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Formulation and Evaluation of Gel by using Natural Gelling Agent

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ABSTRACT

Natural products are the source of synthetic and traditional herbal medicine. Plant extracts have been in spot light for their extreme ability to natural gel including other gel preparation .

Natural formulation gel has gained a drastic importance in the field of natural formulation. The present study deals with an environment friendly and formulation and evaluation gel by using natural gelling agents. The formulation gel using *Annona squamosa* leaf extract was determined by FTIR, and DSC. Antibacterial efficacy of gel was also investigated by disc diffusion method and it was found that the antibacterial activity of natural formulation gel is impressive in hampering the growth of *E. coli* and Bacillus subtilis

Key words: *Annona squamosa* leaf extract, carbopol 940 and Xantham gum., Antimicrobial, Disc diffusion.

INTRODUCTION

Medicinal plants have been a major source of cure for human diseases since time immemorial. It is no wonder that the world's one-fourth population i.e. 1.42 billion people, are dependent on traditional medicines for the treatment of various ailments. It is documented that most of the world's population has taken in traditional medicine, particularly plant drug for the primary health care. as therapeutic remedies in many developing countries. Plants are a rich source of secondary metabolites with interesting biological activities. Secondary metabolites like flavonoids, phenols, phenolic glycosides, saponins and glycosides are an important source with a variety of structural arrangements and properties.[3] The improvement of health after herbal treatment, low cost of the drugs, non-availability of synthetic rugs particularly in the rural areas, where available were either fake or expired drugs and in some cases the people are more accustomed to and comfortable with traditional healing.[4] *Annona squamosa (L.)* is a small ever green tree is cultivated throughout India for its fruits, different parts of *Annona squamosa (L.)* are used in folkloric medicine for the treatment of various diseases.[5] This plant is commonly called custard apple in english & sharifa in hindi & sitaphalam in telgu in india. [6] In Indian folk medicine, various species of *Annona* have been used as vermifuges, anti-inflammatory agents, in wound healing, as antimalarial agents and in the treatment of diarrhoea and dysentery.[7] The bark of the plant *Annona squamosa* is a powerful astringent and given as tonic. The leaves of *Annona squamosa* has been used as an anti-inflammatory agent in wound healing, anti-anxiety, anti-stress, anti-mutagenic, and spasmolytic agent. Leaf and stem extract shows inotropic, positive chronotropic and spasmolytic activities. The plant is reported tocontain acetogenins mainly *cis*- and *trans*-isomurisolenin annoreticuin, bullatacin, squamosine, rolliniastatin, reticullacinone, rolliniastatin-2, molvizarin

^{14}-hydroxy-25-deoxy-rollinicin. Reticulatacin and kauranediterpenes were also identified from the bark of the plant. Other terpene ssuch as spathenelol, muurolene, copaene and eudesmol were reported in the previous literature.

The main objectives of this study was to formulation and evaluvation natural gel by using aqueous extract of *Annona squamosa*. to characterize the gel by using FT-IR, DSC, and also to analyze antimicrobial properties against Gram-positive and Gram-negative bacteria.

MATERIALS AND METHOD

Collection and authentication of plant materials

The leaves of *Annona Squamosa* were collected from S.R.T.M. University campus and authenticated in School of Life science, S.R.T.M.U. Nanded.

Preparation of plant extract: From Annona Squamosa leaves

100gm of Annona squamosa leaves are washed with distilled water dried and chopped into small pieces and grinded to form powder .The powder was subjected to extraction using methanol as solvent in soxhlet apparatus for 6hrs at 400°C the resultant extract dried to get powder. The powdered extract was used for study.

- **Method of preparation**

A) Preparation of leaves extract *Annona squamosa*

The collected fresh leaves of *Annona squamosa* were washed with water and dried in shade. After drying plant leaves were coarsely powderd and kept in well closed container. About 100gm of coarse powder of leaf was weighed and soaked in 500 ml of ethanol and left for maceration for about 4-5 days. After maceration the extract was concentrated and used for further formulations.

Phytochemical screening test:

All the preliminary phytochemical tests *Annona Squamosa L* were performed.

1) Detection of alkaloids

Methanol extracts were dissolved individually in dil. hydrochloric acid (10 ml) and then filtered and referred as test solution.

- **Mayer's test:** To 1 ml of test solution of methanolic extract added few drops of Mayer's reagent (Potassium Mercuric Iodide Solution). Cream precipitate indicated the presence of alkaloids.
- **Wagner's test:** To 1 ml of test solution of methanolic extract added equal volumes of Wagner's reagent (Iodine in Potassium Iodide). Reddish precipitate indicated the presence of alkaloids.
- **Hager's test:** To 2 ml of test solution of methanolic extract added few drops of Hager's reagent (Saturated Picric Acid Solution). Bright yellow precipitate indicated the presence of alkaloids**.**
- **Dragendroff's test:** To 1 ml of test solution of methanolic extract added few drops of Dragendroff's reagent (Solution of Potassium Bismuth Iodide). Formation of red precipitate indicated the presence of alkaloids.
- **2) Detection of glycosides:** Methanol extracts were treated individually with dil. hydrochloric acid (10 ml), and then filtered and referred as test solution.
  - **Modified Borntrager's test:** Methanol extract were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicated the presence of anthranol glycosides.
  - **Legal's test:** Methanol extract were treated with sodium nitropruside in pyridine and NaOH. Formation of pink to blood red color indicated the presence of cardiac glycosides.
  - **Keller-killani test:** Methanol extrac (50 mg) were treated with 2 ml of glacial acetic acid containing one drop of 5% ferric chloride, followed by addition of 1 ml of concentrated sulphuric acid. A brown ring at the interface is the feature of cardenolidedeoxy sugar. Appearance of the violet ring below the brown ring and greenish ring in acetic acid layer indicated the presence of cardiac glycoside.

3) Detection of Saponins

- **Froth test:** Methanol extract were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicated the presence of saponins.
- **Foam test:** 0.5 gm of Methanol extract were shaken with 2 ml of water. If foam produced and persists for 10 minutes it indicates the presence of saponins.

4) Detection of phenols

- **Ferric chloride test:** Methanolic extract were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenols.

5) Detection of flavonoids

- **Alkaline reagent test:** Methanol extract were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.
- **Lead acetate test:** Methanol extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicated the presence of flavonoids.

6) Detection of proteins and amino acids

- **Xanthoproteic test:** Methanol extract were treated with few drops of conc. nitric acid. Formation of yellow color indicated the presence of proteins.

- **Ninhydrin test:** To Methanol extract 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicated the presence of amino acid.

7) Detection of phytosterols

- **Salkowski's test:** Methanol extract were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenes.
- **Libermann Burchard's test:** Methanol extract were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride then boiled and cooled. After that conc. Sulphuric acid (0.5 ml) was added. Formation of brown ring at the junction indicated the presence of phytosterols.

8) Detection of tannins

- **Ferric chloride test:** Methanol extract were dissolved in 5 ml of distilled water and few drops of 5% ferric chloride were added. Bluish black color indicated the presence of tannins.

9) Detection of carbohydrates

Methanol extract were dissolved individually in 5 ml of distilled water then filtered and referred as test solution.

- **Benedict's test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicated the presence of reducing sugars.
- **Molisch's test:** Filtrates were treated with 2 drops of alcoholic a-naphthol solution in a test tube. Formation of the violet ring at the junction indicated the presence of carbohydrates.
- **Formulation of Placebo Gel (Control formulation):**

For the preparation of gel formulation, firstly take carbopol 940 and Xantham gum which was then dispersed in distilled water along with methyl paraben, propyl paraben and glycerine kept for overnight. Take the leaves extract of *Annona squamosa* in propylene glycol which was then added in polymer dispersion. Remaining quantity of water was then added and neutralized to pH 7 with triethanolamine by constant stirring for 10 minutes.

Table 1: Control batch formulation of herbal gels

Ingredients	**Quantity**
Carbopol 940	1.0 gm
Xanthum gum	1.0 gm
Propylene glycol	10 ml
Methyl paraben (0.5 %)	0.2 ml
Propyl paraben (0.2 %)	0.1 ml
Glycerine	1 ml
Triethanolamine (to maintain pH)	q.s.
Distilled water	100 ml

A) **Development of Herbal gel formulations**

For the preparation of gel formulation, firstly take carbopol 940 which was then dispersed in distilled water then methyl paraben, propyl paraben and glycerine were added and kept for overnight. Take the leaf extract of *Annona squamosa* in propylene glycol which was then added in polymer dispersion. Remaining quantity of water was then added and neutralized to pH 7 with triethanolamine by constant stirring.

Table 2: Development of Herbal gel formulations

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**
Annona squamosa leaf Extract	0.5 gm	1.0 gm	1.5 gm	2.5 gm	3.0 gm
Carbopol 940	1.0 gm	1.0 gm	1.0gm	1.0gm	1.0 gm
Xantham gum	1.0 gm	1.0 gm	1.0 gm	1.0 gm	1.0 gm
Propylene glycol	10 ml	10 ml	10 ml	10 ml	10 ml
Methyl paraben (0.5 %)	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Propyl paraben (0.2 %)	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Glycerine	1 ml	1 ml	1 ml	1 ml	1 ml
Triethanolamine (to adjust pH)	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water	100 ml	100 ml	100 ml	100 ml	100 ml

Evaluation of Herbal gels:

Physical evaluation:

All the formulated herbal gels were checked for colour and homogeneity by visual observation.

pH:

The pH of all the formulated herbal gels was measured by using digital pH meter.

Viscosity:

Viscosity of herbal gels was determined by using Brookfield rotational viscometer at 100 rpm using spindle no.64.

Spreadability:

The spreadability of gel formulations was determined by measuring the spreading diameter of 1g of gel between two horizontal plates.

Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powders of different solvent extracts of each plant material were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}

Differential Scanning Calorimetry Analysis:

The DSC was used to measure the occurrence of exothermic or endothermic changes with increase in temperature. The DSC because of its sensitivity and accuracy has been extensively used to study the phase transition of polymer. Differential Scanning Calorimetry (DSC) measures the temperature and heat in the material. It determines time function and temperature in a controlled atmosphere. These measurements provide quantitative and qualitative information about physical and chemical changes that involve during endothermic or exothermic processes, or changes in heat capacity. The onset, peak and conclusion temperatures of base transition were observed to be moderate. The knowledge of glass transition temperature is essential in production processes and storage glass transition temperature is affected by moisture and other additives, facilitating conversion to the rubbery state and hence fasciltating crystallization through molecular rearrangement.

Procedure:

To carry out DSC testing a required quantity of sample was taken. Then this sample was placed in the pan. This pan is carefully handled by using forceps. This was covered by using cap and pressed well. After this crucible is kept in the instrument where it comes in between length path and graph is obtained. The x-axis shows the time and energy change is given on y-axis. The DSC of synthesized silver nanoparticles in are shows in figure.

Antimicrobial Study

Microorganisms used for Antimicrobial Activity

The strains used for screening antibacterial activity were performed from S.R.T.U.Nanded. The bacterial strains like *Staphylococcus aureus, Becillus subtilis, Escherichia coli* were obtained from school of life science.

Antibacterial activity:

The antibacterial screening of herbal gels was done by disc diffusion method. The gels were tested against bacterial agents namely *B. subtilus, S. aureus* and *E. coli.* A loopful of the pure bacterial culture was suspended in nutrient broth and incubated for 24 hours. Nutrient agar media was sterilized and poured into petri plates. After solidification, 0.1ml of the inoculum was spread over the agar evenly using a rod. 6mm diameter cavity was prepared and formulated gel is placed in the cavity. A standard antibiotic was used as the control. The inoculated plates are incubated for 24 hours. Later, the zone of inhibition around the disc was measured and recorded.

RESULT AND DISCUSSION:

Phytochemical Analysis

The aqueous leaf extract of *Annona squamosa* is reported to contain glycoside, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols, and amino acids. The aqueous leaf extract of *Annona squamosa* was found to contain major phytochemicals. Phenolic compounds, flavonoid and tannin were present. The preliminary phytochemical tests that the leaves of the plant possess alkaloids, glycosides, flavonoids,[19] tannins etc. The flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving the vascularity. Hence any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibers, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis.[20] Flavonoids[21] and triterpenoids [22] are also known to promote the wound healing property which seems to be responsible for wound contraction and increased rate of epithelialization. Tannins the main component of many plant extract acts as free radical scavenger.[23] *Annona squamosa* has many alkaloids such as glaucin and annonaine in different part of the plants.

Table 3 : Phytochemicals in aqueous leaf extract of *Annona squamosa* .

Sr.No.	**Name of the phytochemical test**	***Annona squamosa* leaf extract**
1	Alkaloid test	+
2	Carbohydrate test	+
3	Saponin test	+
4	Flavonoid test	+
5	Tannins test	+
6	Phenol test	+
7	Glycosides test	+
8	Protein and amino acid test	-

FTIR ANALYSIS:

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the compoud. The FTIR spectrum of annona squamosa leaf extract ,natural geling agent carbapol 940 and xantham gum in case showed the band between 3490-3500 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peak found around 1500-1550 cm^{-1} showed a stretch for C-H bond, peak around 1450-1500 cm^{-1} showed the bond stretch for N-H. C=O showed the bond stretch for 1730-1750 cm^{-1}.

Whereas the stretch for were found around 500-550 cm^{-1} From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that extract to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of in the aqueous medium. Flavanones or terpenoids could be adsorbed on the surface of, possibly by interaction through carbonyl groups or π-electrons in the absence of other strong legating agents in sufficient concentration. The presence of reducing sugars in the solution could be responsible for the reduction of metal ions and formation. These issues can be addressed once the various fractions of the *Annona Squamosa* leaf extract are separated, identified and individually assayed for reduction of the metal ions.

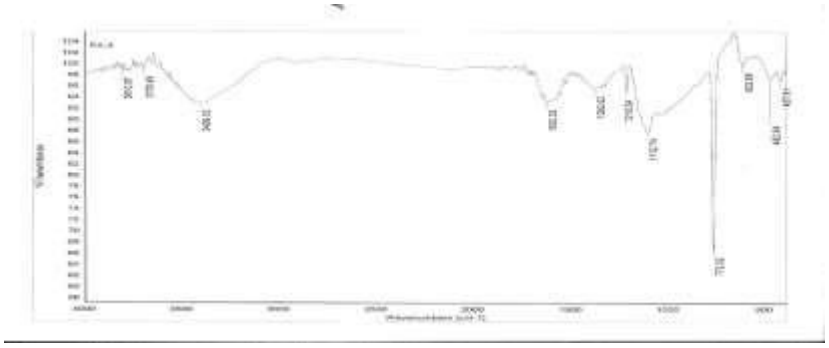

Fig.1: FT-IR of *Annona squamosa* leaf Extract

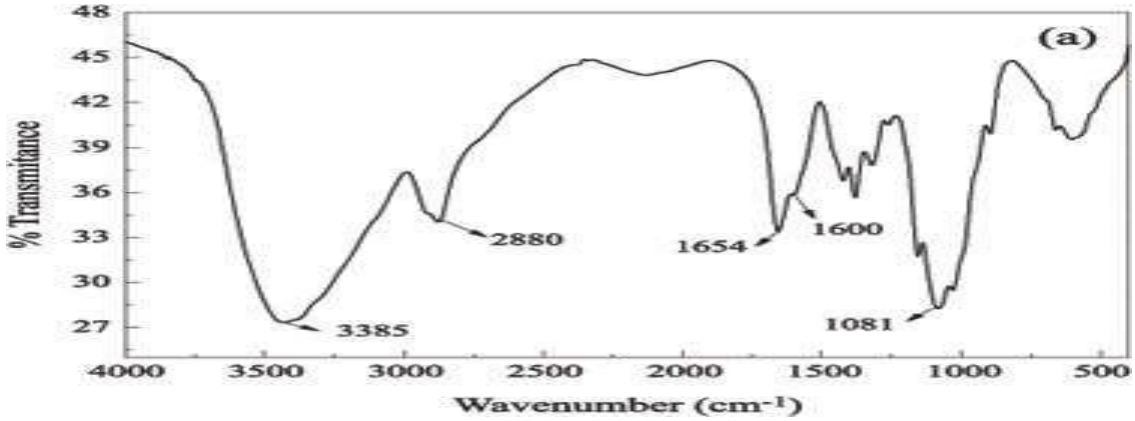

Fig. 2: FT-IR of Carbapol 940

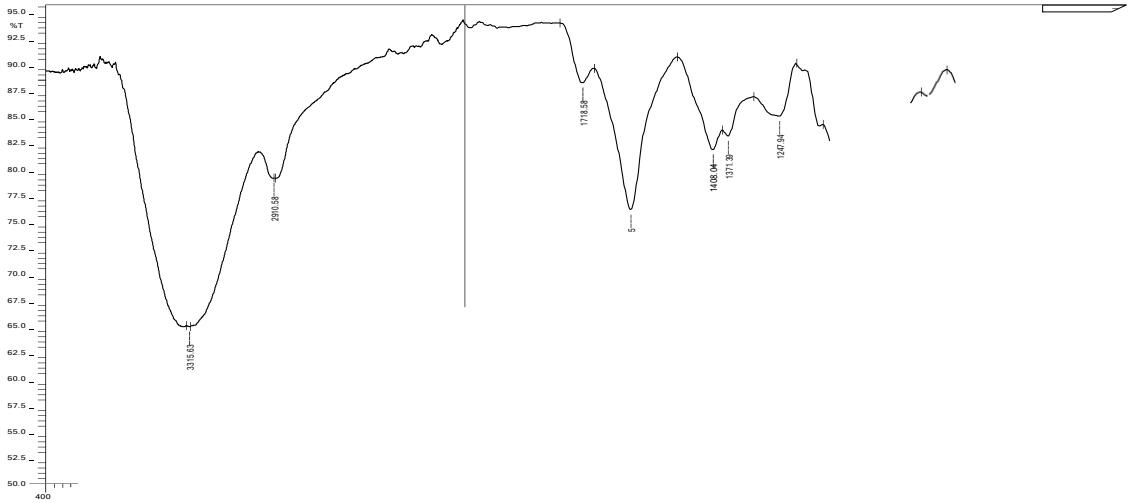

Fig. 3: FT-IR of A*nnona Squamosa* leaf extract +Carbapol 940 +Xntham gum gel formulation

DSC ANALYSIS

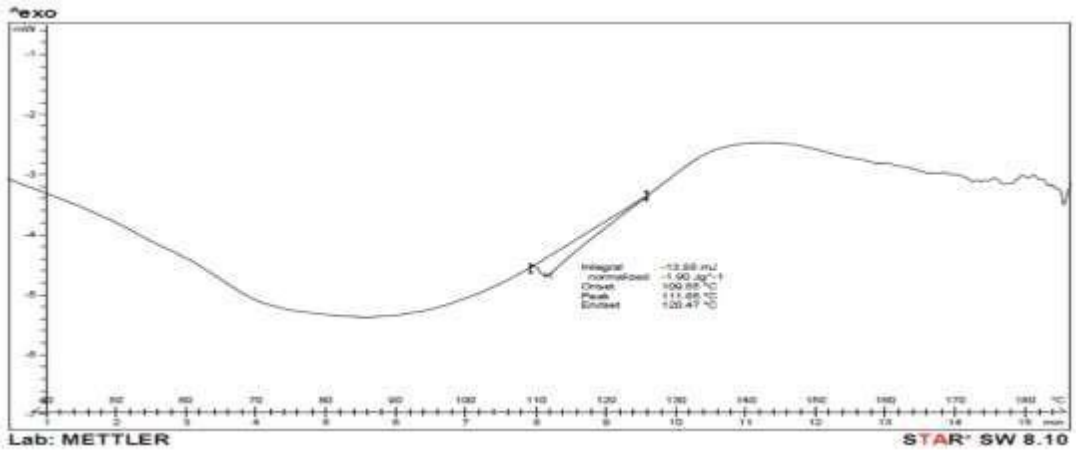

Fig. 4: DSC of A*nnona Squamosa* leaf extract

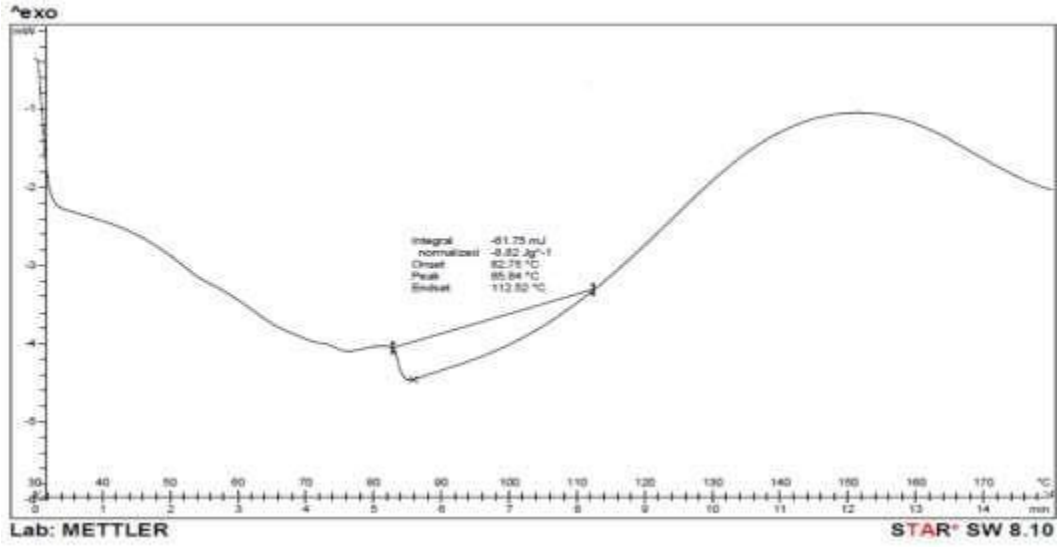

Fig. 5: DSC of A*nnona Squamosa* leaf extract +Carbapol 940 +Xntham gum gel formulation

In the above fig.4 DSC of *Annona Squamosa* plant extract graph shows melting temperature i.e. onset-109.65˚C, Peak-111.65˚C & Endset-120.47˚C which is maximum temperature at which extract degraded. And in the fig.5 DSC graph A*nnona Squamosa* leaf extrac+Carbapol 940 +Xntham gum gel formulation extract shows melting temperature i.e. onset-82.75˚C Peak-85.84˚C & Endset-112.52˚C which is maximum temperature.

Table 4: DSC Parameter of *Annona Squamosa* leaf Extract.

Sr. No.	**Parameter**	***Annona Squamosa* leaf Extract**	**A*nnona Squamosa* leaf extract +Carbapol 940 +Xntham gum gel formulation**
1.	Onset Temperature (˚C)	109.65˚C	82.75˚C
2.	Peak Temperature (˚C)	111.65˚C	85.84˚C
3.	Endset Temperature (˚C)	120.47˚C	112.52˚C

- **Evaluation of prepared formulation**

The topical gels were prepared and subjected to physical evaluations such as appearance, pH, viscosity, spreadability and skin irritation (Table-5). The gels were clear throughout the evaluation. The pH was constant (6.8 – 7.0) and did not produce any type of irritation when applied on the skin. Viscosity, and spreadability were excellent.

Table 5: Evaluation of prepared formulation

Formul ation Code	**Appearance**	**pH**	**Viscosity (cps x 10^3)**	**Spreadability (g.cm/sec)**	**Skin irritation**
F1	Light green	6.8	79	15.65	nil
F2	Light green	6.8	82	13.45	nil
F3	Light green	6.9	85	12.36	nil
F4	Light green	6.8	81	14.60	nil
F5	Dark greenish	7.0	84	13.00	nil

- **Evaluation of Antimicrobial activity**

The results of antibacterial activity of all formulated herbal gels against some pathogenic microorganisms is shown in below Table 6

Fig . 8 Antibacterial activity against *Bacillus subtilis*

Fig. 9: Antibacterial activity against *Escherichia coli*

Fig . 10 Antibacterial activity Staphylococcus aureus

Table 6: Antibacterial activity of herbal gels

Micro-organism culture	Zone of inhibition of Herbal gels (mm)					
	Standard drug	F1	F2	F3	F4	F5
S. aureus	28	11	14	17	21	25
E. coli	30	10	13	16	20	24
B. subtilus	36	12	18	24	28	32

The antibacterial activity of all the formulated herbal gels showed good results of zone of inhibition against skin pathogens.

DISCUSSION:

The colour of all the formulated herbal gels was Light greenish to dark greenish and all the herbal gels were good in homogeneity. The pH of all the formulated gels was in the range of 6.4- 7.1 matching with skin pH range. Viscosity of all the herbal gels was ranging from 79- 84 cp x 10^3 at 100 rpm measured with Brookfield viscometer. The spreadability of all herbal gels was in the range of 12.36-15.65 (g.cm/sec). The antibacterial activity of all the formulated herbal gels showed good results of zone of inhibition against skin pathogens.

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