR spectroscopy

Plant compounds can be analyzed using an automatic recording IR spectrophotometer, such as the Thermo-Nicolet Avtar 370 FT-IR Instrument, to get IR spectra. in the solid state, when combined with potassium bromide. The spectral bands observed in the region of observations from 4000 to 667 cm-1 are a result of the vibration of specific bonds or functional groups inside the molecule being analyzed. The identification of functional groups can be accomplished by analyzing their distinctive vibration frequencies, making the infrared (IR) spectrum the most straightforward and dependable method for classifying a chemical.

The absorption spectra of plant ingredients can be recorded in highly diluted solutions compared to a solvent blank (Methanol) using an automated recording UV-VIS spectrophotometer (Systronics Double Beam UV-VIS Spectrophotometer 2201). Measurements are conducted within the wavelength range of 200-400nm. The recorded values of the wavelength corresponding to the peaks and valleys of the absorption spectrum are expressed in nanometers (nm)[20].

FINDING RHYNCHOSIA ROTHII's THE CONCEPTS OF MINIMAL INHIBITORY CONCENTRATION (MIC) AND MINIMAL BACTERICIDAL CONCENTRATION (MBC)

The micro-broth dilution method was used to determine the minimum inhibitory concentration (MIC). Mueller-Hinton broth medium was used to dilute the reconstituted medication twice. For every dilution, ranging from 0.025 µg/ml to 25.6 µg/ml and from 0.025 µg/ml to 1024 µg/ml, two sets of tubes were made. Five times as many cells (cfu) of the test bacterial strain were added to each batch. After that, the cultures were incubated for eighteen hours at 37 °C. Both common strains of bacteria and multidrug-resistant (MDR) species were treated with this particular approach. The medication's most diluted form that showed no discernible growth was determined to be the minimum inhibitory concentration, or MIC[21,22].

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Spectrophotometric Method Development and Validation for *Rhynchosia Rothii* in Gel Formulations Getting the Standard Stock Solution Ready

After carefully weighing one milligram of *Rhynchosia Rothii*, the material was transferred to a 10milliliter volumetric flask. It was dissolved in 10 milliliters of methanol to yield a standard solution containing 100 micrograms per milliliter. To help with the dissolution process, the volumetric flask could, if necessary, be submerged in an ultrasonic bath.

Determination of λ max.

One milliliter of a standard solution having 100µg/ml of concentration was added to a ten-milliliter volumetric flask. After that, the flask was filled to the desired level with the necessary volume of methanol. The absorbance of the resulting solution was measured in the ultraviolet (UV) range (200-400nm) to determine the maximum absorbance after taking into consideration the presence of methanol in that specific range.

Procedure for Plotting Calibration Curve

The primary stock solution was diluted with methanol to form standard solutions, which were intended to serve as working standards within the concentration range of 1–10 µg/ml. The absorbance at a wavelength of 225 nm was measured using a solvent blank as a reference. A calibration curve was then created after that. Furthermore, the absorbance of the sample solution was measured, and the amount of Rhynchosia Rothi present was ascertained using the calibration curve.

Estimation of Rhynchosia Rothii Gel Formulation.

To determine the amount of medication in the gels, 1 gram of each gel formulation was subjected to a 30-minute *rhynchosia Rothii* extraction process using 50 milliliters of methanol. Subsequently, the extract was passed through a 0.45µm-pore-size membrane filter. A pipette was used to remove 2.5 ml of the extract, and 10 ml of diluted fluid remained after that. The resulting solution was then utilized one milliliter at a time. The sample was diluted to a volume of 10 ml using a spectrophotometer that was calibrated to detect absorbance at a wavelength of 225 nm. *Rhynchosia Rothii* concentration was determined using the regression equation derived from the calibration curve. Sometimes High-Performance Liquid Chromatography is the best option.

Preparation of Gels

Carbopol 940 NF50, 114 was used to create *Rhynchosia Rothii* hydrogels (0.1%, w/w) For two hours, the Carbopol resin was soaked in a solution of water, glycerin, and preservative. To achieve a smooth dispersion, the mixture was then agitated at a speed of about 600 rpm using a mechanical stirrer. To allow any trapped air to escape, the stirring was halted and the liquid was left to stand. An aqueous triethanolamine solution was slowly stirred into this produced dispersion. At this point, the enhancers and *Rhynchosia Rothii* were added to the prepared base after being dissolved in ethanol. Glycerine and filtered water are also added to the gel to get the product's.

Evaluation of Rhynchosia Rothi Gel pH:

Digital pH meters were used for direct measurements.

Determination of Viscosity: With a cone and plate viscometer (Digital Rheometer model DV11, Brookfield), the viscosities of the gels that formed were determined. Ten revolutions per minute were produced by a spindle (number 7). Measuring gel samples weighing 0.5 grams involved leaving them at 37 °C for 30 minutes as the test temperature. Viscosities were measured using the mean of three copies in cps.

Spread ability: A spread ability apparatus was used to determine spread ability . The number of seconds needed to separate the slides after adding weight was recorded. Every formulation's spread ability was reported in terms of seconds. The formula was then used to determine spread ability.:

$S=M.L/T$

Where,

S=Spread ability,

M = Weight tide to upper slide ,L=Length of glass slide and
T=Time taken to separate the slide completely from each other.

Extrudability

A piece of extrudability equipment (132) was used to evaluate the substance's extrudability. At the sealed end of the collapsible tube, the formulation was securely compressed. Expelling the formulation until the pressure was released occurred when the cap was removed. In 10 seconds, the weight in grams required to extrude a 0.5 cm wide ribbon of the formulation was determined. Documentation was done on the mean extrusion pressure in grams.

Test for Homogeneity:

Once the gels had solidified in the container, the formulations were visually examined to ensure consistency. In addition, a small amount of each gel is pushed between the thumb and index finger in order to evaluate the consistency uniformity of the gel. To determine the drug's presence in the gels, 1g of each gel formulation was extracted for 30 minutes using 50ml of methanol to contain *Rhynchosia Rothii*. After that, the extract was run through a membrane filter with 0.45µm pore size. To make 10 ml, 2.5 ml was extracted using a pipette. Spectrophotometric measurements were made of the sample's absorbance at 225 nm after it had been diluted from 1 ml to 10 ml. The concentration of *Rhynchosia* was estimated using the regression equation of the calibration curve[23].

***In-vitro* Drug Release Studies:** Using Franz-type diffusion cells, which had cellulose membranes and an effective diffusion surface area of 1.54 cm2, the drug release from gel formulations was studied in vitro. The donor and receptor compartments of the diffusion cell were separated by a 0.45µ cellulose acetate membrane, commonly referred to as a cellophane membrane. To create a sink state, 15 milliliters of a 7.4 pH phosphate buffer solution were supplied to the receiver compartment. One gramme of the vehicle solution containing the test medication was added to the cell's donor compartment. The system was kept at 370°C±0.50°C using a jacket that surrounded the cell and a water bath circulator. It was necessary to stir continuously at 600 rpm for the experiment. For a duration of twelve hours necessary for the experiment was performed in duplicate, and the average cumulative percentage release from three batches was computed. Throughout the course of twelve hours.

PHARMACODYNAMIC EVALUATION OF GELS Wound Infection and Dressing Bacteria Tested

The strains of Pseudomonas aeruginosa (ATCC9027), Group A-Streptococcus, and Staphylococcus aureus (ATCC29737) were applied as test organisms.

In oculum:

Following injection into Soybean Casein Broth (SBCB), the bacteria were incubated at 35 ± 2°C for four hours. The resulting turbidity suspension was diluted with SBCB until it met the 1

McFarland turbidity threshold. This level of turbidity is equivalent to approximately 3.0 × 108 CFU/ml.

An excision wound model was utilized in this study. Six albino rats (Wistar strain) of different weights (150–200 g) and both sexes were randomly assigned to three groups. On the rats' shaved dorsal side, full thickness incisions of 1.5 X 1.5 cm were created using a sterile surgical blade. Wounds were injected with the test organisms at 108 CFU (0.1 ml) between the paraspinus muscle and the thin skin muscle, and the wounds were permitted to infect for 24 hours.

Wound Healing Rate

The percentage of wound closure was calculated using the starting and end areas that were created on glass slides during the testing, as

$$\% \text{ of wound closure} = \frac{\text{Wound area in day 0} - \text{Wound area on day n weight upto10g.}}{\text{Wound area on day}} \times 100$$

RESULTS AND DISCUSSION:

Physicochemical Evaluation: Table 1 presents the results of the physicochemical examination, including the ash value, extractive value, and loss on drying. The report demonstrates that every value is within the range specified in the official monograph.

Table 1: Physicochemical Evaluation (Mean±SD,n=6)

Plant Names & Parts Used	ASH VALUE		EXTRACTIVE VALUE		LOD (%)
	Total Ash percentage	insoluble Acid percentage	soluble Water percentage	soluble Alcohol percentage	
Rhynchosia rothii	6.0±0.08	0.6 ±0.02	215.0±0.02	17.0±0.007	3.0±0.3

Evaluation of Microbial Content

Table 2 reports on the microbial type and microbial contamination in the plant medications.According to the second report, every sample passes the test.

Table 2: Evaluation of Microbial Content (Mean±SD,n=3)

Plant Names & Parts Used	Total Aerobic Organisams (cfu/g)	Total Viable count (cfu/g)			
		Staph. auereus	*E.coli*	*Salmonella*	*Pseudo-monads*
Rhynchosia rothii	$4x10^2$	-	-	-	-

Items containing herbal medicines are often contaminated by mildew, germs, and dust. 105 CFU/g for total aerobic bacteria, 103 CFU/g for yeasts and molds, 103 CFU/g for Enterobacteria and other Gram-negative organisms, and 0 CFU/g for Salmonella and E. Coli are the maximum amounts of bacteria that can be found in a sample. Non-sterile pharmaceutical medicines that contain microbiological contamination may harm users and lose some or all of their therapeutic efficacy. The usage of highly polluted raw materials derived from natural sources has been linked to several viral outbreaks. The microbiological integrity

of pharmaceuticals is affected by the outside environment as well as the caliber of the raw materials utilized in the formulation process. To ensure the proper quality, safety, and efficacy of the final products, manufacturers must ensure that the raw materials they use have the fewest bacteria feasible.

Toxic Heavy Metal Analysis

The metal concentrations in the plant samples are displayed in Table 3. According to the report, every sample that was examined fell under the official monograph's prescribed level.

Table 3: Toxic Heavy Metal Analysis (Mean±SD,n=2)

Name of Plant & Part used	MERCURY	Concentration in µg/g.	
		CADMIUM (LIMIT=0.3 µg/g)	LEAD (LIMIT=10 µg/g)
Rhynchosia Rothii	NIL	0.10±0.02	5.2±0.01

Poisons associated with the presence of heavy metals in medicinal plants have been reported in several Asian, European, and American regions. Throughout their growth, plants have the capacity to absorb heavy metals from the soil, water, and air around them. High amounts of dangerous metals can occur when plants are grown in polluted areas, including next to highways, metal mines, or smelting industries. Additionally, when specific compounds are used in agricultural practices—such as cadmium-containing fertilizers like Rajphose, leadcontaining herbicides, and contaminated irrigation water—higher amounts of contaminants can be found. Human nephrotoxicity can result from prolonged exposure to cadmium, mostly because of abnormalities in tubular reabsorption. Lead can be hazardous to the neurological and renal systems, and because it can cross the placental barrier, it may have negative effects on the developing foetus.

Preparation of plant extract

Particular plant samples were extracted using the Soxhlet method. After that, the extract was completely dried out by distilling the solvent in a rotary evaporator at low pressure. A percentage yield was computed for each extract (Table 4).

Table 4: Soxhlet extraction of plants studied with three different solvents

Botanical source	Organ tested	Solvent used	Weight of samples taken (gms)	Time of extraction (hrs)	Time of distillation (hrs)	Yield (%w / w)	Colour and consistency of extract
Rhynchosia rothii	Bulb	Aqueous	230	4.5	7	20.8	Pale yellow powder

DETERMINATION OF ANTIMICROBIAL ACTION

Table 5: Antimicrobial activity of plants studied

Botanical Name	Family	Organ tested	Yield(% W/W)	ZONE of Inhibition in mm			
				Staphylococcus Aurous	Escherichia coli	Psudeomonous	Candida
Rhynchosia rothii	Fabaceae	BULB	23.8	10.1+0.0	7.6+0.00	11.3+0.0	0

The preliminary screening and comparison of plant extracts are the subjects of this investigation. They have been tested against a few common human diseases, including S. aureus, P. aeruginosa, E. coli, and C. albicans. 87 percent of the plant extracts tested had antibacterial action against S. aureus. Coliform E.

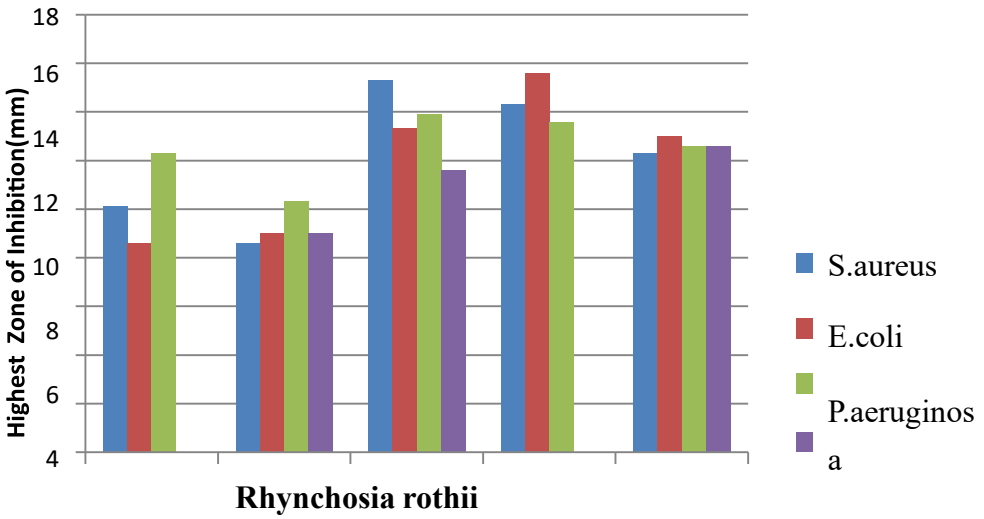


Fig 1: Antimicrobial Activity

Infrared Spectroscopy (IR)

The isolated metabolite's infrared spectrum revealed two carbonyl group absorption bands at 1624 (chelated CO) and 1737 (ester-CO) cm-1, as well as a prominent hydroxyl group absorption band at 3435 cm-1.

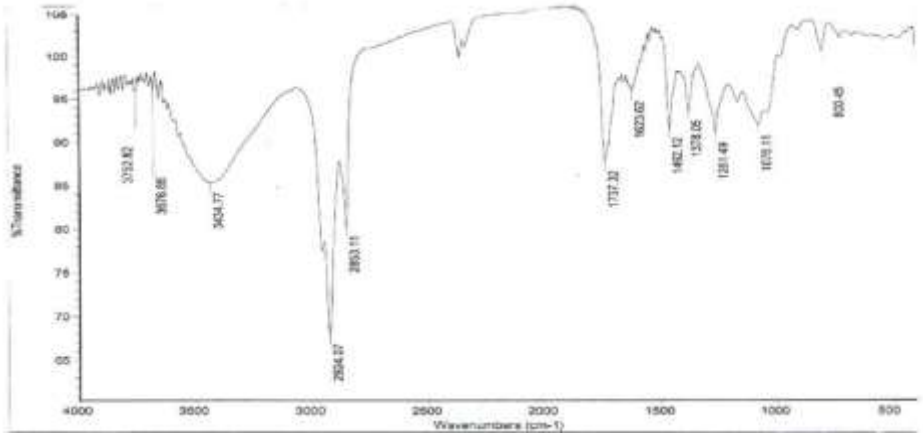


Figure No8.3: Infrared Spectroscopy

Table 6: IR Signals of a separate substance

OH	**3435cm^{-1}**
CO(chelated)	**1624cm^{-1}**
CO(ester)	**1737cm^{-1}**

8.4.1.2. Ultra Violet Spectroscopy (UV)

UV absorption peaking at **225 nm**

The antibacterial metabolite *Rhynchosia rothii* was found by matching these spectra with those documented in the literature.

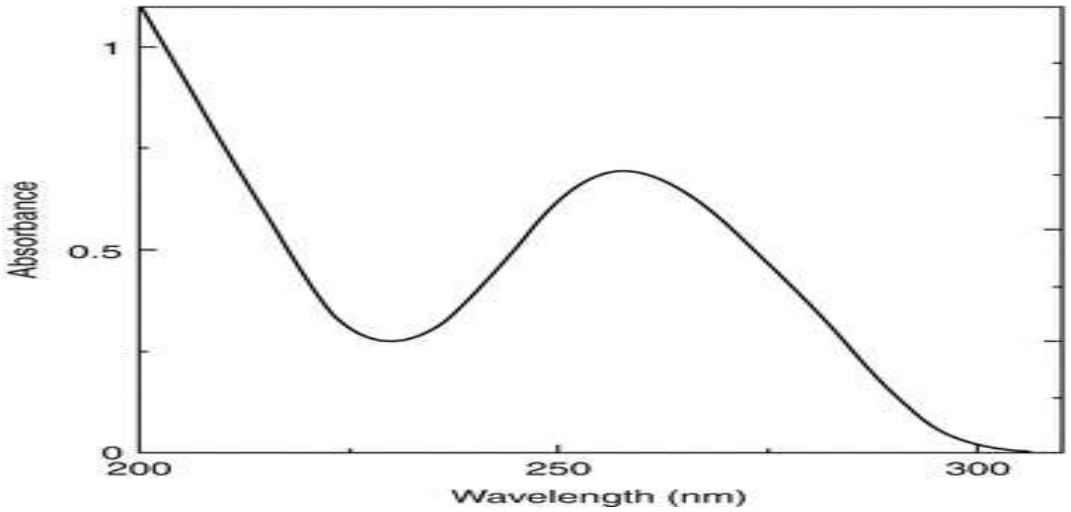


Fig:8.4 Ultra Violet Spectra of Isolated Compound (UV)

Rhynchosia rothii: Minimal Bactericidal Concentration (MBC) and Minimal Inhibitory Concentration (MIC) determination

The MIC was determined using the micro-broth dilution method. The highest dilution at which no appreciable growth was observed was the medication's lowest concentration, or MIC. MBC was determined by subculturing the test dilution onto a fresh, drug-free solid medium and letting it incubate for a further 18 to 24 hours. The lowest concentration, or maximum dilution, that failed to result in even a single bacterial colony on a solid medium was known as MBC. The results of our study showed that Rhynchosia rothii inhibited Grampositive bacteria more effectively than Gram-negative bacteria, as shown in Table 8.6.

Table 7: MIC and MBC Values

Bacterial strains	**MIC** Rhynchosia rothii **(µg/ml) ±SD**	**MBC** Rhynchosia rothii **(µg/ml) ±SD**	**MIC Standard drug-Gentamycine**
S. aureus	0.9±0.001	1.5±0.00	3±0.001
P. aeruginosa	3±0.00	3.5±0.001	1±0.001
E. coli	2±0.003	2±0.002	4±0.00
Group A strep	2±0.001	2±0.001	4±0.001

Table 8: Validation parameters for UV method of analysis of Rhynchosia rothii

Validation parameters	***Rhynchosia rothii***
lamda max (λmax).	225nm
Regression equation(y=a+bx)	y=0.134+6.98x
Slope.(S.E.) a	6.98(0.0412)
Y- intercept.(S.E.) a	0.134(0.0012)
Range(µg/ml)	1-10
Coefficient of correlation (r)	0.999
Correlation coefficient (*r*2)	0.998
Limit of detection (µg/ml)	0.57
Limit of quantification (µg/ml)	1.7

a=Standard error of mean

Table 9: Accuracy and precision data for the developed method(n=6)

Levels	**Estimated concentration by proposed method(µg/ml)a**		**Mean % Recovery ±S.D**	**Accuracy (%)b**
	Mean±S.D	**%R.S.D**		
LC(4 µg/ml)	4.01±0.3368	0.842	100.25±0.257	0.025
IC(5 µg/ml)	4.98±0.3696	0.738	99.6±0.498	-0.4
HC(6 µg/ml)	5.99±0.5487	0.915	99.83±0.392	-0.16

a. Estimated concentration of Rhynchosia rothii was calculated by linear regression equation.

b. Accuracy is given in %relative error

(=100x(predicted concentration-nominal concentration)/Nominal concentration)

Table 10: Standard Addition of *Rhynchosia rothii* for Accuracy(n=6).

Drug in formulation (µg/ml)	Pure drug added (µg/ml)	Total drug found by proposed method(µg/ml)	Total drug found by HPLC(µg/ml)	%Recovery	%RSD
5	0	5.02	5.04	100.40	0.915
5	1	5.89	6.03	98.90	1.010
5	2	7.09	7.09	100.67	0.804
5	3	8.04	8.02	100.20	0.674

Table 11: System Precision Study/Stability Profile (n=6)

Sr. No.	Concentration (µg/ml)	Absorbance at 225 nm at time intervals in minutes Mean±SD				
		0	15	30	45	60
1	4	0.554±0.001	0.553±0.003	0.556±0.005	0.554±0.003	0.555±0.001
2	5	0.710±0.001	0.709±0.002	0.710±0.004	0.7108±0.001	0.711±0.002
3	6	0.865±0.001	0.864±0.003	0.865±0.003	0.866±0.002	0.864±0.002

DEVELOPMENT AND EVALUATION OF RHYNCHOSIA ROTHII GEL FORMULATION

The created gel exhibited high homogeneity and was free of lumps. The pH of all the formulations was determined to be between 6.5 and 6.9, indicating that they were suitable for topical application. It was discovered that the created gel formulations' rheological qualities, such as viscosity, spreadability, and extrudability, were comparable to those of the commercial gel.

Table 12: Physicochemical Evaluation of Gels (Mean±SD,n=6)

Formulation code	Drug content ±SD	Extrudeability, g±SD	pH ±SD	Homo-genicity	Viscosity in cp at 10 (RPM) ±SD	Sprea-Dability (gm.cm/sec)±SD
C1	99.1 ±0.04	254 ±0.2	6.9 ±0.00	Homogenous	85610 ±0.00	6.3 ±0.5
C2	99.1 ±0.02	243 ±0.7	6.9 ±0.00	Homogenous	85646 ±0.00	6.1 ±0.7
C3	98.9 ±0.02	254 ±0.3	6.9 ± 0.00	Homogenous	85645 ±0.00	6.2 ±0.5

Irritancy test

Gel formulations' medication contents ranged from 98.7-99.6%, indicating content homogeneity. Rhynchosia rothii gel was shown in a main skin irritation investigation to not be a primary irritant of the skin.

Table13: Dermal Observations

Sr no	Formulation	Results
1.	C1	Non-irritancy
2.	C2	Non-irritancy
3.	C3	Non-irritancy

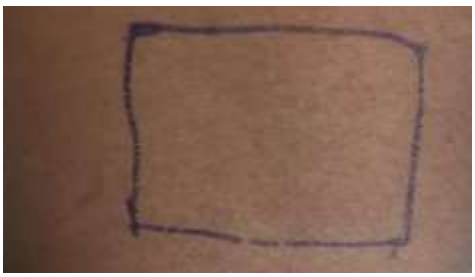
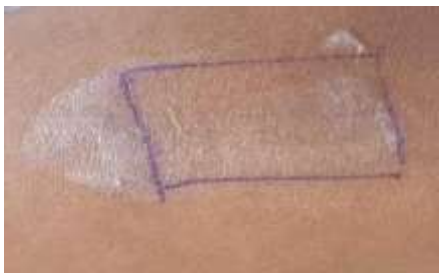
Before apply on skin	After Apply on skin
	

Fig 2: Skin Irritancy Test

Table 14:Evaluation of Primary Irritation Index (PII)

Index	Evaluation
0.00	No irritation
0.02 to 0.99	Irritation barely perceptible
1.00 to 1.99	Slight irritation
2.00 to 2.99	Mild irritation
3.00 to 5.99	Moderate irritation
6.00 to 8.00	Severe irritation

During the stability trials, it was found that the drug contents of all the formulations were in good agreement with the theoretical value. The drug's stability in those forms is demonstrated by the consistent appearance and the absence of a noticeable time-dependent fluctuation in pH and viscosity for all formulations.

Table 15: Every formulation, demonstrates the medication's stability in each formulation.

Code of Formulation	Flux (µgcm^{-2}h^{-1})	Diffusivity (µ^{2}h$^{-1/2}$)	Slope(n)	Ratio of Enhancement (ER)
C1	257.96	856.71	0.99	1.00
C2	505.69	1734.22	1.07	1.96
C3	453.56	1568.00	1.00	1.76

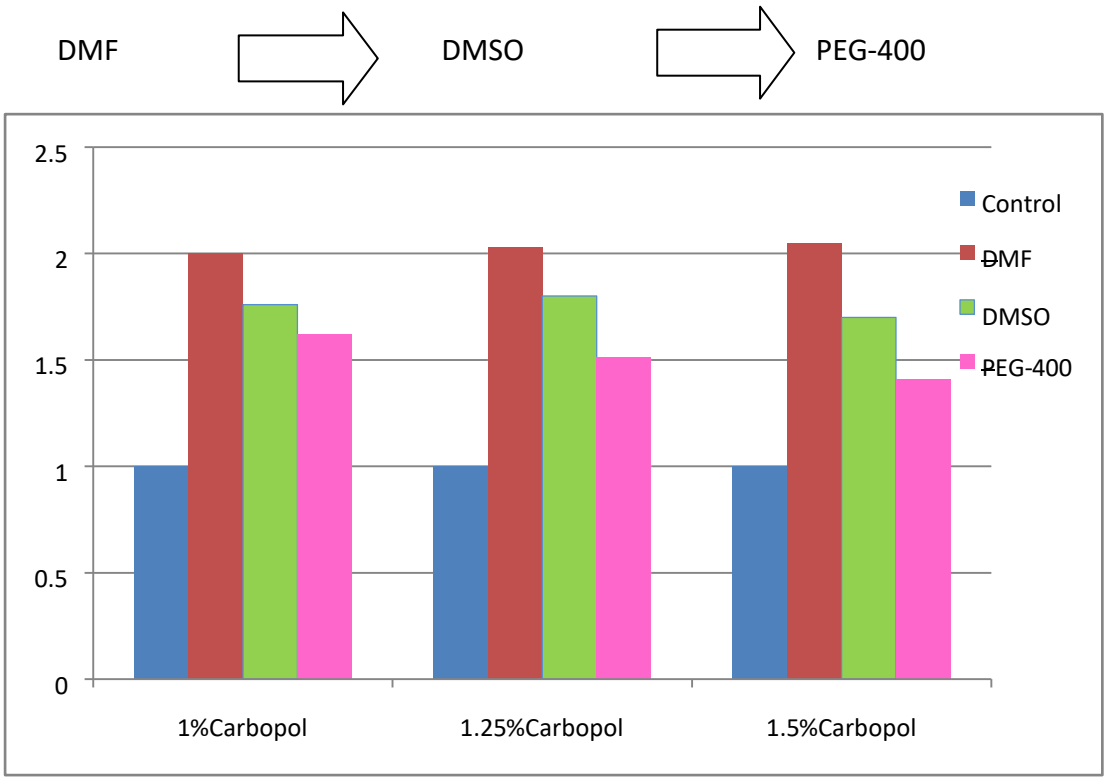


FIG 3: Gel Formulations Based on Carbopol and Their Enhancement Ratio (ER)

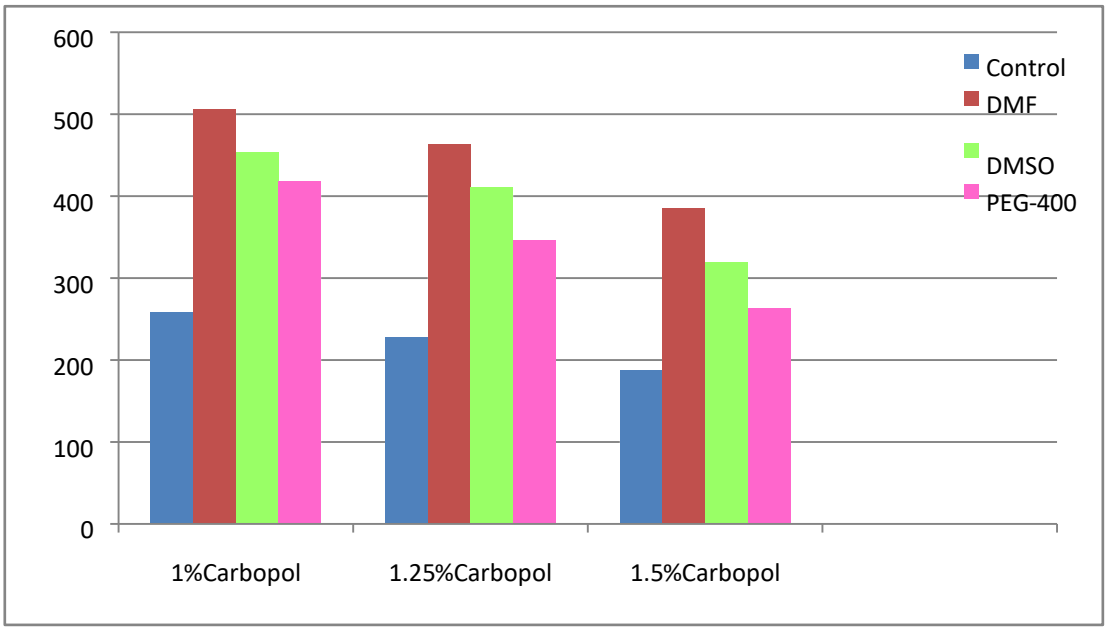


FIG 4: Flux (µgcm^{-2}h^{-1}) Value of Carbopol Based Gel Formulations

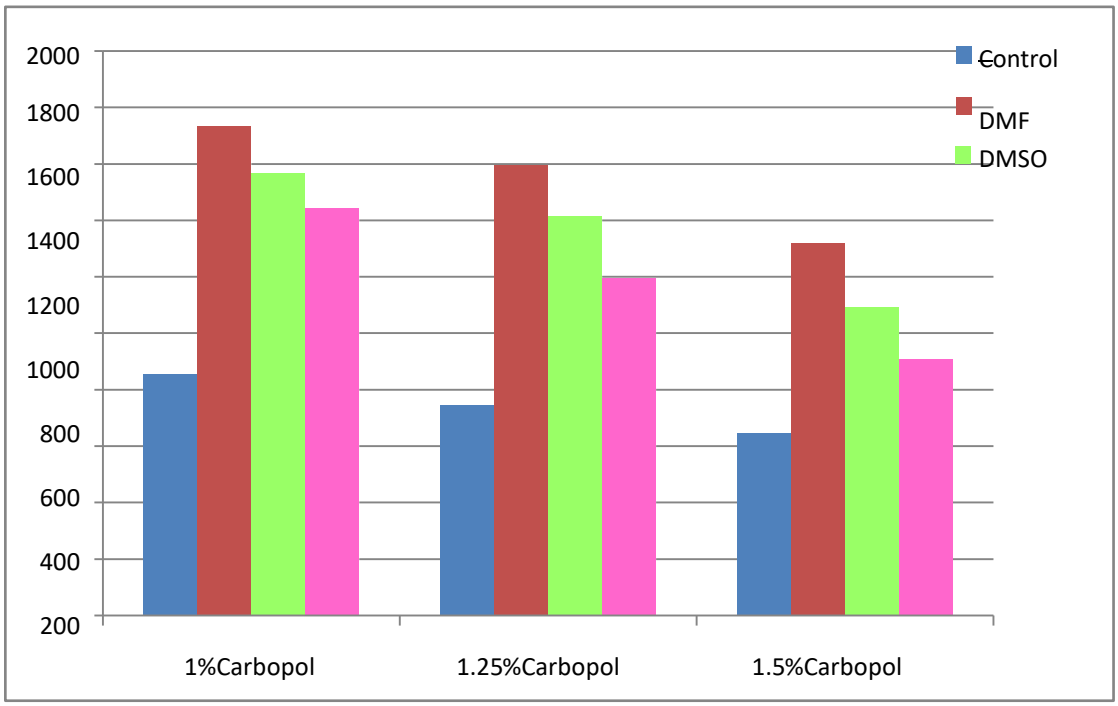


FIG 5: Diffusivity (µgcm-2h-1/2) Carbopol-Based Gel Formulations: Their Relevance

It was discovered that the drug molecules' diffusivity and flux decreased in the previously stated order. This is most likely related to the ensuing decline in the permeation enhancers' capacity to raise drug solubility, or thermodynamic activity.

It is easy to compare the changes in the flux and diffusivity values since the formulations under research were divided into three groups, each with polymer concentrations of 1%, 1.5%, and 1.5%w/w of carbopol and the same concentration of various enhancers of penetration with a control. Based on the fact that Flux values drop with increasing polymer concentration and that the nature of permeation enhancers changes, it is reasonable to assume that the Flux would be considerable at low polymer concentrations. The permeation enhancers can be arranged in the

DMF> DMSO> PEG-400.

Wound Healing Rate

When compared to the untreated group, the treated wounds were shown to contract at the highest rate. On day eight, there was a significant difference ($p<0.05$) in the percentage of wound contraction between the treated and untreated groups. In 16 days, 98% wound healing was possible for the Rhynchosia rothii-treated groups, while 96% wound closure was possible for the standard drug-treated groups. On the other hand, untreated control wounds healed more slowly, and after 16 days, only roughly 59% of the wounds were closed.According to these

results, the untreated control group's wound healing was noticeably slower than that of the Rhynchosia rothii and Neosporin treated groups.

Bacteriological examinations

On day 4, the administration of Rhynchosia rothii gel reduced the total bacterial count in the infected wound from 3×108 to 2.6×104 CFU/g, and a comparable reduction was observed in the control group. The control group captured images between 2.5×108 and 3.9×108.When compared to the control group, the Rhynchosia rothii ($p<0.05$) and Neosporin treated groups ($p<0.05$) had a considerably lower bacterial count on days 4 and 8. By day 12, both the Rhynchosia rothii treated group and the control group had zero bacteria. At all time points after the 12-day mark, there was no statistically significant difference observed between the Rhynchosia rothii and Neosporin-treated groups ($p>0.05$), indicating that the gel's ability to manage wound infections is on par with commercially available standard formulations th

Table 16: Rate of Wound Healing on Infected Albino Rat Model(Mean ± SD, n=6,N=3)

Parameters	Day4	Day8	Day12	Day16
% Wound closure				
Control	15.21±2.1	26.71±2.6	45.32±3.3	59.29±2.8
Test	58.45±2.2	79.76±2.4	89.32±3.2	99.42±3.1
Standard	57.33±1.9	80.21±1.6	89.56±2.2	98.01±2.7
Total bacterial Count (CFU/g)				
Control	3.9±0.6×109	4.7±0.5×106	4.1±0.5×104	5.2±0.4×103
Test	2.5±0.4×104	2.6±0.3×102	0	0
Standard	2.2±0.2×104	1.9±0.2×102	0	0

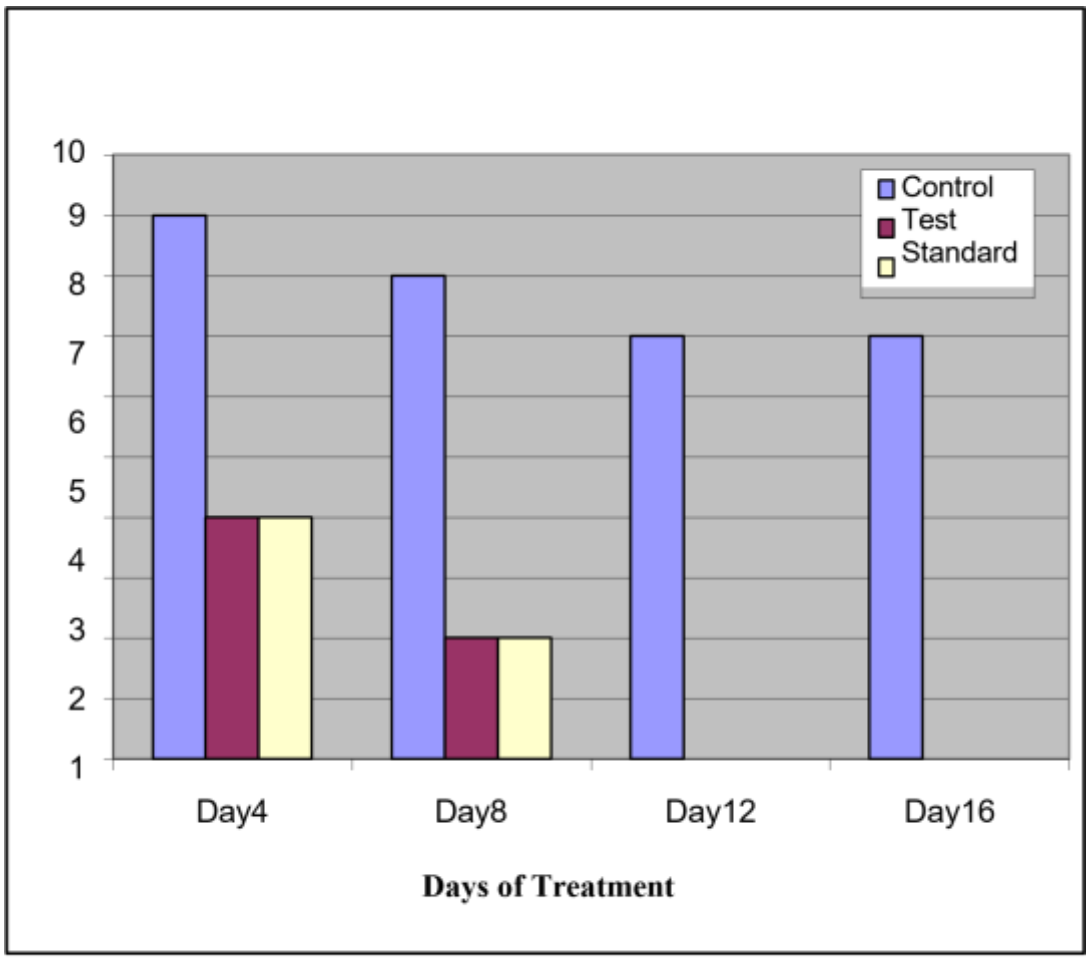


Fig 6: Effect on total Bacterial Count

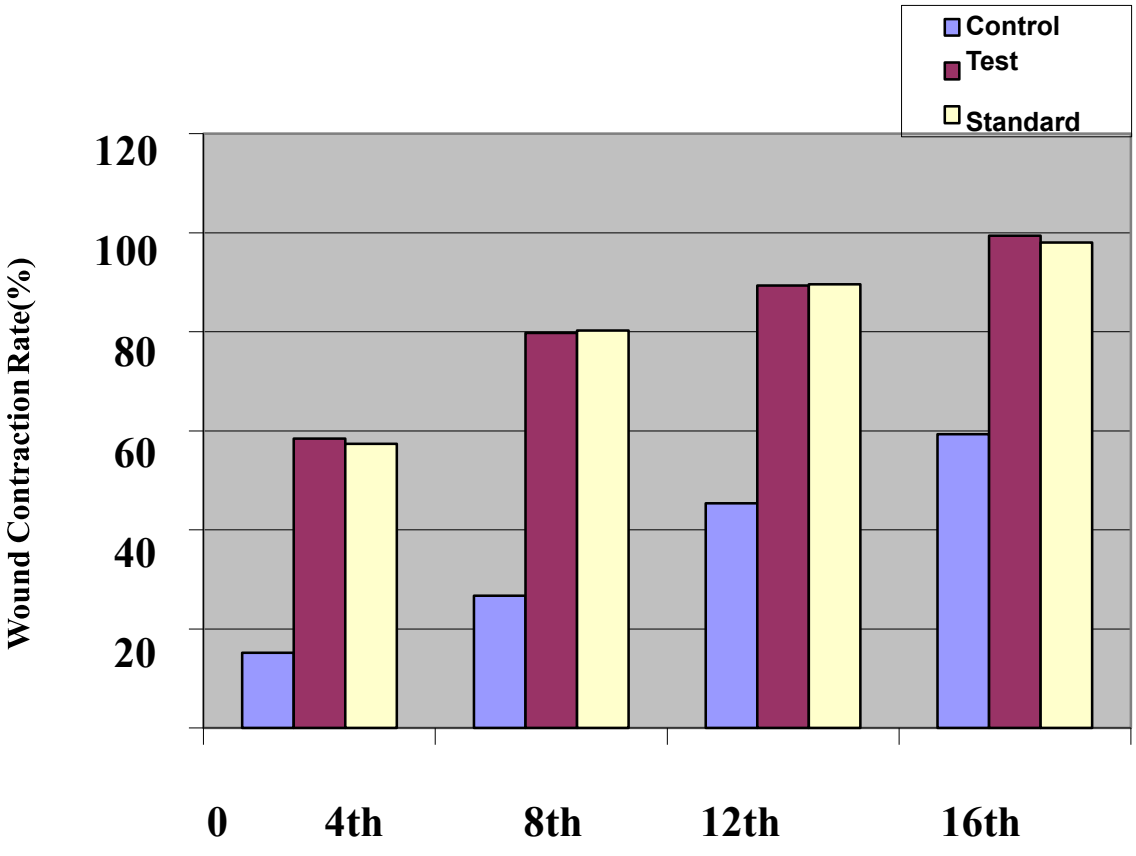


Fig 7: Wound Healing Rate

CONCLUSION:

The formulated herbal gel containing Rhynchosia rothii leaves extract demonstrated significant antibacterial and wound healing activities. The evaluation of physical properties such as homogeneity, pH, viscosity, and spreadability confirmed the suitability of the gel for topical application. The antibacterial studies showed that the gel was effective against common pathogens, indicating its potential as a natural antibacterial agent. Additionally, the wound healing studies conducted on an animal model revealed that the gel accelerated the healing process, likely due to the bioactive compounds present in the Rhynchosia rothii extract. These findings suggest that the herbal gel can be a promising natural alternative for treating wounds and preventing infections, offering an effective and safe option for topical therapy. Further studies are recommended to explore the long-term stability and clinical efficacy of the gel.

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