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FORMULATION AND EVALUATION OF POLYHERBAL GEL WITH ANTIFUNGAL PROPERTIES

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Abstract: Herbal medicines is still the mainstay of about 75-80% of the world's population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with human body and lesser side effects. Herbal medicines consist of plant or its part to treat injuries, disease or illnesses and are used to prevent and treat diseases and ailments or to promote health and healing. it is a drug or preparation made from a plant or plants and used for any to such purpose. The aim of present study was to prepare herbal gel formulation containing ethanolic extract of antifungal herbal gel containing ethanolic extract of senna alata, murraya koenigi cassia tora and aloevera. Topical gel formulation was designed by using ethanolic extract antifungal herbal gel containing ethanolic extract of senna alata, murraya koenigii and aloevera in varied concentrations. The gel was prepared by using carbopol 940(1%w/v),ethanol,propylene glycol, methyl paraben, propylparaben, edta disodium, tri-ethanolamine and required amount of distilled water. the prepared gels were evaluated for physical appearance, ph, spread ability, drug content, swelling index, diffusion study, viscosity, homogeneity and grittiness. it was inferred from results that gel formulations were good in appearance and homogeneity. Antifungal herbal gel containing ethanolic extract of senna alata, murraya koenigii anda loevera based gel proved to the formula of choice, since it showed the highest percentage of extrudability, g ood spreadability and rheological *Antifungal activity of some components of* Senna alata*flower was examined against five fungi. Themethanolic crude extract and the partially purified fractions were both active against standard strains of* E.coli and Streptococcus Aurueus *, but at different concentrations.*

Index Terms **- Component, formatting, style, styling, insert.**

INTRODUCTION

Most of the time, the human species live in peaceful coexistence with the microorganisms that surround them and only when the defense system is damaged orthe concentration of pathogens reach an exceptionally high density, an infection mayemerge. Most infections pass by unrecognized but sometimes the infecting agents doelicit a response of the body, which leads to clinically manifest signs and symptoms,a condition known as infectious disease. As strategies to control bacterial infections in patients improved, fungi be the most hazardous pathogens. Yeasts and moulds nowrank amongst the 10 most frequently isolated pathogens among patients in Intensive Care Units. On the contrary, modern treatment modalities may even facilitate the growth of fungi through negative interference with the remaining components of theimmune system. Let's have a closer look at these peculiar infective agents, called fungi or mycoses.

In India, drugs of herbal origin have been used in traditional systems of medicines such as Ayurveda, Unani, Siddha and Folk (tribal) medicines since ancienttimes. Among these systems, Ayurveda is most practiced and widely accepted alternative system of medicine in India. The most noticeable change towards herbal medicine in the developed countries of this century has been because of the interest shown by the ordinary people. From being regarded as "old fashioned" and "distrusted", herbs such as ginseng and guarana which are now hailed as wonder drugs. The change in attitude began in the 1960s, when the 'hippie' movement advocated a nature living, initiating "alternative"' medicine and therapies. The growthof the conservation movement and the founding of companies using only natural products in an environmentally friendly way were also major factors. As a result, increasingly wide ranges of herbs are now available as fresh, dried, as ingredients of cosmetics, perfumes, and over-the-counter medicines. Besides the advances and advantages of conventional medicine, or biomedicine as it is also known, it is clear that herbal medicine has much to offer. We tend to forget that in all but the last fiftyyears or so humans have relied almost entirely on plants to treat all types of illnesses,from minor problems such as coughs and colds to life threatening diseases such as tuberculosis and malaria. Today, herbal remedies are coming back into prominence because the efficacy of conventional medicines such as antibiotics, which once had near-universal Tectiveness against serious infections, is on the wane.[1]

Difference between herbal and synthetic preparation

Traditional medicines play an important role in health services around the globe. About three quarters of the world population relies on plants and plant extractsfor health care. A large number of Indian medicinal plants are attributed with variouspharmacological activities as they contain diversified classes of photochemical. Theantibiotics , azoles drugs, widely used to reduce the fungal infection of various types,suffer from severe side effects like redness, itching etc. As a result, a search for otheralternatives seems to be necessary which would be more beneficial. The literature survey revealed that various plants scattered throughout the plant kingdom exhibit anti-fungal activity. Few well known examples are *cassia torra, murraya koenii, senna alata e*tc. which contain flavonoids and are reported for their anti-fungal activity. The plants selected for present work are *Cassia tora* and *senna alata* whichcontain high percentage of flavonoids saponoid alkaloids and other component responsible for antifungal activity.

Thus, an attempt was made to study the anti-fungal activity of individual as well as combination of extracts in a single dosage form which may show synergisticantifungal activity

Gel formulations are used to deliver the drug topically because of easy application, increase contact time and minimum side effects as compare to other topical preparation and oral administration.[2]

What is herbal gel

A gel is a solid or semisolid system of at least two constituents, consisting of acondensed mass enclosing and interpenetrated by a liquid. Gels and jellies are composed of small amount of solids dispersed in relatively large amount of liquid, yet they posses more solid-like than liquid-like character. The characteristic of gel and jelly is the presence of some form of cutaneous structure, which provides solid- like properties.

ADVANTAGES :

- Non greasy application
- Being easy to formulate with active ingredients
- Adhering well to application sites
- Being washable and non-toxic
- Stability over time
- Ability to target affected area for rapid treatment and relief.
- Preventing unwanted side effects through bypassing the digestive system.
- Easy spreading
- Skin retention
- A colling effect on the skin

DISADVANTAGES :

- Some aren't absorb easily through the skin
- There is a posiibility of an allergic reaction.
- The effect of gels initiates slower. (slower but last longer)
- Additives in the gels may irritate skin.
- Application site must be monitored for reaction.
- Effevtiveness may be impacted by temperature, humidity and other environmentalfactors. [3]

TYPES OF SKIN LAYER

Skin is the thick, protective covering of body which comprises of the following layers:

1) Epidermis: It is the outermost covering of stratified squamous epithelial tissue, lacking blood vessels. The major portion of epidermis is made up of keratinocytes (cells). These keratinocytes synthesise a protein called keratin. Desmosomes are protein structures that act like bridges for keratinocytes. These keratinocytes constantly move towards the periphery of the skin. Thickness of epidermis varies from 0.05 mm (at eyelids) to 0.8-1.5mm (on soles of feet and palms of hands). The epidermis is further differentiated into four distinct layers made up of keratin and is distinguished by the various maturation stages. Starting from the lowest lying layer and moving towards the superficial layer, the layers of the epidermis are as follows:

i) Stratum Basale: It is the deepest layer of epidermis present just above the dermis, and consists of dividing and non-dividing keratinocytes. The inner basal surface of epidermal hearationcytes consists of small structures called hemidesmosomes. Gradually these keratinocytes undergo division followedby differentiation and move towards the upper surface. Basal cells consist ofa pigment called melanin, produced by melanocytes.

ii) Stratum Spinosum: As the cells from stratum basale mature, they move upwards to form the next layer, which is stratum spinosum. The cells in thislayer are connected by intercellular bridges called desmosomes. At microscopic level, these desmosomes appear as ‘prickles' . Stratum spinosumcontains Langerhans cells which are dendrites, and are formed in the bone marrow. Langerhan s cells play a crucial role in immunological reactions related to skin.

iii) Stratum Granulosum: The cells now move upwards to reach stratum granulosum where they become Hat and anucleated. The cytoplasm of the cells appears granular.

iv) Stratum Corneum: The stratum corneum is composed of several layers of hexagonal shaped non-viable, comified cells called corneocytes. These corneocytes are keratinocytes that are in their last phase of maturation. The corneocytes form around 10-30 layers in almost every part of the skin; but, the thickest layers are found in the palms and soles. Every corneocyte is enclosed by a protein casing which is filled with keratin proteins having water retaining property. The main strength to stratum corneum is providedby the shape of the cells and orientation of keratin proteins. The corneocytes are sandwiched between the stacked layers of bi-layered lipid in the extracellular space . Corneocyte (Filled with keratin protein filaments)

v) : Corneocyte Lipid Bilaver The corneocyte-bilayered lipid structure acts as a physical barrier. It also retains water for the skin. The absorption capacity of corneocyte is 300% more than its weight. Epidermal cells take 28 days approximately to reach stratum corneum and this time of movement is known as epidermal transit time. 34 Cosmetic Science There is another thinlayer of translucent cells called stratum lucidum. It generally exists in the thick epidermis. Stratum lucidum represents the transition phase between stratum granulosum and stratum comeum. Few scientists refer to stratum spinosum and stratum

granulosum as the Malpighian layer.

2) Dermoepidermal Junction/Basement Membrane: The dermal-epidermal junction represents the acellular zone present between the dermis and the epidermis. It is also referred to as the cutaneous basement membrane. The epidermis gets its nutrition by diffusion and the waste is also discarded by the process of diffusion at these junctions. As age increases, the dermoepidermal junction gets flattened, which is a visible sign of ageing.

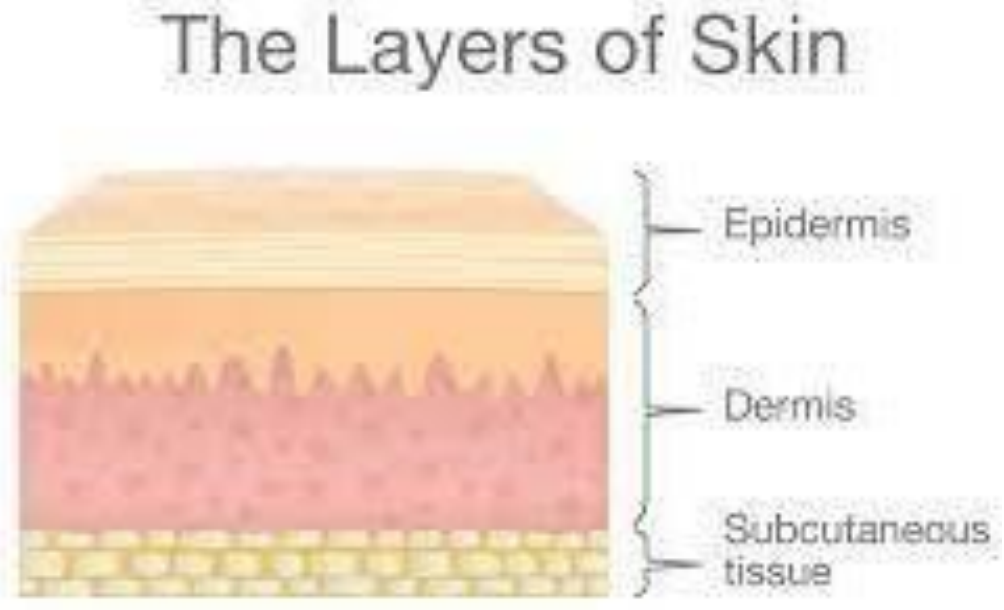
3) Dermis: Thickness of dermis varies in different parts of the body, for example,on the eyelids, it is up to 0.6mm and on the back, palms and soles it is up to 3mm.Dermis lies below the epidermis and is made up of a hard, supportive cellular matrix. It consists of the following two layers:

i) A thin papillary layer, and ii) A thicker reticular layer.

4) Subcutaneous Tissue: The subcutis is composed of a loose connective tissue and adipose tissue. It can be up to 3cm in thickness on the abdomen. This sheet ofadipose tissue is also known as superficial fascia. It contains a layer of areolar tissue having fat and helps in the attachment of the dermis to the underlying structure. H.nr shall Sweat pore Epidermis Epidermal ridge Dermal papilla

• Arrector pili muscle Sebaceous (oil) gland Sweat gland duct Mcmcnne sweat gland Papillary . layer Dermis - Reticular - Artery Subcutaneous layer Adipose connective tissue Hair follicle Sensory nerve fibre Sensory receptors Areolarconnective tissue

Blood vessels present in dermis layer supply the glands, hair follicle of dermis;and the metabolically active stratum basale of the epidermis. A characteristic feature of blood supply of skin is the presence of numerous arteriovenous anastomoses. These anastomoses help in maintaining the body temperature by regulating blood flow through the capillary bed. Lymphatic meshes arise from thepapillae and drain lymph from the skin, which is then drained into the lymph nodesvia larger lymphatic vessels.



Nerve Supply Nerves of the skin are known as cutaneous nerves. Axons of thecutaneous nerves lie in the dorsal root ganglia. They can either be myelinated or non-myelinated. The dermis contains open sensory nerve endings that detect various stimulations

(e.g., pain, itching and temperature). Special kind of corpuscular receptor cells known as Meissner's corpuscles, are present in the skinthat detect touch while the Pacinian corpuscles help in detection of pressure and vibration. Motor receptors of the skin are affected by autonomic nervous system in which the blood vessels and hair erector muscles are supplied by adrenergic neurons and the eccrine sweat glands are supplied by cholinergic nerve fibres. Theendocrine system (and not the autonomic neurons) regulates the sebaceous glands.[4]

LITERATURE REVIEW

1. **Sanjay Krishna Pandey et al. (2020)** – "FORMULATION AND EVALUATION OF ANTIFUNGAL TOPICAL GEL OF C. tora SEED EXTRACT." The researcher in this article has formulated a novel topical antifungal gel containing seed extract of c.tora which shows pH range of 6.25±0.05 to 6.45±0.15, viscosity range 386±27.90 to 680±45.50cp, spread ability 40±2.5 to 38±2 mm and shows antifungal activity against candida albicans. [41]

2. **Fazila Shireen (2015) –** " Anti fungal activity of Aloe Vera : In vitro study."The researcher in this research paper investigate the anti-fungal activity of Aloevera extract on Candida albicans. A. vera extract at 1000 µg/ml concentration effectively inhibit the growth of C. albicans (14 mm) compared with the positive control-amphotericin B (15 mm). [42]

3. **O.Adedayo (1999) –** "Antifungal properties of some components of senna alata flower." The researcher in this research paper investigated antifungal activity of Senna alata extracts exhibited a relatively high antifungal activity against mycelial growth. [43]

4. **Shubhangi Vishwakarma et al. (2019) -** "Formulation And Evaluation Of Antifungal Herbal Gel Containing Ethanolic Extract Of Senna Alata, Murraya Koenigii And Aloe Vera" The researcher in this research paper formulated antifungal herbal gel containing ethanolic extract of senna alata, murraya koenigi iand aloevera which showed the highest percentage of extrudability, good spreadability and rheological properties. [44]

PLAN OF WORK

- Literature review
- Collection of herbs
- Authentication of herbals
- Extraction of herbals
  - Maceration
  - Hot continuous percolation
  - Centrifugation
- Phytochemical screening test
- Formulation of antifungal herbal gel
- Evaluation of antifungal herbal gel
  - Physical aearance
  - pH
  - Viscosity
  - Extrudability
  - Stability
  - Homogenicity
  - Spreadibility
  - Antimicrobial study

Collection and Authentication

- Senna alata :

S. alata, also known as Cassia alata, is a widely distributed herb of the Leguminosae family. It is commonly known as candle bush, craw-craw plant,acapulo, ringworm bush, or ringworm plant. plant is commonly found in Asia and Africa, and has many local names [5]. It has arrays of bioactive chemical compounds.Some of the reported chemical constituents are phenolics (rhein, chrysaphanol, kaempferol, aloeemodin, and glycosides), anthraquinones (alatinone and alatonal), fatty acids (oleic, palmitic, and linoleic acids), steroids, and terpenoids (sitosterol, stigmasterol, and campesterol) [6, 7]. *ese secondary metabolites are reported to display numerous biological activities [8–13].

Geographical Distribution.

S. alata is widely distributed in Ghana, Brazil, Australia, Egypt, India, Somalia,Sri Lanka, and all over Africa [14]. It is an ornamental plant native to the Amazon

Rainforest [15]. Like other Senna species, it is cultivated in humid and tropic regionsof Africa, Asia, West Indices, Mexico, Australia, South America, the Caribbean Islands, Polynesia, Hawaii, Melanesia, and different parts of India [16]. In Philippines, *ailand, and Indonesia, this shrub is widely dispersed and is cultivated for medicinal purposes [17

Dermatophytic Activities. Currently, the leaves, flowers, and bark of S. alata are used for treating various kinds of skin infections and diseases. In *ailand, the plant was mentioned as one of the 54 medicinal plants used for treating scabies, shingles, urticarial, itching, pityriasis versicolor, and ringworm [18]. *e dermatophytic activities displayed by S. alata are linked to the bioactive compoundssuch as anthranols, anthrones, flavonoids, phenols, tannins, and anthracene derivatives [19]. Leaves decoction displayed strong inhibitory activities against S. pyogenes, S. aureus, K. pneurnoniae, E. coli, S. rnarcescens, P. cepacia, and P. aeruginosa [20, 21].

- Murraya koenjii

Murraya koenigii, commonly known as curry leaf or kari patta in Indian dialects, belonging to Family Rutaceae which represent more than 150 genera and 1600 species [22]. It is widely used as a spice and condiment in India and other tropical countries. The leaves have a slightly pungent, bitter and feebly acidic taste, and theyretain their flavour and other qualities even after drying. A scrutiny of literature reveals some notable pharmacological activities of the plant such as activity on heart,Anti diabetic and cholesterol reducing property, antimicrobial activity, antiulcer activity, antioxidative property,

cytotoxic activity, anti diarrhea activity, phagocytic activity. Various parts of Murraya koenigii have been used in traditional or folk medicine for the treatment ofrheumatism, traumatic injury and snake bite [23]. The Murraya species has richest source of carbazole alkaloids. Further, Carbazole alkaloids has been reported for theirvarious pharmacological activities such as anticonvulsant, antitumor, anti- inflammatory, diuretic, antiviral and activities [24]. The leaves of the plants are full of antioxidants, namely, tocopherol, β-carotene, and lutein, and possess antioxidativeand anti-lipid peroxidative activities, providing protection against oxidative stress [25] .

Origins Curry leaf trees are naturalised in forests and waste land throughout theIndian subcontinent except in the higher parts of the Himalayas. From the Ravi Riverin Pakistan its distribution extends eastwards towards Assam in India and Chittagongin Bangladesh, and southwards to Tamil Nadu in India. The plants were spread to Malaysia, South Africa and Reunion Island with South Asian immigrants [26]

- Cassia Tora

Cassia tora Linn (Family: Leguminosae) is annual under shrub grows all over thetropical countries (throughout India, Pakistan, Bangladesh and west China) and grows well in wasteland as a rainy season weed [27]. It grows in low lying coastal area,river banks, abundant in waste places and other moist places like uncultivated fields,up to 1000-1400 meters. It is also known as ‘Chakramard’ in Ayurveda, ‘Panwar’ inUnani and ‘Jue Ming Zi’ in Chinese system of medicine. It is most commonly knownas ‘Sickle pod’ due to Sickle shape of pods.

The leaf extract has shown the significant antifungal activity to inhibit the growth of Candida albicans, Aspergillus niger, Sachharomyces cerevisiae and Trichophyton mentagrophyte [28]. It shows antifungal activity due to chrysophenol and crysophanic acid-9- anthrone and other anthraquinones such as emodine, physcion and rhein [29 30]

Several compounds belonging to anthraquinone and naphthopyrone groups have been isolated from seeds of this plant. Three crystalline substances have been isolated from seeds of C. tora known as tora substance A, B and C. From properties of these substances and some typical derivatives, it appeared that tora substance C might be identical with rubrofusarin a metabolic product of the fungus, Fusarium culmorum and tora substance B with norrubrofusarin the demethylation product of rubrofusarin [31 32 33]

The seeds of C. tora yielded sitosterol from petroleum ether extract, chrysophanol, physion emodin and rubrofusarine from chloroform extract and two glycosides, rubrofusarin -6- β -gentiobioside and 8- Hydroxy-3-methylanthraquinone -1- β -gentiobioside have been found in ethanolic extract 4, 17, 20. Three naphthopyrone glucoside, cassiaside, rubrofusarin -6- O- β-D-gentiobioside and toralactone -9-O- β-D- - gentiobioside isolated from butanol soluble extract of seed [34]

PHYTOCHEMICAL TEST

- **TEST FOR SAPONINS**

Foam test

A small amount of extract taken in a test tube with little quantity of water. Shake vigorously. Appearance of foam persisting for 10 minutes indicates presence ofSaponin.

- *TEST FOR ALKALOIDS*

1. Dragendroff's test**:** Dissolve extract of the herbal drug in chloroform. Evaporate chloroform and acidify the residue by adding few drops of Dragendroff's reagent (Potassium Bismuth Iodide). Appearance of orange red precipitate indicates presence of alkaloids.

2. Mayer's test**:** 2-3 ml of filtrate with few drops of Mayer's reagent gives ppt.

3. Wagner's test**:** 2-3 ml of filtrate with few drops of Wagner's reagent givesreddish brown colour.

4. Murexide test for purine alkaloid**:** To 3-4 ml. test solution add 3-4 drops of conc.HNO3. Evaporate to dryness. Cool and add 2 drops of NH4OH. Purplecolour is observed.

- *TEST FOR FLAVANOIDS*

i. Ferric chloride test**:** To the alcoholic solution of the extract add few drops of neutralferric chloride solution. Appearance of green colour indicates presence of flavanoids.

ii. Shinoda Test**:** To dry extract, add 5 ml. of 95% ethanol, few drops conc. HCL and0.5 g magnesium turnings. Pink colour observed.

iii. Zinc-hydrochloric acid-reduction test: Test solution with zinc dust and few dropsof HCL shows magneta red colour

iv. Alkaline reagent test**:** Test solution when treated with sodium hydroxide solutionshows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

v. Lead acetate solution test: Test solution with few drops of lead acetate solution(10%) gives yellow precipitate.

- *TEST FOR GLYCOSIDES*

i. Baljets test**:** Treat the extract with sodium picrate solution. Appearance of yellowto orange colour indicates presence of glycoside with lactone ring.

ii. Keller-killiani test**:** The test solution with few drops of glacial acetic acid in 2 ml of ferric chloride solution and conc. sulphuric acid is added from the sides of test tubewhich

shows the separation between two layers, lower layer shows reddish brown and upperlayer turns bluish green.

iii. Bromine water test**:**Test solution dissolved in Bromine water gives yellowprecipitate.

iv. Legal's test**:** Test solution when treated with pyridine (made alkaline by addingsodium nitroprusside solution) gives pink to red colour.

- *TEST FOR STEROIDS*

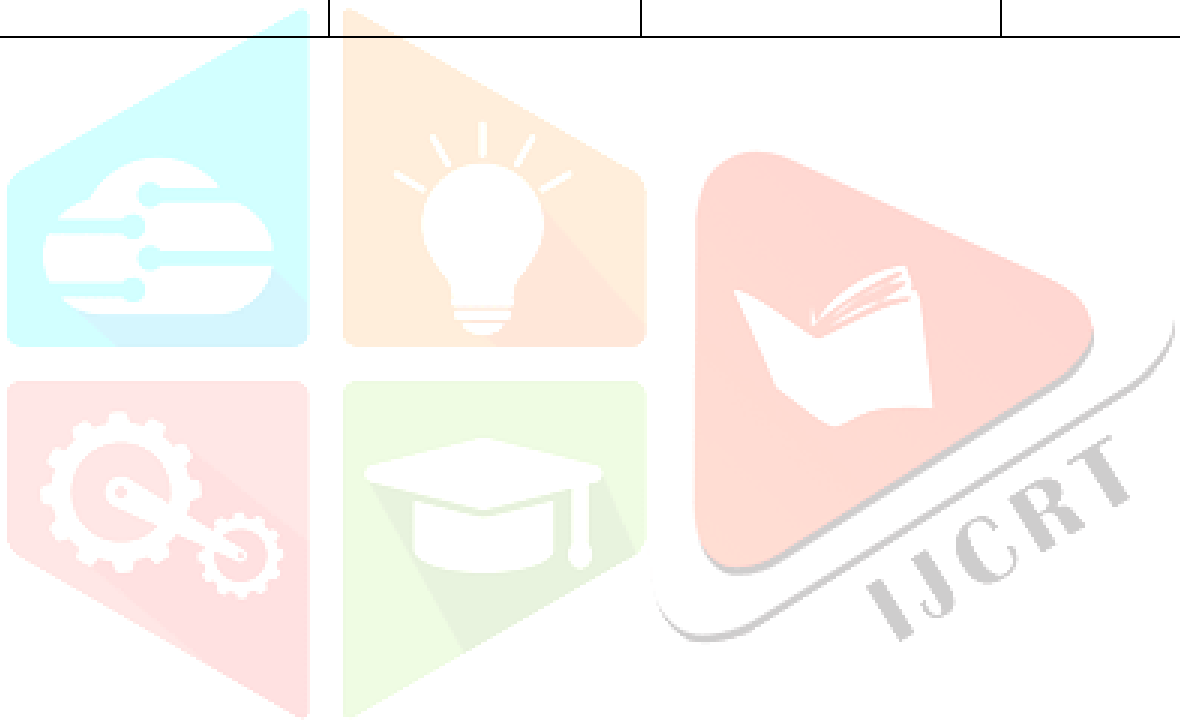
i. Salkowski reaction**:** To 2 ml of extract, add 2 ml chloroform and 2 ml of conc.H2SO4. Shake well. Chloroform layer appears red and acid layer shows greenishyellow fluorescence.

ii. Liebermann's reaction**:** Mix 3 ml extract with 3 ml acetic anhydride. Heat andcool. Add few drops of conc. H2SO4. Blue color appears. [35]

Table result

TEST	Senna alata	Murraya koenigii	Cassia tora
For saponins Foam test	-	-	-
For alkaloids Dragandroff test	+	+	-
Mayers test	-	-	+
Wagner test	+	+	+
Hager's test	+	+	-
Murexide test	+	-	+

Test for flavonoids Ferric chloride test	+	+	+
Shinoda test	+	+	+
Zinc hydrochloric acid	-	-	-
Alkaline reagent test	+	+	+
Lead acetate solution test	+	-	-



Test for glycoside Baljet test	+	+	-
Keller killiani test	+	+	+
Bromine water test	+	+	+
Legal test	+	+	-
Test for steroids Salkowsky test	+	+	+
Lieberman test	-	+-	-

Formula for 50gm of 1 % Herbal Gel

Formulation Ingredients	Quantity In gm/ml
Extract of senna alata	1 gm
Extract of murrya koeinigii	1 gm
Extract of Cassia Tora	1 gm
Carbopol	1 gm
Polyethyleneglcol	5 ml
Methyl paraben	0.5 ml
Propyl paraben	0.05 ml
Triethanolame	1.5 ml
Water	Q. S

METHOD OF PREPARATION

Firstly carbopol 934 was dispersed in distilled water and purified water. kept the beaker aside to swell the carbopol 934 for half an hour and then stirring should be done to mix the carbopol 934 to form gel. In another beaker weight and transfer the required quantity of extracted drug powder and dissolved in polyethylene glycol400 and go for sonication for 10 mint sand the solution was added and mixed to the first solution. 5ml of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath and solution was cooled. Finally full mixed ingredients were mixed properly to the carbopol 934 gel with continuous stirring and lastly triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8 - 7) and to obtain the gel at required consistency. By using this method we prepared 4 formulations with 4 different concentration of carbopol i.e 1% 1.5% 2% 2.5% respectively. [36]

INSTRUMENTS

Sr. no.	INSTRUMENTS	BRAND NAME
1.	**Analytical balance**	
2.	**Digital PH meter**	
3.	**Brook field Viscometer**	DV-I, LV-I spindle, USA
4.	**Digital autoclave**	
5.	**UV spectrophotometer**	UV 1700, Shimadzu, Japan

PHYSICOCHEMICAL EVALUATIONS

- *Physical appearance*

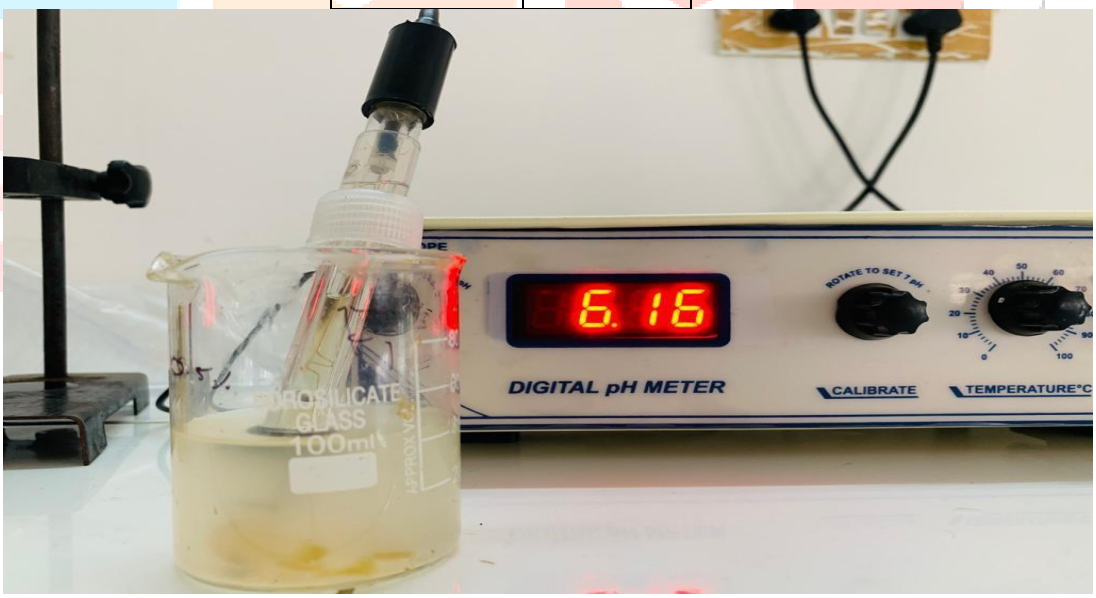
The prepared gel formulations containing Clerodendrum Serratum were inspected visually for their color, homogeneity, consistency and phase separation.

- *Measurement of pH*

The pH of developed gel formulations was determined using digital pH meter. 1 gm of gel was dissolved in 100 ml distilled water and kept aside for two hours. The

measurement of pH of each formulation was done in triplicate and average values arecalculated.

sample	PH
F1 (0.5g)	6.16
F2 (1%)	6.12
F3 (2%)	5.74
F4 (3%)	5.36



- *Spreadability*

Spreadability was determined by the apparatus which consists of a wooden block,which was provided by a pulley at one end. By this method spreadability was measured onthe basis on slip and drag characteristics of gels. An excess of gel (about2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed

ground slideand provided with the hook. A one kg weighted was placed on the top of the two slides for 5 min. to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gm. With the help of string attached to the hookand the time (in sec.) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability.Spreadability was calculated using the following formula**:**

S= M × L/ T

Where, S= Spreadability,

M= weight in the pan (tied to upper slide),L= Length moved by the slide,

T= Time (in sec.)

- *Rheological Study*

The viscosity of the developed gel formulations was determined by using Brookfieldviscometer (Brookfield viscometer RVT) with spindle No. 7.

Result of viscosity

F1	1832
F2	2558
F3	1472
F4	2159

- *Extrudability*

The gel formulations were filled in standerd capped collapsible aluminum tubes

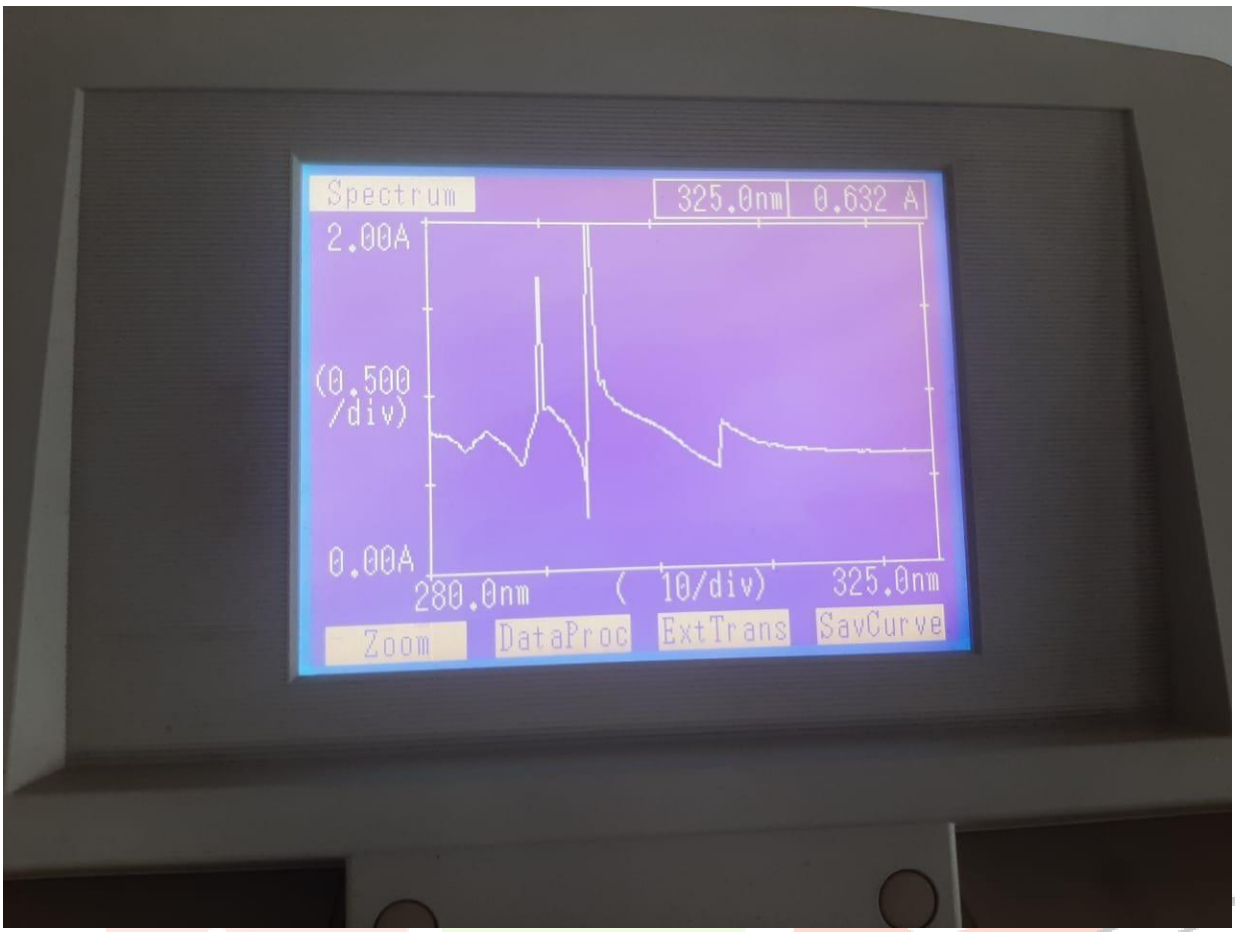
and sealed by crimping to the end. Weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 gm was placed over theslides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percentage of the extruded gel was calculated (>90% extrudability:excellent, >80% extrudability: good, >70% extrudability: fair).

In-vitro Drug Diffusion Study:

For deducing this parameter for all gel formulations, the Franz-Diffusion cell apparatus was used. For making study, egg-membrane was fastened in between the donor and receiver compartment of the apparatus. The receptor compartment was maintained at a temperature of 37 ± 10 C and was filled with 10.0 ml of phosphate buffer ph 6.8. For testing, 0.1 g of gel formulation was placed over egg-membrane and solution of phosphate buffer ph 6.8 in the receptor compartment with stirring at 50 rpm. Then the sample was

withdrawn at regular time interval of 0, 1, 2, 3, 4, 5 and6 hrs. and diluted with 10.0 ml of blank solution and sink condition was maintained..Diffusion study of formulation was carried out in triplicate and average value ± standard deviation was calculated. [37 to 40]

sample	uv range
10 min	0.457
20 min	0.649
30 min	0.563
40 min	0.599
50 min	0.675
60 min	0.785
2 hr	0.819



Antimicrobial screening

Preparation of Fluconazole:

Fluconazole was taken as standard drug and 0.1 g of it was dissolved in 100 ml of DMSO (dimethyl sulfoxide) to obtain the ultimate concentration of 10 mg/ml.

Assessment of Antifungal activity:

The antifungal efficacy of Methanolic plant extract was evaluated by Agar cup bioassay method.

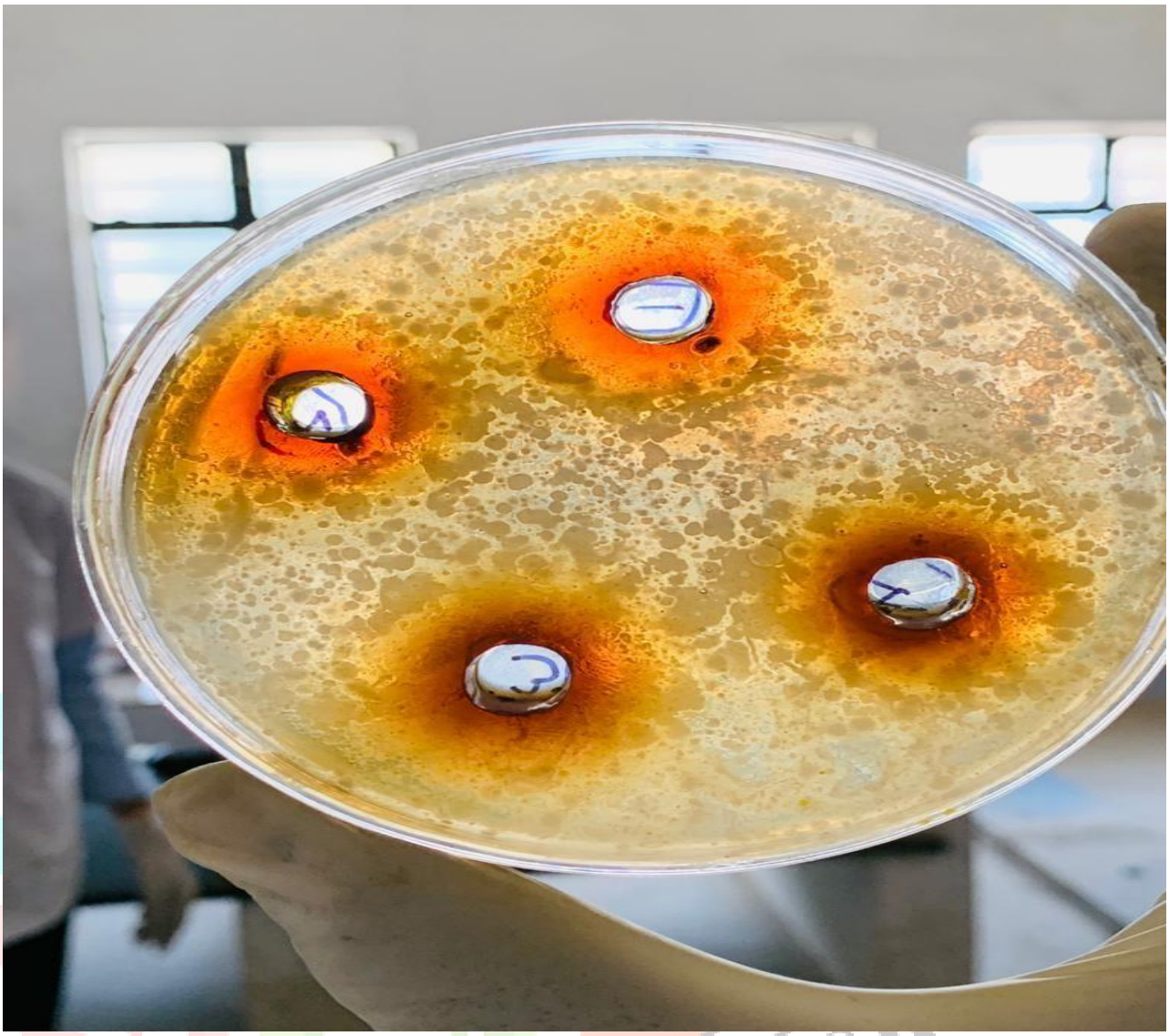
Preparation of plates for inoculation:

The petri plates used for inoculation purpose were made aseptic using oven at 1600 C for 1-1/2 hr. The plates were filled with molten SDA (20 ml) aseptically in laminarair flow. After 30 min, keeping the plates at room temperature, the plates were inoculated with another layer of 5 ml of molten SDA containing 0.05 ml of normal cell lines of E. Coli . The hole was made in each agar plate with the help of cork borerno. 4.

Determination of zone of inhibition:

The required quantity of the gel was transferred into the cavities of petri plates, theseplates were then refrigerated for 1h for pre incubation diffusion. After refrigeration the plates were normalized at room temperature and then incubated at 37±10 c for 24hrs. The same experiment was carried out with standard drug Fluconazole. For accuracy of the result, the experiment had been conducted threefold and medianvalues for zone of inhibition were calculated (Nascimento et al., 2000).

concentration	Zone of inhibition
1 %	7mm
2%	6mm
3%	6mm
standard	5mm



Homogeneity:

To check this parameter, all gel formulation was taken in suitable container and allowed to settle. The homogeneity was tested by visual inspection (Nawaz A et al.,2013, Ubaid M et al., 2016).

Conclusion

As many traditional healers are using this *sennaalata, CASISA TORA and murrya kaunigii* for treating number of fungal and bacterial infections, we made a formulation by using the senna *alata ,CASSIA TORA and murry kainigii* extractions.No change was observed in its pH and other physical parameters and skin irritation studies were observed with all the four formulations. Along with the above the gel formulation is also have good antimicrobial activity. When compared with the standard drug our formulation gels are showing better antimicrobial activity.

By this study results we are concluding that all these four formulations are best in their stability and anti-microbial activity so we can use this formulation for treating microbial infections. Antifungal activity of the herbal gel formulation was also planned to perform by using the isolated fungal strains as early as possible

Result

the antifungal activity of cassia tora senna alata murray koenjii was quantitatively ASSESED by the presence or absence of the inhibition zone and by measuring the diameter of zone of inhibition around the wells or disks .the antifungalactivity was done by using E.colii.and S.Aurues. the rsullts of antimicrobial activity is shown below.this result help us to choose more effective formulation.

concentration	Zone of inhibition
1 %	7mm
2%	6mm
3%	6mm
Standard	5mm

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