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DEVELOPMENT AND CHARACTERIZATION OF HERBAL GEL FROM LEAF EXTRACT OF BRYOPHYLLUM PINNATA

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Abstract : The present study aimed to formulate & evaluate. Herb gel of bryophyllum pinnatum leaf extract. The extraction was done by the aqueous and then the was done by Mon-aqueous method. Then the antimicrobial and antioxidant study of leaf extract was done The gel was formulated by using different concentration of leaf extract and optimized gel was evaluated for physical appearance ph. Spread ability and in vitro study by to France Franz diffusion cell and it was observed that by using different excipient we can prepared a stable gel.

Key words- Bryophyllum Pinnatum, antimicrobial, antioxidant, gel

1.Introduction [6,7,16,35]

Bryophyllum pinnatum is a succulent perennial plant that grows 3-5 feet tall. Commonly known as 'air plant,' it has tall hollow stems, fleshy dark green leaves that are distinctively scalloped and trimmed in red, and bell-like pendulous flowers. Kalanchoe is botanically classified with two main Latin names which refer to the same plant. Bryophyllum pinnatum /Kalanchoe pinnatum *Bryophyllum pinnatum* is a medicinal plant largely used in Folk medicine (Traditional medicine) for the treatment of kidney stones, gastric ulcer, pulmonary infection, rheumatoid arthritis etc. Kalanchoe pinnata has become naturalized in temperate regions of Asia, Australia, New Zealand, West Indies, Macaronesia, Mascarenes, Galapagos, Melanesia, Polynesia, and Hawai In many of these, such as Hawaii, it is regarded as an invasive species. In French Polynesia, Kalanchoe **pinnata has been declared a threat to biodiversity. It is also widely distributed in the Philippines** and it is known as katakataka or kataka-taka India it is cultivated in gardens and wild on the hills of North-Western India, Deccan and Bengal. The leaves of this species are thick, fleshy, elliptical in shape, curved, with a crenate or serrated margin, often reddish. Simple at the base of the stem, the leaves are imparipinnate at the top, 10–30 cm (4–12 in) long, with three to five pairs of fleshy limb lobes. The leaves are remarkable for their ability to produce Bulbils. At their margin, between the teeth, adventitious buds appear, which produce roots, stems and leaves. When the plantlets fall to the ground, they root and can become larger plants. This is a fairly common trait in the section Bryophyllum**.**

1.1 Taxonomy

1) Kingdom: Plantae (Plants)
2) Subkingdom: Tracheobionta (Vascular plants)
3) Super division: Spermatophyta (Seed plants)
4) Division: Magnoliophyta (Flowering plant)
5) Class: Magnoliopsida (Dicotyledonous)
6) ubclass: Rosidae
7) Order: Saxifragales
8) Family: Crassulaceae Stonecrop family
9) Genus: Kalanchoe (Species: Kalanchoe pinnata)

1.2 Synonyms

1) Bryophyllum calycinum,
2) B. germinans,

3) B. pinnatum
4) Cotyledon calycina,
5) C. calyculata
6) C. pinnata,
7) C. rhizophilla,
8) Crassuvia floripendia
9) Crassula pinnata
10) Sedum madagascariense,
11) Verea pinnata

1.3 Pharmacognostical Studies :-

The macroscopic studied showed that leaves are opposite, simple or compound, 12-18cm and 6-8cm in size, apex is obtuse, ovate or elliptic in shape, crenate or serrate margin, asymmetric base, reticulate venation, petiole is long, surface is glabrous, upper epidermis dark green in color and lower epidermis lighter in color and with a characteristic odors and bitter test. The microscopic studies of leaves of plant showed xylem, phloem, mesophyll tissue, midrib, while the trichomes absent both sides i.e., adaxial side and abaxial side. It is broadly shallow on the adaxial side and convex on the abaxial side.It has thin adaxial epidermal layer of small, less prominent cells. The abaxial epidermis is also nary thin and less distinct. The ground tissue of midrib is parenchymatous and homogenous. The cells are circular or angular and compact. The vascular strand is single, collateral, small and hemispherical in shape. It consists of thick horizontal band of xylem and fairly wide band of phloem. The vascular bundle is 100µm in vertical plane and 170 µm in horizontal plane. The lamina is uniformly flat with even surface. The mesophyll tissue is not differentiated into palisade and spongy parenchyma. The stomata are abundant, these are 18-20 stomata per mm², having anisocytic in nature.

1.4 Chemical Composition :-

The leaves of ***Bryophyllum pinnatum*** were collected from biological garden in D. D. Vispute College of Pharmacy and Research Center. B. Pinnatum is rich in alkaloids, triterpenes, glycosides, flavonoids, cardienolides, steroid The leaves contain a group of chemicals called bufadienolides which are very active. Bufadienolides like bryotoxin A, B, C which are very similar in structure and activity as two other cardiac glycosides, digoxin and digitoxin and possesses antibacterial, antitumorous, cancer preventative and insecticidal actions Bufadienolides-Bryophyllin A (bryotoxin); Bryophyllin B; Bryophyllol; Bryophollone; Bryophollenone; Bryophynol.**Phenols, Phenylpropanoids and Flavonoid**: Syringic acid, caffeic acid 10, 4-hydroxy-3- methoxy-cinnamic acid, 4-hydroxybenzoic acid, p-hydroxycinnamic acid, paracoumaric acid, ferulic acid, protocatechuic acid, phosphoenolpyruvate, protocatechuic acid.**Triterpenoids and Steroids**: α-amyrin, α-amyrinacetate, β-amyrin, β-amyrinacetate, bryophollenone, bryophollone, taraxerol, Ψ-taraxasterol, pseudo taraxasterol, 18-α-oleanane, friedelin, glutinol.

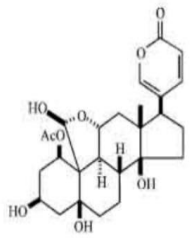


Fig 1. Bryophyllin

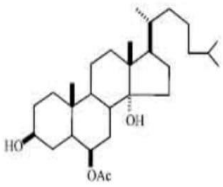


Fig 2 Bryophyllol

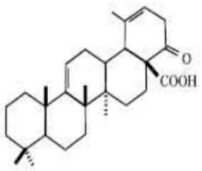


Fig 3 Bryophollone

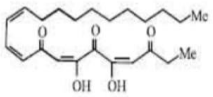


Fig 4 Bryophollenone

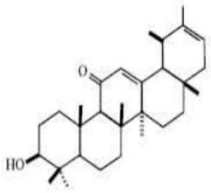


Fig 5 Bryophynol

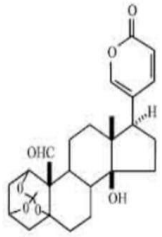


Fig 6 Bersaldegenin

1.5 Pharmacological Activities [6,18] :-

1.5.1 Antibacterial activity: The presence of phenolic compounds indicate that the plant possesses **anti-microbial** activity. Ofokansi et al. (2005) reported that plant is effective in the treatment of typhoid fever and other bacterial infections, particularly those caused by S. aureus, E. coli, B. subtilis, P. aeruginosa, K. aerogenes, K. pneumoniae and S. typhi. In his study antibacterial activities of the infusion and methanolic extracts against S. aureusi ATCC 13709, E. coli ATCC 9637, Bacillus, P. aeroginosa, K. pneumonia and S. typhi using the agar diffusion method; also against S. aureus, E. coli, S.typhi, Klebsiella spp and P.aeruginosa using a modification of checkerboard method. These findings supported its use in treating the placenta and navel of newborn baby, which not only heals fast but also prevent the formation of infections. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects.

1.5.2 Antioxidant activity: The medicinal plant is tested forits anti-oxidant activity by metal chelating assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. Study outcomes have indicated that the ethanolic extract has marked anti-oxidant activity. Roots extracts have also exhibited the anti-oxidant effects when analyzed by DPPH assay. The leaves were reported to show maximum scavenging effects than stems and the ethanolic extract showed more total phenolic and flavonoid content than other extracts. The high number of phenols and flavonoids in the extracts may be the reason for their high antioxidative activity K. pinnata has strong protective potential than standard antioxidants against oxidative stress in both aqueous and lipid phases. They isolated seven kaempferol rhamnoses derivatives from ethyl acetate extract and tested for their antioxidant ability. And also **Analgesic, Anti-inflammatory and Wound Healing activity**, **Anticancer**, **Anti-allergic activity** Antipyretic **activity, Hepatoprotective activity**

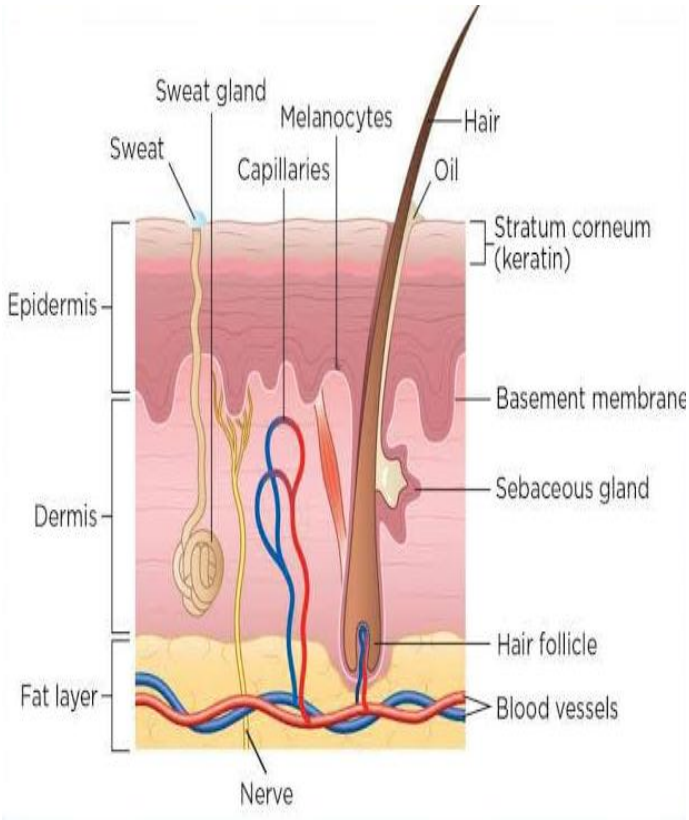
1.6 HUMAN SKIN [39]

The skin is not only protective wrap for the body; it is busy frontier which mediates between the human body and environment. It not only controls the loss of valuable fluids, prevents the penetration of noxious foreign materials and radiation and cushions against mechanical

shock but also regulates heat loss and transduces incoming stimuli. Structure of skin is continuous sheet covering the entire body surface. It is composed of two main layers i.e. Epidermis, Dermis

1.6.1 Epidermis: Epidermis is the surface of the skin. Cells in the epidermis form a multi- layer system and forms barrier. Epidermis is composed of five distinct layers Stratum corneum-horny layer, Stratum lucidum - refractile layer, Stratum granulosum- granular layer**,** Stratum spinosum-spiny layer, Stratum basale - basal layer.

1.6.2 Dermis: Dermis is a thick layer of tough, viscoelastic tissue below the epidermis that contains blood vessels, nerve endings, hair follicles and sweat glands The structure of dermis comprises of relatively small number of cells, located within a network of protein fibers and surrounded by an amorphous "jelly" called ground substance. The major cells with the dermis are fibroblasts. These cells synthesize main two types of fibrous proteins-Collagen, Elastin



1.7 Transdermal drug delivery systems [21] :- Transdermal drug delivery systems are topically administered medicaments in the form of patches that deliver drugs for systemic effects at a predetermined and controlled rate. A transdermal drug delivery device, which may be of an active or a passive design, is a device which provides an alternative route for administering medication. These devices allow for pharmaceuticals to be delivered across the skin barrier.In theory, transdermal patches work very simply. A drug is applied in a relatively high dosage to the inside of a patch, which is worn on the skin for an extended period of time. Through a diffusion process, the drug enters the bloodstream directly through the skin. Since, there is high concentration on the patch and low concentration in the blood, the drug will keep diffusing into the blood for a long period of time, maintaining the constant concentration of drug in the blood flow.

1.7.1 ***Advantages*** This approach to drug delivery offers many advantages over traditional methods

- a substitute for the oral route
- Transdermal drug delivery enables the avoidance of gastrointestinal absorption, with its associated pitfalls of enzymatic and pH
- associated deactivation.
- This method also allows for reduced pharmacological dosing due to the shortened metabolization pathway of the transdermal route versus the gastrointestinal pathway.
- The patch also permits constant dosing rather than the peaks and valleys in medication level associated with orally administered medications. Multi-day therapy with a single application,
- Rapid notification of medication in the event of emergency, as well as the capacity to terminate drug effects rapidly via patch removal

1.7.2 Disadvantages

- The drug that requires high blood levels cannot be administered and may even cause irritation or sensitization of the skin.
- The adhesives may not adhere well to all types of skin and may be uncomfortable to wear.
- High cost of the product is also a major drawback for the wide acceptance of this product.

1.7.3 Properties that influence Transdermal Delivery Release of the medicament from the vehicle, Penetration through the skin barrier., Activation of the pharmacological response

1.7.4 Basic Components of Transdermal Drug Delivery Systems Polymer matrix or matrices., The drug Permeation enhancers, other excipients

1.8 Introduction Of Gel [10]:- Gels are defined as semi rigid systems in which the movement of the dispersing medium is restricted by an interlacing three-dimensional network of particles or solvated macromolecules of the dispersed phase. Gels are generally considered to be more rigid than jellies because gels contain more covalent crosslinks, a higher density of physical bonds, or simply less liquid. Gel-forming polymers produce materials that span a range of rigidities, beginning with a sol and increasing in rigidity to a mucilage, jelly, gel, and hydrogel. Some gel systems are as clear as water, and others are turbid because the ingredients may not be completely molecularly dispersed (soluble or insoluble), or they may form aggregates, which disperse light. The concentration of the gelling agents is mostly less than 10%, usually in 0.5% to 2.0% range, with some exceptions.

1.8.1 Properties of Gels [10]

1. Ideally, the gelling agent must be inert, safe and cannot react with other formulation constituents.
2. The gelling agent should produce a sensible solid-like nature at the time of storage which is easily broken when exposed to shear forces produced by squeezing the tube, trembling the bottle or at the time of topical application.
3. It should have suitable anti-microbial agent.
4. The topical gel must not be sticky.
5. The ophthalmic gel must be sterile.

6. The apparent viscosity or gel strength increases with an increase in the effective crosslink density of the gel. However, a rise in temperature may increase or decrease the apparent viscosity, depending on the molecular interactions between the polymer and solvent.
7. They exhibit the mechanical characteristics of the solid state.
8. Each component is continuous throughout the system.
9. There is high degree of attraction amongst the dispersed phase and water medium so the gels remain equally uniform upon standing and doesn't freely settle.

1.8.2 Advantages of Gel

Gels are non-sticky, greaseless, emollient, non-staining, water soluble or miscible, easily spreadable and removable.
Ease of self-administration, higher residence time, increased bioavailability, site specific drug delivery with predictable, controlled and sustained duration of action, therefore greater patient compliance due to reduced dosage frequency.

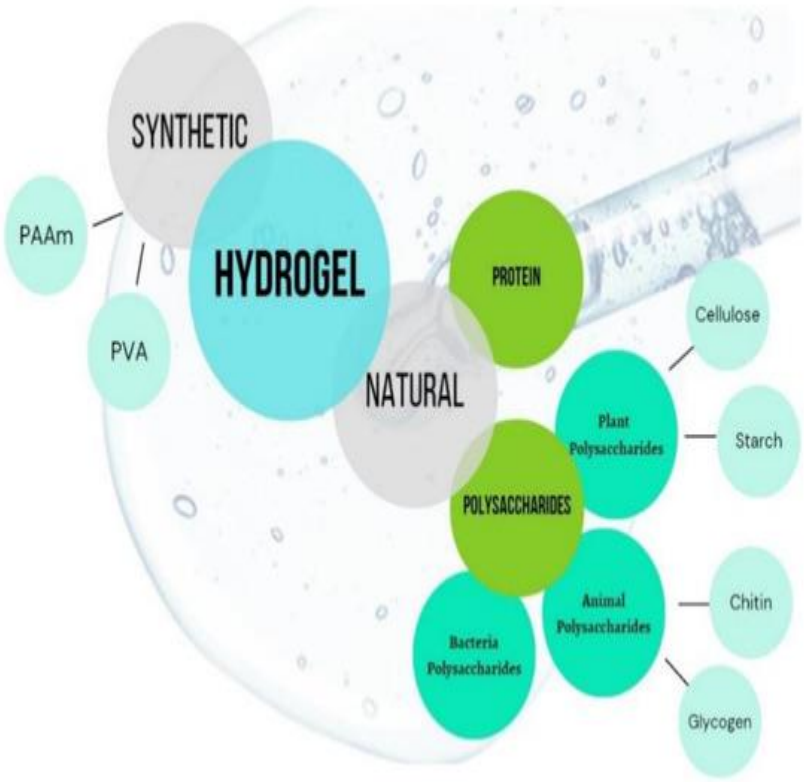
1.8.3 Routes of Administration of Gel [14]

Topical routes of administration:

Topical gels are applied directly to an external body surface to produce local or systemic effect; for treatment of a variety of dermatological infections. Example: Sandoz Metronidazole Gel USP (1%) and Sandoz Metronidazole Topical Gel USP (0.75%).

Vaginal routes of administration:

Vaginal gels are intended for both local as well as systemic diseases. It is advantageous due to its large surface area, rich blood supply, avoidance of the first-pass effect and relatively high permeability (as it is covered with mucous membrane). Example: Sandoz Metronidazole Vaginal Gel USP (0.75%).



1.8.4 Classification of Gel: [10]

Based on nature of solvent

-Hydrogels: water is dispersion medium.
-Organogels: organic solvent is dispersion medium.
-Xerogels: highly porous solid formed from a gel by drying or removal of solvent.

Based on rheological properties

-Usually, gels exhibit non-Newtonian flow properties.

They are classified into:

Plastic gels: exhibit plastic flow
Pseudo plastic gels exhibits pseudo-plastic flow
Thixotropic gels

Based on physical nature

Elastic gels: exhibit an elastic behavior
Rigid gels.

1.8.5 Methods of Preparation of Gel [10,12]

Fusion Method: In this method various waxy materials employed as gelling agent in non-polar media. Drug is added when waxy materials melted by fusion. Stir slowly until uniform gel is formed.

Cold Method: Water is cooled to 4-10°C and placed it in mixing container. Gelling agent is added slowly by agitation. Maintain temperature below 10°C. Drug is added slowly in solution form with gentle mixing.

Dispersion Method: Gelling agent is dispersed in water with stirring. Drug is dissolved in the non-aqueous solvent along with the preservative. This solution is added in above gel with continuous Stirring.

2 PLAN OF WORK

2.1 Literature review

- Materials and Method
- Identification and confirmation of herbal drug
- Organoleptic properties
- Solubility
- Authentication
- Fourier transform Infra-red (FTIR) Spectroscopy study

2.2. Preformulation studies

- Ultraviolet visible (UV-VIS) Spectrophotometry
- Calibration curve
- Drug- Excipient compatibility

2.3. Extraction of plant leaves

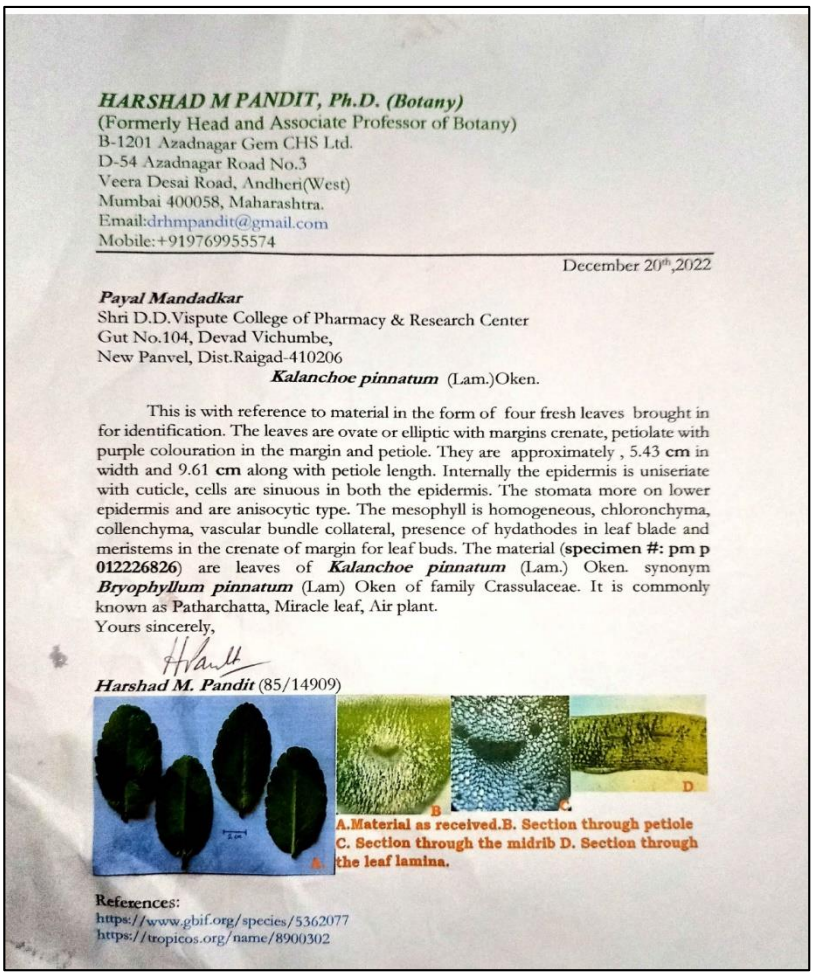
- Alcoholic Extraction
- Aqueous Extraction
- Phytochemical tests

2.4. Confirmatory test

- Antimicrobial activity
- DPPH Assay

2.5.Preparation of herbal gel

- By dispersion method
- Evaluation of Herbal gel
- In- Vitro Diffusion Study
- Stability study



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December 20th,2022

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Shri D.D.Vispute College of Pharmacy & Research Center
Gut No.104, Devad Vichumbe,
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Kalanchoe pinnatum (Lam.)Oken.

This is with reference to material in the form of four fresh leaves brought in for identification. The leaves are ovate or elliptic with margins crenate, petiolate with purple colouration in the margin and petiole. They are approximately , 5.43 **cm** in width and 9.61 **cm** along with petiole length. Internally the epidermis is uniseriate with cuticle, cells are sinuous in both the epidermis. The stomata more on lower epidermis and are anisocytic type. The mesophyll is homogeneous, chloronchyma, collenchyma, vascular bundle collateral, presence of hydathodes in leaf blade and meristems in the crenate of margin for leaf buds. The material (**specimen #: pm p 012226826**) are leaves of ***Kalanchoe pinnatum*** (Lam.) Oken. synonym ***Bryophyllum pinnatum*** (Lam) Oken of family Crassulaceae. It is commonly known as Patharchatta, Miracle leaf, Air plant.

Yours sincerely,

Harshad M. Pandit (85/14909)

A.Material as received.B. Section through petiole C. Section through the midrib D. Section through the leaf lamina.

References:
https://www.gbif.org/species/5362077
https://tropicos.org/name/8900302

3.1 Materials and Method: Leaves of *Bryophyllum pinnat*a are collected from the herbal garden of Shri D.D Vispute college of Pharmacy and research center, New Panvel Maharashtra, Dist.; Raigad. The chemicals required for study is provided by the institute Shri D.D Vispute college of Pharmacy and research center, new Panvel.

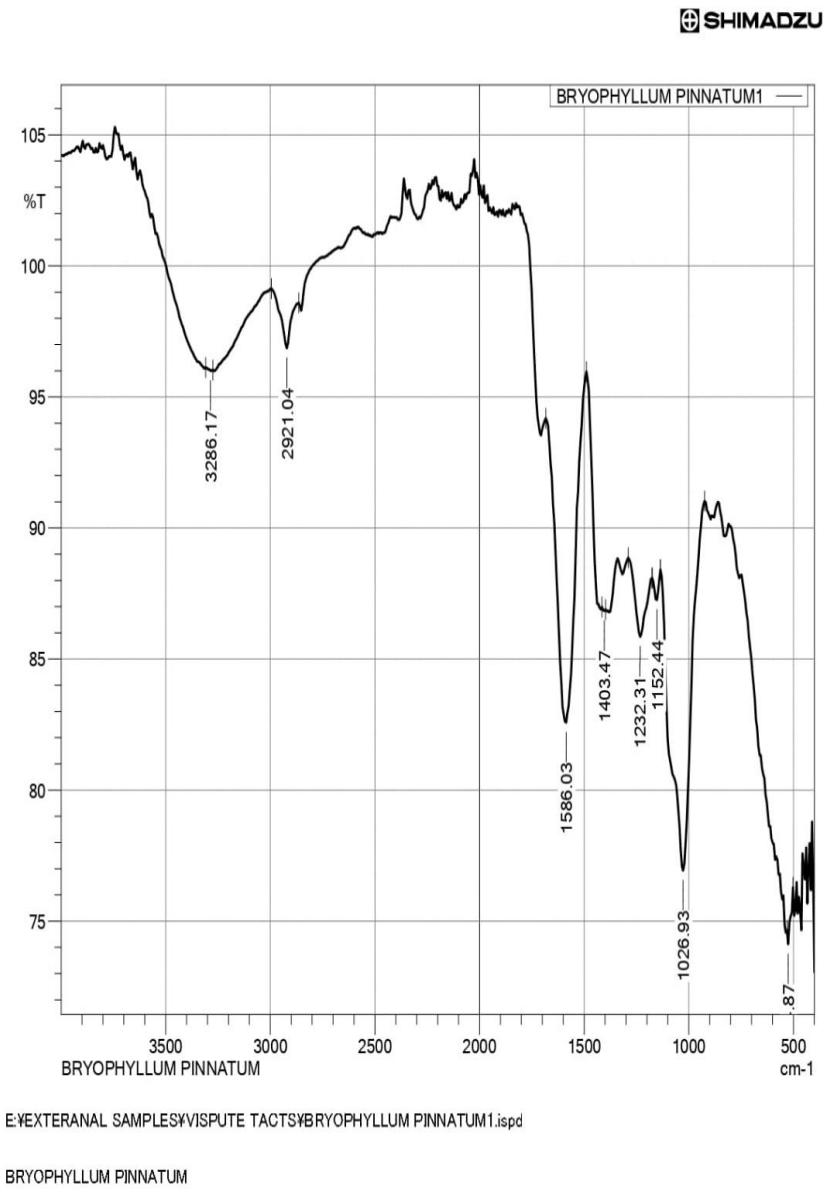
3.2 Authentication Letter: -

3.3 List of Materials and Chemicals

Sr .no	Materials and chemicals	Suppliers
1	***Bryophyllum Pinnatum***	**Shri D. D. Vispute College Of Pharmacy And Research Center**
2	**Carbopol 934**	**Shri D. D. Vispute College Of Pharmacy And Research Center**
3	**Polyethylene glycol**	**Shri D. D. Vispute College Of Pharmacy And Research Center**
4	**Disodium hydrogen phosphate / sodium phosphate**	**Shri D. D. Vispute College Of Pharmacy And Research Center**
5	**Potassium hydrogen phosphate / potassium phosphate**	**Shri D. D. Vispute College Of Pharmacy And Research Center**
6	**Chloroform**	**Shri D. D. Vispute College Of Pharmacy And Research Center**
7	**Methanol**	**Shri D. D. Vispute College Of Pharmacy And Research Center**
8	**Cellophane**	**Shri D. D. Vispute College Of Pharmacy And Research Center**

3.4 List of Instruments and Equipment

Sr.no	Instrument and equipment
1	Electronic Precision Balance
2	Water Filtration Unit
3	Fourier Transform Infra-Red (FTIR) Spectrophotometer ingle Reflection ATR Accessory
4	Ultraviolet Visible (UV-Vis) Spectrophotometer
5	Ultra Sonication with Heater
6	Optical Microscopy (compound microscopy)
7	Digital PH Meter
8	Brookfield Viscometer
9	Digital Magnetic Stirrer with Hot Plate



10	Refrigerator
11	Franz Diffusion Cell
12	Stability Chamber

3.5 Identification and Conformation of compound: -

- **Organoleptic properties: -** properties such as appearance, colour and Odors were checked
- **Ultraviolet Visible (UV-Vis) Scanning Spectrophotometry** [37]
  Test solution: Dissolve 1 g of the drug in water and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with water. -Spectral range: 400-800 nm. Absorption maximum: at 474 nm, about 1.039
- **Fourier Transform Infra-Red (FTIR) Spectroscopy study**
- IR spectrum of the Drug was recorded using FTIR in the region of 4000–500cm−1compared with the reference spectrum respectively
- **Ultraviolet Visible (UV-Vis): Calibration curse plot by using UV-Vis Spectrophotometry**

3.5.1 Preparation of standard stock solution of drug:
Drug 10 mg was accurately weighed and transferred to 100 ml volumetric flask. It was dissolved and diluted up to the mark with water to obtain 100pg/ml as standard stock solution.

3.5.2 Spectrophotometric scanning and determination of max of drug:
From the standard stock solution 1 ml was pipetted out and dilated up to 10 ml using water and was scanned between wavelength 200 nm to 400 nm

3.5.3 Plotting of calibration curve of drug:
From the standard stock solution series of dilution were made by pipetting out 1.0 and 2.5 ml of standard stock solution and diluting up to 10 ml by water in order to obtain 2. 4, 6, 8, and 10 ppm solution respectively Absorbance was measured at 284 mm by using UV-Vi Spectrophotometer. This experiment was performed in triplicate and calibration curve was plotted in order to check the linearity

3.5.4 Preparation of PBS (Phosphate Buffer Solution) pH 6.8:
Wight accurately 28.80 gm of disodium hydrogen phosphate and 11.45 gm of potassium dihydrogen phosphate and transfer to 1000ml volumetric flask Dissolved in 100 ml distilled water and make up the volume with distilled water Use concentrated hydrochloric acid to adjust pH of solution.

3.5.5 Preparation of standard stock solution of drug (in FBS pH 6.8):
Drug 10 mg was accurately weighed and transferred to 100 ml volumetric flask. It was dissolved and diluted up to the mark with Phosphate Buffered Solution (PBS) pH 6.8 to obtain 100pg/ml as standard stock solution

3.5.6 Spectrophotometric scanning and determination of max of drug (in PBS pH 6.8):
From the standard stock solution, 1 ml was pipetted out and diluted up to 10 ml using PBS pH 6.8 and it was scanned between wavelength 200 nm to 400 nm

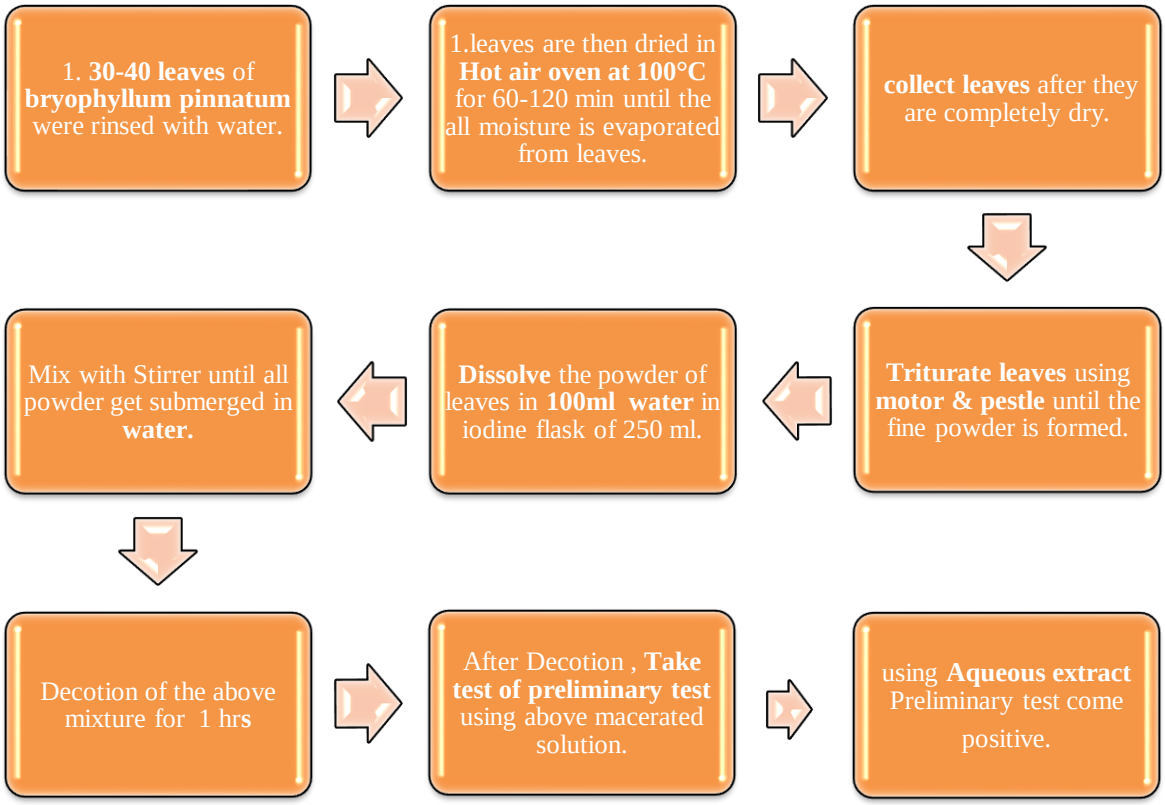
Plotting of calibration curve of drug: From the standard stock solution, series of dilution were made by pipetting out 1,2,3,4 ml of standard stock solution and diluting up to 10 ml by PBS pH 6.8 in order to obtain 1000,2000,3000,4000 ppm solution respectively. Absorbance was measured at 340 nm by using UV-Vis Spectrophotometer. calibration curve was plotted in order to check the linearity.

3.5.7 Drug-Excipient Compatibility Study by Fourier Transform Infra-Red (FTIR) Spectroscopy:

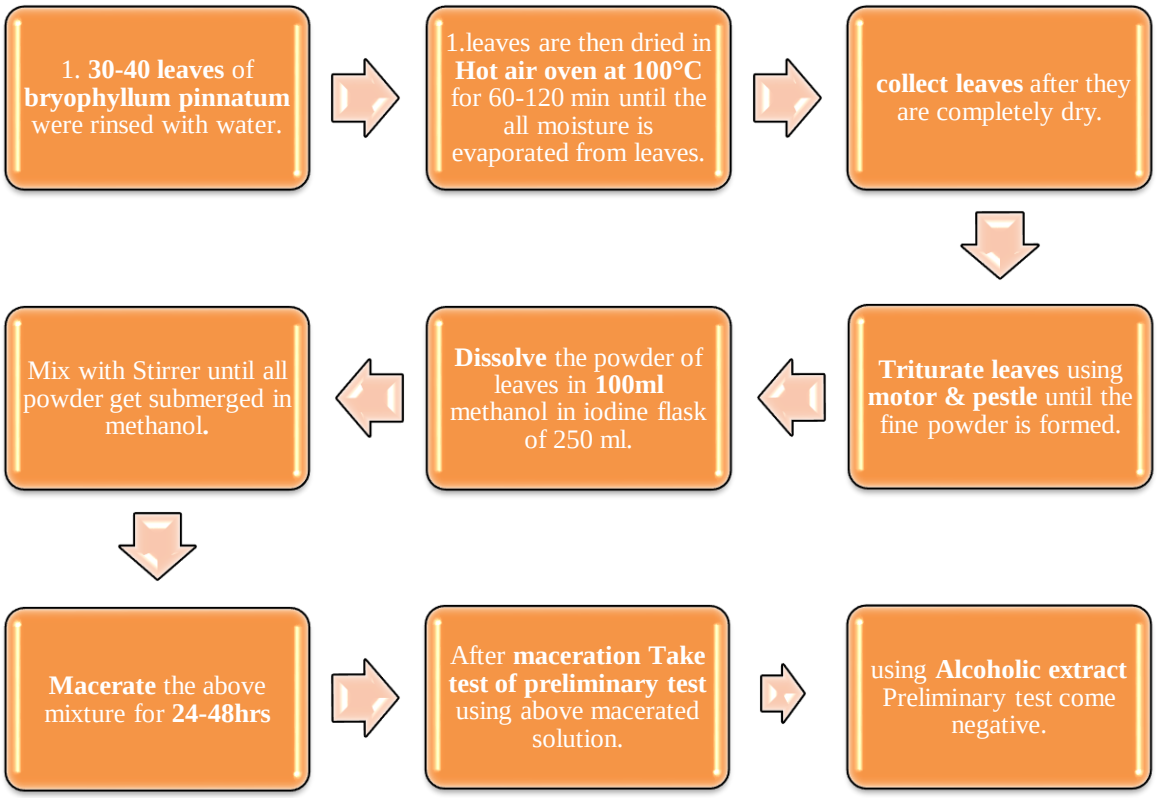
IR spectrum of pure drug and excipients along with mixture of drug and excipients were recorded by FTIR and the compatibility of drug and excipients was checked by comparing the spectra.

3.6 Extraction method: [40,41]

3.6.1.Aqueous extraction



3.6.2.Alcoholic Extract:

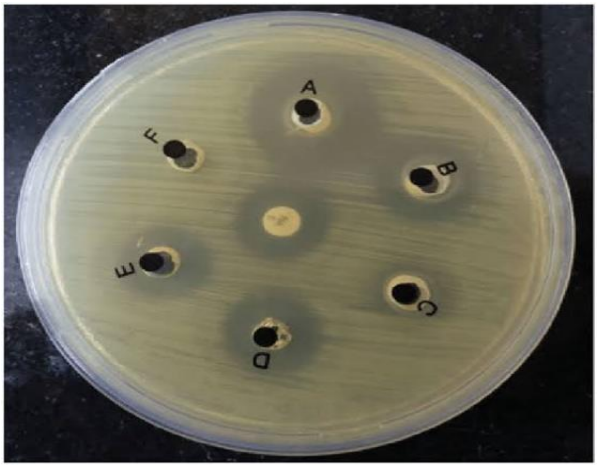


3.7 Phytochemical tests: [25]

sr.no	test name	Aqueous extract	Alcoholic extract
1	**Test for flavonoids**		
	Shinoda Test	Positive	Negative
	Sulphuric Acid Test	Positive	Negative
2	**TESTS FOR TANNINS AND PHENOLIC COMPOUNDS**		
	(a) 5% $FeCl_3$ solution→ deep blue-black colour	Positive	Negative
	(b) Lead acetate solution→ white ppt	Positive	Negative
	(c) Gelatin solution→ white ppt	Positive	Negative
	(d) Bromine water → decolouration of bromine water	Positive	Negative
	(e) Dilute iodine solution → transient red colour	Positive	Negative
3	**TESTS FOR SAPONIN GLYCOSIDES:**		

	Foam test	Positive	Negative
	Liebermann - Burchard reaction	Positive	Negative
4	**TESTS FOR CARBOHYDRATES**	Positive	Negative
5	**TESTS FOR REDUCING SUGARS**		
	Fehling's test	negative	Negative
	Benedict's test	negative	Negative
6	**TEST FOR PROTEINS**		
	Biuret test (General test)	negative	Negative
	Xanthoprotein test	negative	Negative
	Test for proteins containing sulphur	negative	Negative
	Precipitation test	negative	Negative
7	**TESTS FOR AMINO ACIDS**		
	Ninhydrin test (General test)	negative	Negative
	Test for tyrosine	negative	Negative
	Test for tryptophan	negative	Negative
	Test for cysteine	Negative	Negative
8	**TESTS FOR STEROID**		
	Salkowski reaction	negative	Negative
	Liebermann	negative	Negative
	Liebermann's reaction	negative	Negative
9	**TESTS FOR VOLATILE OILS**		
	(a) Volatile oils have characteristic odour	negative	Negative
	(b) Filter paper is not permanently stained with volatile oil	negative	Negative
	(c) Solubility test: Volatile oils are soluble in 90% alcohol	negative	Negative
10	**TESTS FOR GLYCOSIDES**	negative	Negative
11	**TESTS FOR CARDIAC GLYCOSIDES**		
	Balfet's test	negative	Negative
	Legal's test (Test for cardenoloids)	negative	Negative
	Test for deoxysugars (Keller-Killiani test)	negative	Negative
	Liebermann's test	negative	Negative
	Kedde's test	negative	Negative
12	**TESTS FOR ANTHRAQUINONE GLYCOSIDES**		
	Borntrager's test for anthraquinone glycosides	negative	Negative
	Modified Borntrager's test for C-glycosides	negative	Negative
13	**TESTS FOR SAPONIN GLYCOSIDES**:		
	Foam test	negative	Negative
	Heamolytic test	negative	Negative

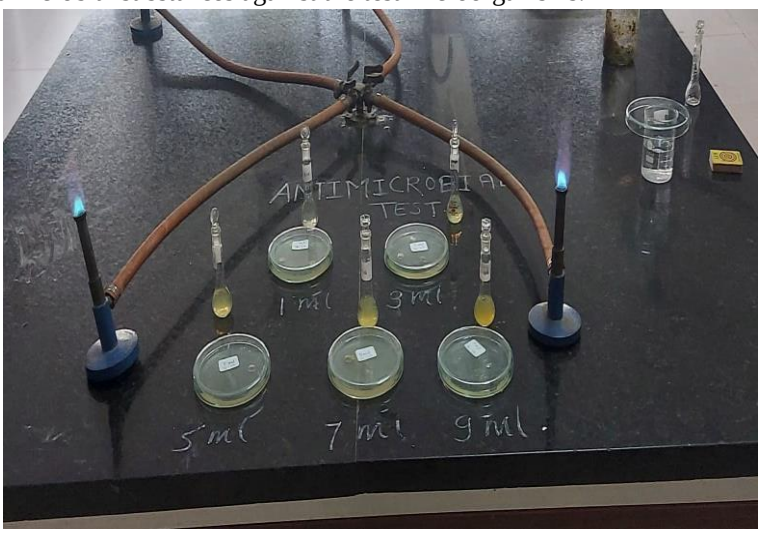
15	**TESTS FOR CYANOGENETIC GLYCOSIDE**		
	Guignard reaction or sodium picrate test	negative	Negative
	To dry drug powder or extract, add 3% aqueous mercurous nitrate solution Metal mercury form	negative	Negative
	Dip a piece of filter paper in guaiacum resin and moist it with dilute copper sulphate solution. Expose it to freshly cut surface of drug, blue stain is produced	negative	Negative
16	**TESTS FOR COUMARIN GLYCOSIDES**		
	Coumarin glycosides have aromatic odour	negative	Negative
	Alcoholic extract when made alkaline, shows blue or green fluorescence.	negative	Negative
	Take moistened dry powder in test tube. Cover test tube with filter paper soaked i dilute NaOH. Keep in water bath. After sometime expose filter paper to ultra violet light. It shows yellowish-green fluorescence	negative	Negative
17	**TESTS FOR ALKALOIDS**		
	Dragendorff's test	negative	Negative
	Mayer's test	negative	Negative
	Wagner's test	negative	Negative
	Murexide test for purine alkaloids	negative	Negative

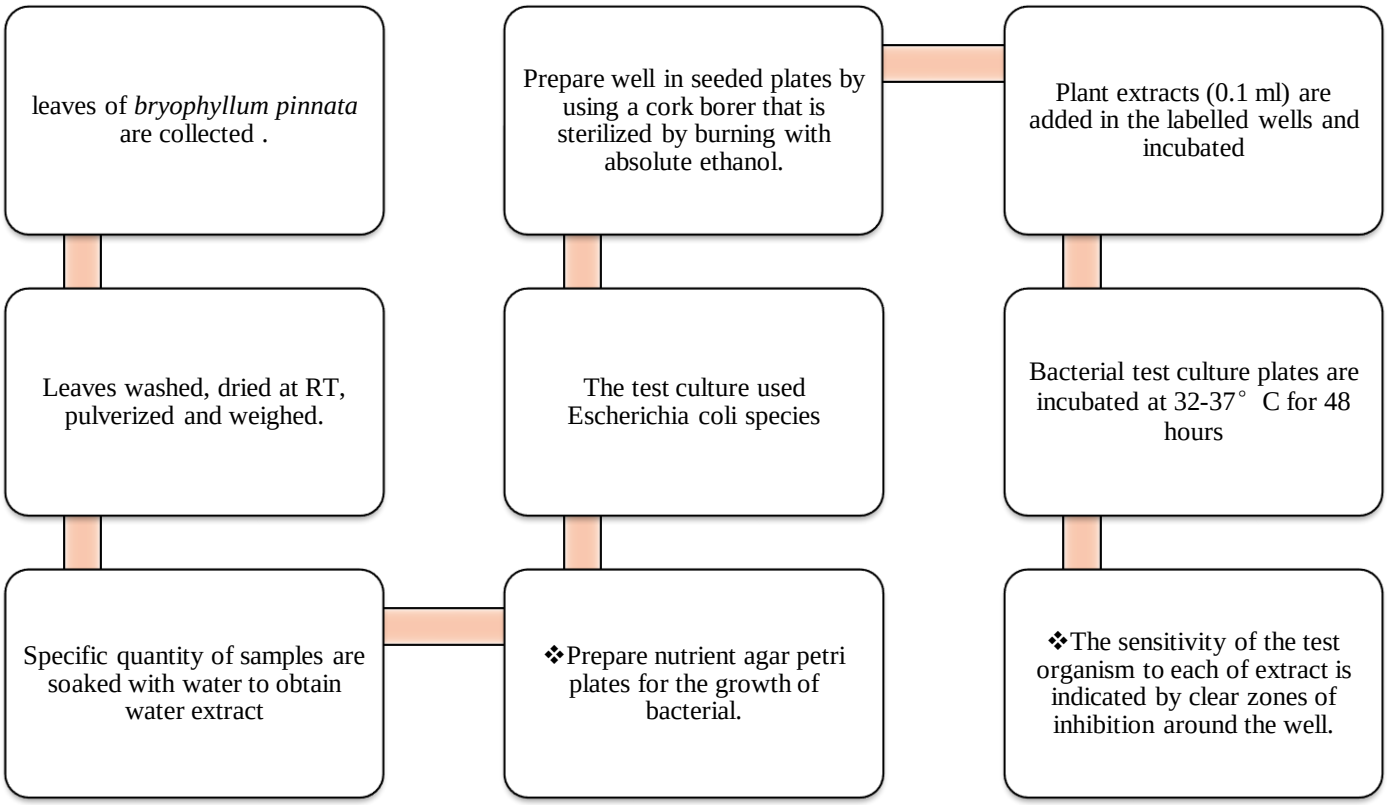


4. Antimicrobial Activity:[38]

- Requirements:
- Micro-organism: Escherichia coli and Streptomycin aurus
- Cultures Media: Nutrient agar plates,
- Solvents: Water
- Apparatus Test tubes, flask, pipette, Petri plates, cork borer.
- Equipment: Hot air oven, incubator, water bath.

4.1 Procedure: - In these methods, the agar is melted, cooled at 45°C, inoculated with the microorganisms and poured into a sterile Petri plate. In the cup-plate method, when the inoculated agar has solidified, holes about 9 mm in diameter are cut in the medium with a sterile cork borer. The antimicrobial agent is directly placed in the holes in the cylinder plate method, The zone of inhibition is observed after incubation at 30 to 35°C for 2 to 3 days. The diameter of the one of inhibition gives an indication of the relative activities of different antimicrobial substances against the test microorganisms.





5. DPPH ASSAY: [15,27]

The free radical scavenging activity of the different extracts was measured in vitro by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) method as described. solution of DPPH was added to sample solutions at different concentrations A control (Abs Control) containing methanol and DPPH solution was also prepared. All solutions obtained were then incubated for 1 hour at room temperature. Absorbance was measured at 517 nm. Vitamin C was used as standard and the same concentrations of it were prepared as the test solutions. The percentage of inhibition of samples was calculated from obtained absorbance by the equation:

$$Percentage\ of\ Inhibition = Abs\ control - Abs\ test/standard\ Abs\ control \times 100$$

6. Preparation and optimization of Herbal base : [2]

Generally, the water-soluble excipients are firstly dissolved in vehicle, in a mixing vessel by using mechanical stirrer. To prevent aggregation, add hydrophilic polymer to the stirred mixture slowly. Stirring is continued until the dissolution of the polymer has occurred.The excessive stirring results in entrapment of air. The mixing rate not be extreme or a mixing vessel may be used to which a vacuum may be pulled, to prevent the entrapment of air

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Carbapol 934	0.5gm	1gm	1.5gm	-	-	-	-	-	-
Sodium CMC	-	-	-	0.5gm	1gm	1.5gm	-	-	
HPMC	-	-	-	-	-	-	0.5gm	1gm	1.5gm
Span 80	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
PEG 400	0.25ml	0.25ml	0.25ml	0.25ml	0.25ml	0.25ml	0.25ml	0.25ml	0.25ml
Methyl Paraben	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm
Dis. water	30ml	30ml	30ml	30ml	30ml	30ml	30ml	30ml	30ml

The ***F2 formulation*** prepared using 1% carbopol 934 was selected for further preparation of herbal gel as it exhibited better quality

INGREDIENTS (g)	f1	f2	f3
CARBOPOL 934	0.5 gm	1 gm	1.5 gm
EXTRACT	5%	5%	5%
PEG 400	0.25ml	0.25ml	0.25ml
SPAN 80	0.2ml	0.2ml	0.2ml
METHYL PARABEN	0.2 gm	0.2 gm	0.2 gm
DIL. WATER	30ml	30ml	30ml

characteristics

7.EVALUATION OF HERBAL GEL: [3,9,12]

a) Measurement of pH:

The pH of Herbal gel formulations was determined by digital pH meter. pH was measured by dispersing one gram of gel in 100 ml distilled water.

b) Spread ability

Spreadability denotes the extent of area to which the gel readily spread on application to skin or the affected part. The bioavailability efficiency of a gel formulation also depends on its spreading value. The Spreadability is expressed in terms of time in seconds taken by two slides slip off from the gel, placed in between the slides, under certain load. Lesser the time taken for separation of two slides, better the spread ability. Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 6 cm along the slide. A 30gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated three times both formulated gels and marketed gel and the meantime taken for calculation.

Formula: $S=M\times L/T$

S=Spreadability

M= Mass in gm (30gm)

L=Length of the glass (6cm)

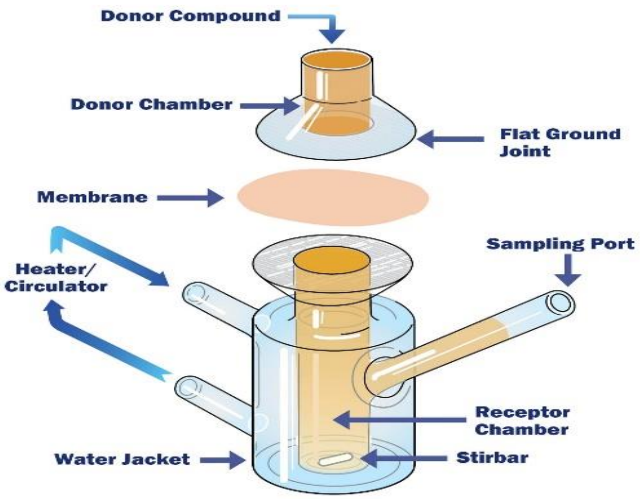
T= Time in sec.

c) **Viscosity of Gel:**

Viscosity of the gels was measured by using cup and bob type of viscometer i.e., by using Brookfield viscometer at different rpm and torque.

d) In vitro drug diffusion study from the Herbal gel formulation through cellulose membrane:

Modified Franz diffusion cell were used for the study of drug permeation through Herbal gel. 1 gram of Herbal gel was kept in the donor compartment. In the receiver compartment P.B.S. pH 6.8 and water (in 80:20 ratio) was kept. 3 ml sample was withdrawn every hr. from the receiver compartment and replaced by the fresh medium of equal volume



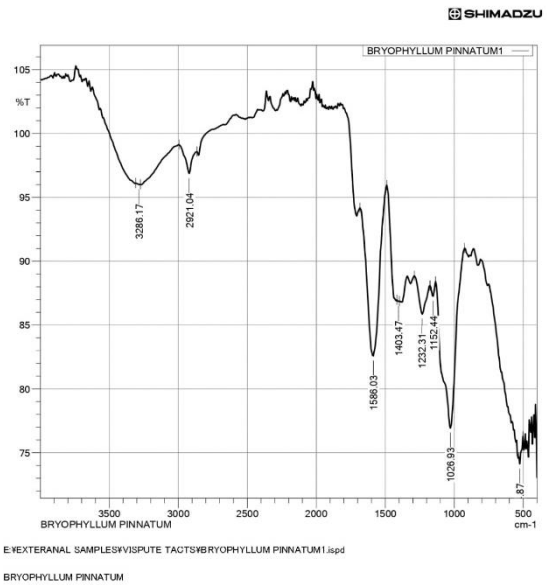
e) Stability Study

The stability study of formulation was performed as per International Council for Harmonization (ICH) guidelines. Freshly prepared formulation was divided into groups and kept at specified storage condition as per ICH guidelines. Sample were withdrawn periodically and tested for various evaluation parameters mentioned above. Stable formulation must retain the evaluation parameters at specified storage conditions over a period of time.

8. RESULT AND DISCUSSION

8.1 Organoleptic properties: Bryophyllum pinnatum was found to be green powder with slight and characteristic odor.

8.2 Fourier Transform Infra-Red (FTIR) of drug :



8.3 Preformulation study

Plotting of calibration curve of Bryophyllum pinnatum (in water).: The concentration from 4000 ppm to 6000 ppm of Bryophyllum pinnatum in water was selected for calibration curve. The value of R was found to be 0.9908 indicating the relation of drug concentration and absorbance was linear in the selected range. The absorbance of different concentrations of drug in water is given in the table and the standard calibration curve is represented in the figure.

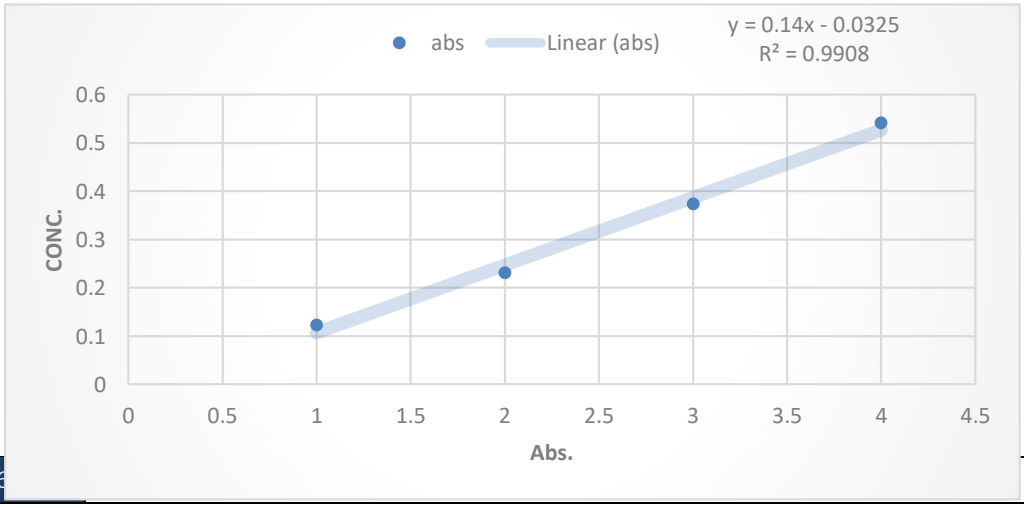
The equation y = 0.14x - 0.0325 where y is absorbance and x are concentration

$$y = 0.14x - 0.0325$$

$$R^2 = 0.9908$$

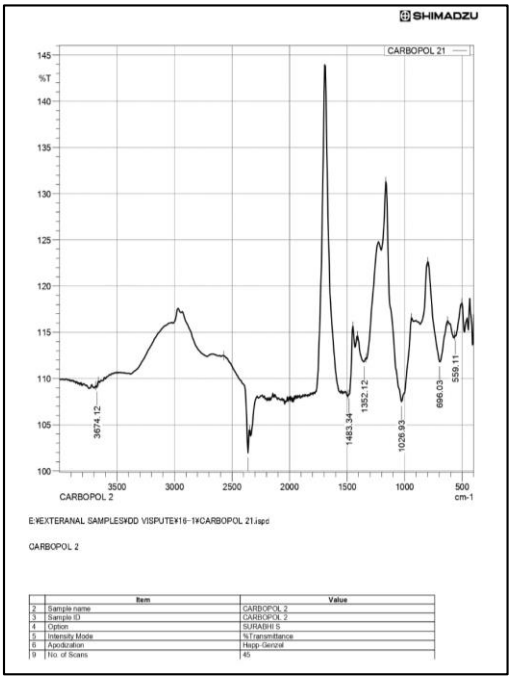
Conc.	abs
1	0.123
2	0.231
3	0.374
4	0.542

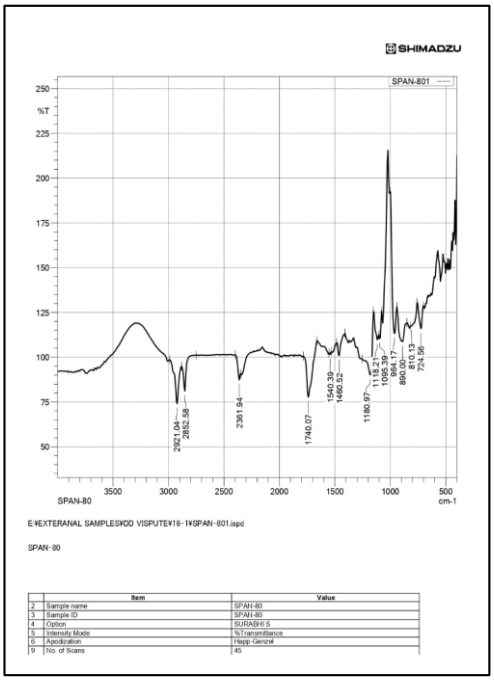
Calibration Curve of *Bryophyllum Pinnatum* in water

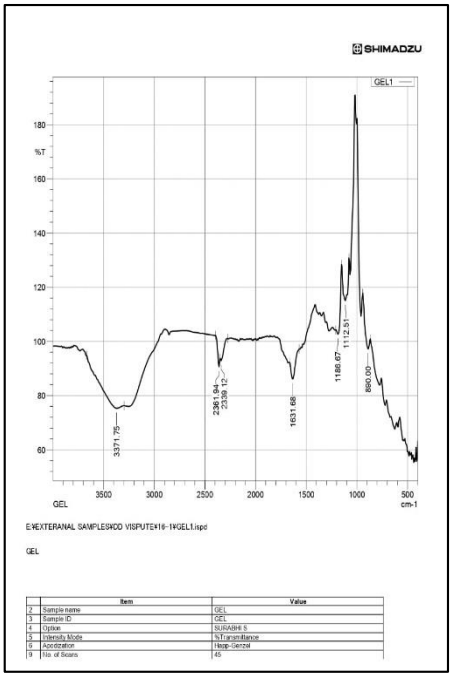


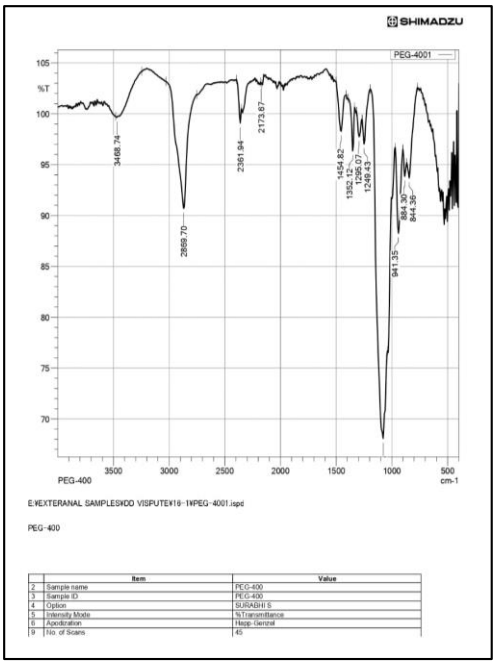
8.4 Drug- Excipient Compatibility Study by Fourier Transform Infra-Red (FTIR):

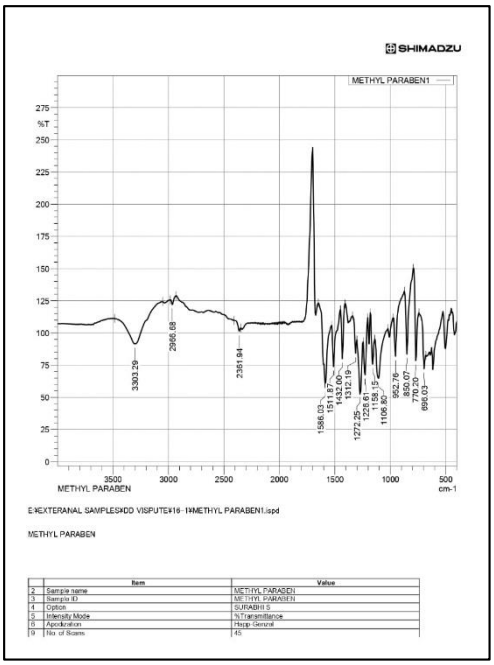
Spectroscopy IR spectrum of pure drug and excipients along with the mixture of drug and excipients were recorded by FTIR and the compatibility of drug and excipients was checked by comparing the spectra. The FTIR of all the samples is represented. along with spectrum peaks All the characteristic peaks were found in the sample. The FTIR study concluded that there was no interaction between Bryophyllum pinnate and any of the excipients











8.5 ANTIMICROBIAL ACTIVITY :-

As from obtained results of antimicrobial activity, using different microorganisms of Escheriachia Coli, Staphylococcus Aureus obtained various zone of inhibition in diameter for different concentration.

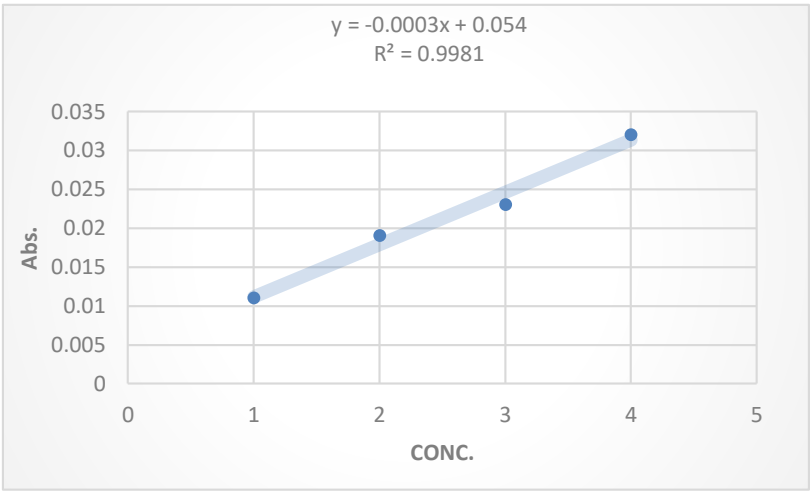
The antimicrobial activity testing was performed by measuring and comparing the diameter of zones of inhibition (in mm)

SR.	Microorganism strain	Drug concentration	Zone of Inhibition in mm
1	Escherichia coli	5ml	19mm
2	Escherichia coli	3ml	11mm
3	Escherichia coli	2ml	7mm
4	Staphylococcus aureus	5ml	12mm
5	Staphylococcus aureus	2ml	3mm

8.6 Antioxidant activity:

The result of DPPH radical scavenging assay has been presented in table

Concentration (ppm)C	Abs. of Control	Abs. of Sample	%RSA
10	0.132	0.051	61.36%
20	0.132	0.048	63.63%
30	0.132	0.043	67.42%
40	0.132	0.042	68.18%
50	0.132	0.05	62.12%



8.7 Preformulating study of Herbal gel :-

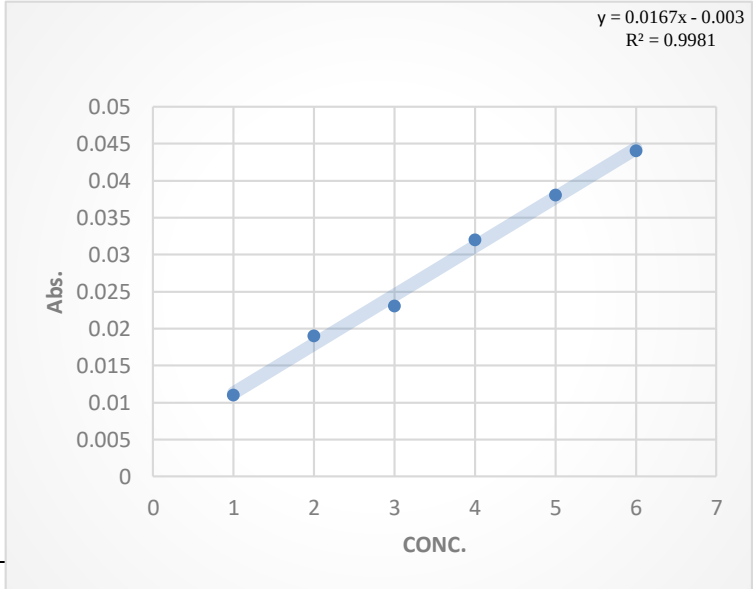
Plotting of calibration curve of Herbal gel of *Bryophyllum pinnatum* (in water).:

The concentration from 1000 ppm to 4000 ppm of *Bryophyllum pinnatum* in water was selected for calibration curve. The value of R was found to be 0.9962 indicating the relation of drug concentration and absorbance was linear in the selected range. The absorbance of different concentrations of drug in water is given in the table and the standard calibration curve is represented in the figure

The equation y = 0.0167x - 0.003

where y is absorbance and x is concentration

Sr.no	Con.	abs
1	1	0.011
2	2	0.019
3	3	0.023



4	4	0.032
5	5	0.038

8.8 Evaluation of Formulated Herbal gel:

All formulations were evaluated for their physical characterization and organoleptic properties, result as follows

S. No.	**Parameters**	**Formulation F1**	**Formulation F2**	**Formulation *F3***
1.	Colour	white	white	white
2.	Odour	Pleasant	Pleasant	Pleasant
3.	Texture	Smooth	Smooth	Smooth
4.	State	Semisolid	Semisolid	Semisolid
5.	Phase separation	NO Phase separation	NO Phase separation	NO Phase separation
6.	Greasiness	No Greasy	No Greasy	No Greasy

Evaluation	**F1**	**F2**	**F3**
ph	6.5	6.5	6.5
appearance	Homogeneous	Homogeneous	Homogeneous
viscosity	105.938 cP	122.400 cP	132.600 cP
spreadability	2.92 sec	8.66 sec	22.87 sec
Skin irritation	Non irritant	Non irritant	Non irritant
Wash ability	Easily Washable	Easily Washable	Easily Washable

8.8.1 **In-vitro Diffusion Study**

Time interval in hrs	**F1**	**F2**	**F3**
1	5%	10%	0%
2	14%	24%	12%
3	23%	35%	20%
4	32%	55%	30%
5	45%	64%	38%
6	55%	75%	48%

Drug content uniformity of F2 was found to be best representing homogeneous distribution of drug throughout the gel. The drug content was found to be in range 70%-90% which is acceptable range.

8.8.2 Stability Study:

The stability study of formulation was performed as per International Council for Harmonization (ICH) guidelines. Freshly prepared formulation was divided into groups and kept at specified storage condition as per ICH guidelines. Sample were withdrawn periodically and tested for various evaluation parameters mentioned above. The formulation is stable and shows not much variation in evaluation parameters.

8.8.3 Comparative study between *Bryophyllum Pinnata* gel and Aloe vera gel: -

The amount of drug diffused through the membrane at particular time interval is tabulated in table

Sr.no	Time interval	*Bryophyllum Pinnata* gel	Aloe vera gel
1	60 min	10%	8%
2	120 min	24%	20%
3	180 min	35%	30%
4	240 min	55%	52%
5	300 min	64%	60%
6	360 min	75%	71%

9. CONCLUSION:

In present study *Bryophyllum pinnata* leaves was selected, whose extract was very useful in the treatment of wounds. It is an attempt made to establish the herbal gel containing pinnata leaves extract at various concentration and study its antibacterial activity against different microbial strain like Streptomycin aurus and Escherichia coli. in addition, antioxidant activity also studied. The herbal gel containing leaves extract at various concentration F1, F2 and F3 were prepared and evaluated for pH, Viscosity and extrudability, Spreadability, Drug content uniformity, In vitro diffusion study, and Drug Polymer Compatibility Studies. The optimized formulation F2 complies with all the parameters. However, In vivo models are required for further studies to evaluate the potential of the herbal gel formulation and then it can be useful for the clinical application.

10. References

1. Mutimer, M. N. Rifkin, C. Hill, J. A., Glickman, M. N: Cyr, ON Modern Ointment BaseTechnology-11-Comparative Evaluation of Bases. J Am Pharm Auoc 1956
2. Gupta, A Mishra, A. K Singh, A. K Gopta, V; Bansal, P. Formulation and Evaluation of Topical Gel of Diclofenac sodium using Different Polymers. Drug Invention Today 2019, 205), 256 253
3. Sarukh et al. FORMULATION AND EVALUATION OF HERBAL GEL CONTAINING Allium cepa EXTRA JOURNAL of drug delivery & therapeutics 2019;9(4-s);492-496
4. EXTRACT Journal of Drug Delivery & Therapeutics. 2019; 9(4-s):492-496
5. Nisar B, Sultan A et.al Comparison of Medicinally Important Natural Products versus Synthetic Drugs-A Short Commentary. January 05, 2018. Volume 6, Issue 2.
6. 2.Shruti Bhavsar et.al A comparative pharmacogenetic and phytochemical analysis of Kalanchoe pinnata (Lam.) Pers. leaf extracts. 2018; 7(5): 1519-1527.
7. 3.Rajsekhar et.al The "Wonder Plant" Kalanchoe pinnata (Linn.) Pers.: A Review. March 2016 Vol. 6 (03), pp. 151-158.
8. Rajasekaran Aiyalu et. Al. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Brazilian Journal of Pharmaceutical Sciences vol. 52, n. 3, jul./sep., 2016
9. Agalu, R. Govindarjan, A., Ramasamy, A Formulation and Evaluation of Topical Herbal Ciel for the Treatment of Arthritis in Animal Model Brazilian J Pharm Sci. 2016/52031493-507
10. 5.Hemendrasinh Rathod era.al A Review on Pharmaceutical Gel. 2015 October.
11. Rathod, H. J Mehta, D., PA Review on Phnical Gal J Phir Se 2015, 1(1) 33-47
12. R. Bhramaramba et. Al. Formulation and Evaluation of Herbal Gel Containing Terminalia chebula Retz., Leaves Extract. Sch. Acad. J. Pharm., 2015; 4(3): 172-176
13. Hastak, V. S. Dosage Form Design. In Pharmaceutical and Applied Sciences: Current Concepts Series: Everest Publishing House: India, 2015; Vol. 17, pp 187.
14. Chakraborty, T.; Saini, V.; Sharma, S.; Kaur, B.; Dhingra, G. Antifungal Gel: For Different Routes of Administration and Different Drug Delivery System. Int. J. Biopharm. 2014, 5(3), 230-240.
15. 7.Akanda Md R, Tareq SM, Zaman S, Khoshnabish Md A, Huq I, Ullah HMA. Evaluation of antioxidant, cytotoxic and thrombolytic activity of Kalanchoe pinnata leaf. World J Pharm Pharm Sci, 2014; 3: 52-62
16. 8.Kaur N, Bains R, Niazi JA. Review on Bryophyllum pinnatum-A medicinal herb. J Med Pharm Innovat, 2014; 1: 13-19
17. Singh, M. P. Nagori, B. P. Shaw, NRT MR. Felation Developme Evaluation of Topical Get Formulations Using Different Gelling Apots and its Comparison with Marketed Gel Formulation but J. Pharm Ersion 2013 (3), 1-10.
18. Pattewar SV, Patil DN, Dahikar SB. Antimicrobial potential of extract from leaves of Kalanchoe pinnata. Int J Pharm Sci Res, 2013; 4: 4577-4580.
19. Sindhu S, Manorama S. Exploration of antioxidant properties in various extracts of Bryophyllum pinnatum (Lank.). Res Pharm, 2013; 3: 01-08.
20. Tavano L, Infante MR, Riya AM, et al. Role of aggregate size in the hemolytic and antimicrobial activity of colloidal solutions based on single and gemini surfactants from arginine. Soft Matter 2013;9:306-19.
21. Virendra Yadav TRANSDERMAL DRUG DELIVERY SYSTEM: REVIEW 22 January, 2012
22. Tatsimo SJN, Tamokou JD, Kuiate JR, Tane P. Antimicrobial and antioxidant activity of kaempferol rhamnoside derivatives from Bryophyllum pinnatum. BMC Res Notes, 2012; 5: 158.
23. Seema V. Pattewar et.al t kalanchoe pinnata: phytochemical and pharmacological Jan-Feb 2012. Vol. 2(1). pp. 1-8.
24. British Pharmacopoeia 2011: Volume 2 British Pharmacopoeia Commission London TSO, 2011 pp 2145
25. Quazi Majaz A. et al. the miracle plant (kalanchoe pinnata): a phytochemical and pharmacological review. May 2011. 2 (5) 1478-1482.
26. A.K. Mishra et.al, herbal cosmeceuticals for photoprotection from ultraviolet b radiation: a review, 2011, 10(3), 351-360.
27. A. D. SATHISHA et al. Evaluation of Antioxidant Activity of Medicinal Plant Extracts Produced for Commercial Purpose**.** 17 July 2010 ISSN: 0973-4945; CODEN ECJHAO E-Journal of Chemistry 2011, 8(2), 882-886.
28. Patel, J; Patel, B.; Banwait. H: Parmar, K.; Patel, M. Formulation and Evaluation of Topical Aceclofenac Gel Using Different Gelling Agent. Ir. J. Drug Dev Res 2011, 31), 156-164.
29. Gupta R, Lohani M, Arora S. Anti-inflammatory activity of the leaf extracts/fractions of Bryophyllum pinnatum Saliv. Syn. Int J Pharm Sci Rev Res, 2010; 3: 16-18
30. Kumar, L.: Verma, R. In vitro Evaluation of Topical Gel Prepared using Natural Polymer. Int. J. Drug Delivery, 2010, 2, 58-63.
31. Walker RB, Smith EW. The role of percutaneous penetration enhancers. Adv Drug Deliv Rev 1996;18:295-301.
32. Demel, R. A.: Kruyff, B. The Function of Sterols in Membranes Biochem Biophys Acta 1976, 457(2), 109-132.
33. Bryophyllum - Wikipedia (Online)

34. Miconazole nitrate gel (Online)
35. Imaobong E. Daniel et. Al. Phytochemical Evaluation, Antioxidant and Antimicrobial Activities of Various Extracts from Leaves and Stems of Bryophyllum pinnatum. Nepal Journal of Biotechnology. 2 0 2 0 Jul y; 8(1):17-28
36. Chandrakant kokare. Pharmaceutical microbiology principles and applications, Nirali Publication
37. Demel, R. A.: Kruyff, B. The Function of Sterols in Membranes Biochem Biophys Acta 1976, 457(2), 109-132.
38. Dr K.R. Khandeiwal et..al PRACTICAL PHARMACOGNOSY Nirali Prakashan ,24th Edition.
39. K.D.Tripathi essentials of medical pharmacology jaypee publication,7th edition, Chapter
40. Text book of Pharmacognosy by C.K. Kokate, Purohit, Gokhlae (2007), 37th Edition, Nirali Prakashan, New Delhi
41. Mohammad Ali. Pharmacognosy and Phytochemistry, CBS Publishers & Distribution, New Delhi