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Formulation and Evaluation of Antibacterial Herbal gels of Murraya koenigii, Psidium guajava, Musa acuminate Leaves Extract

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ABSTRACT:
Herbal medicine has become an item of global importance both medicinal and economical. Herbal remedies are getting increasing patient compliance as they are devoid of typical side effects of allopathic medicines. The present research has been undertaken with the aim to formulate and evaluate the herbal gels containing Murraya koenigii (curry leaves), Psidium guajava (Guava leaves), Musa acuminate (Banana leaves) plant leaf extract. The gel formulations were prepared by using Carbapol 940, Murraya koenigii, Psidium guajava, Musa acuminate leaf extract, propylene glycol, methyl paraben, propyl paraben, glycerine and required amount of distilled water. The skin pH (6.8-7) was maintained by drop wise addition of Tri-ethanolamine. The physical parameters of formulated gels like colour, homogeneity, pH, viscosity and spreadibility were evaluated. The gels were evaluated for antibacterial efficiency by agar diffusion method against some bacterial agents. The herbal gels showed that formulations containing Murraya koenigii, Psidium guajava, Musa acuminate leaves extract have better antibacterial activity.
Musa acuminata, commonly known as banana plant is vastly being consumed across the world. It is known for many antimicrobial activities and reports show that phenolic compounds mainly contribute to this trait. Considering these advantages an herbal gel containing 4% extract obtained from plant leaves was prepared. Extraction of phenolic compound from leaves was carried out using suitable solvent. The phenolic recovery from acetone extract was showing good antimicrobial activity. The physiochemical parameters of formulations (pH, viscosity, Spread ability and homogeneity) were determined. The herbal gel showed that formulation containing Musa acuminata leaves extract have better antimicrobial activity. The antimicrobial activity was carried out against E.coli and Candida albicans.
Nature has endowed Guava with many nutritional and medicinal properties. The fruits are 4-12 cm long with round or oval shape depending on the species (red, strawberry, and off-white). The tree, which belongs to the family, Myrtaceae is chiefly grown in countries with tropical and subtropical climate. The pink variety of guava (when dissected) has the maximum medicinal values. Fruits as well as leaves has many health benefits viz, antidiarrheal, antihypertensive, antilipedemic, anticancer etc.

KEYWORDS: Murraya koenigii, Psidium guajava, Musa acuminate, Carbapol 940, Viscosity, Spreadibility. Herbal gel, Antibacterial activity.

I. 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. Recently considerable attention has been paid to utilize eco-friendly and biofriendly plant based products for the prevention and cure of different human diseases. It is documented that most of the world's population has taken in traditional medicine, particularly plant drug for the primary health care.

Antimicrobial properties of certain Indian medicinal plants were reported based on folklore information and only few reports are available on inhibitory activity against certain pathogenic bacteria and fungi.

Use of plants as source of medicine has been inherited and is an important component of the health care system in India. In these systems of Indian medicine, most practitioners formulate and

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dispense their own recipes; hence this requires proper documentation and research.

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action due to an alarming increase in the influence of new and reemerging infectious diseases and development of resistance to the antibiotics in current clinical use.

Medicinal plants contain numerous biologically active compounds which are helpful in improving the life and treatment of disease. Compounds such as carbohydrates, proteins, enzymes, fats, oils, terpenoids, flavonoids, sterols simple phenolic compounds etc. Natural products are the source of synthetic and traditional herbal medicine and are still the primary health care system. The presence of various life sustaining constituents in plants made scientists to investigate these plants for their uses in treating certain infective diseases and management of chronic wounds.

Murraya koenigii, belongs to the family Rutaceae, commonly known as curry-leaf tree, is a native of India, Sri Lanka and other south Asian countries. It is found almost everywhere in the Indian subcontinent, it shares aromatic nature, more or less deciduous shrub or tree up to 6 m in height and 15-40 cm in diameter with short trunk, thin smooth grey or brown bark and dense shady crown.

The M. koenigii is having grey colour bark, longitudinal striatations on it and beneath it white bark is present. Leaves are bipinnately compound, 15-30 cm long each bearing 11-25 leaflets alternate on rachis, 2.5-3.5 cm long ovate lanceolate with an oblique base. Margin is irregularly creatate, petioles 2-3 mm long, flowers are bisexual, white, funnel shaped sweetly scented, stalked, complete, ebracteate, regular with average diameter of fully opened flower being in average 1.12 cm inflorescence, terminal cymes each bearing 60-90 flowers. Fruits are ovoid to sub globose, wrinkled or rough with glands. It is having the size of 2.5 cm long and 0.3 cm in diameter and gets purplish black when ripen. Fruits are generally biseeded. Seeds generally occur in spinach green colour, 11 mm long, 8 mm in diameter and weighs up to 445 mg. Most part of plant is covered with fine down and has a strong peculiar smell. M. koenigii is genus of tree, native to tropical Asia from Himalaya foothill's of India to Srilanka eastward through Myanmar, Indonesia, Southern China and Hainan.

Murraya koenigii tree leaves are commonly used as spice due to aromatic nature of leaves. Carbazole alkaloids, the major constituents of plant are known to have cytotoxic, antioxidant, antimutagenic and anti-inflammatory activities. The leaves are rich in mono-terpenoids and sesquiterpenoids which exhibited antimicrobial activities.

In the present study, an attempt has been made to formulate and evaluate herbal gels containing M. Koenigii leaves extract for antimicrobial screening against pathogenic microorganisms.

80% of the world population relies on medicinal plants for their primary health care. Such herbal medicines that are easily available, cheaper, time tested and considered safer than most of modern synthetic drugs. Furthermore, evolution has already carried out a screening process whereby plants are more likely to survive if they contain potent compounds, which deter animals or insects from eating them. These potent compounds are secondary metabolites with quite complex structures, in which most of them are biologically active compounds. It is sobering that very few plants were been fully studied and the vast majorities have not been studied at all. Banana is thought to have antibacterial activity, antioxidant activity and other biological activities such as antidiabetic, antidiarrheal, anti-tumoral, antimutagenic, antihelminthic and antiulcerogenic. The phytochemical components like alkaloids, glycosides, flavonoids, saponins, steroids, serotonin and dopamine present inBanana also contribute to pharmacological effects. So a preliminary phytochemical screening of the plant is performed. Then select appropriate extract which give better activity. Then the formulation of gel, the efficacy is often dependent on the composition of the vehicle. The ability of a drug in gel formulation to penetrate the skin and exert its effect depends on to consecutive physical events. The drug must first diffuse out of the vehicle to the skin surface and then, it must penetrate the natural barrier to enter into the site of action.

Carbopol polymers are bearing very good water sorption property. They swell in water up to 1000 times their original volume and 10 times their original diameter to form a gel when exposed to a pH environment above 4.0 to 6.0. Because the pKa of these polymers is 6.0 to 0.5, the carboxylate moiety on the polymer backbone ionizes, resulting in repulsion between the native charges, which adds to the swelling of the polymer. The glass

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transition temperature of Carbopol polymers is 105°C (221°F) in powder form. However, glass transition temperature decreases significantly as the polymer comes into contact of water. The polymer chains start gyrating and radius of gyration becomes increasingly larger. Macroscopically, this phenomenon manifests itself as swelling.

Plants profile:

GUAVA LEAVES

Synonym: Peru, Amrud, jam, pyrifera (L.) Kuntze, Myrtus guajava var pyrifera (L.) Kuntze, Myrtus guajava (L) Kuntze, Psidium aromaticum, Guajava Psidium cujavillus Burm. f. Psidium

Biological source: It consist of dried whole plant of Psidium guajava
Family: Myrtaceae
Geographical source: India, Central America, South America.

Organoleptic characters:
Leaf: Green colour, elliptic long rough textured

Flower: whitish in colour
Fruit: Greenish to yellow
Seed: White or pink
Chemical constituents:
Leaves: Flavonol, morin, morin-3-o-lyxoside, morin-3-o-arabinoside, quercetin, quercetin-3-o-arabinoside.

Uses:
1) It is used in the treatment of bacterial infection.
2) It is used in the treatment of diabetes.
3) It is used as anti-diarrheal agent.
4) It used in treatment of ulcer.

CURRY LEAVES

Synonyms: kadipatta, godlimb, sweet neem, Mitha Neem in Hindi, and Karuveppilei in Tamilnadu and Surabhinimba in Sanskrit.
Biological sources: It is consist of whole plant of Murraya koenigii
Family: Rutaceae
Geographical sources: East and south part of India, Pakistan, Sri Lanka, chaina, Australia, united state
Organoleptic character:
Leaf: green in colour, lanceolate shape,
Chemical Constituents: Cinnamaldehyde, carbozol alkaloids, mahanimbine, girinimbine, mahanine.
Uses:
1) It is act as a powerful antioxidant.
2) It may reduce the risk of cancer.
3) It may reduce the risk of heart diseases.
4) It is act as an antibacterial agent.

BANANA LEAVES

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Synonym: kel, robusta, Musa basjoo, herbaceous plant, herb, banana tree, edible banana,Musa paradisiaca sapientum, Musa,abaca.
Biological Source: It consist of dried leaves of Musa acuminate and Musa balbisiana.
Family: Musaceae
Geographical Sources: India, Africa, America
Organoleptic charecters:
Leaf: Greenish in colour
Fruit: greenish to yellow

Chemical constituents: Carotenoids, biogenic amines, phytosterols, cellulose, hemicellulose.
Uses:
1) It is used as an antibacterial agents.
2) It is used in the treatment of epilepsy.
3) It is used in the treatment of diarrhoea.
4) It is used in the treatment of acute dysentery.

Gel:

Pharmaceutical gels are semisolid systems in which there is interaction (either physical or covalent) between colloidal particles within a liquid vehicle. The vehicle is continuous and interacts with the colloidal particles within the three-
The vehicle may be:
- Aqueous
- Hydroalcoholic
- Alcohol based
- Non Aqueous

➢ **Characteristics of gels:**
Gels should possess the following properties:
- Ideally, the gelling agent for pharmaceutical or cosmetic use should be inert, safe, and should not react with other formulation components.
- The gelling agent included in the preparation should produce a reasonable solid-like nature during storage that can be easily broken when subjected to shear forces generated by shaking the bottle, squeezing the tube, or during topical application.
- It should possess suitable anti-microbial activity against microbial attack. The topical gel should not be tacky.
- The gels intended for ophthalmic use should be sterile.

➢ **Types of Gels:**
1. Based on number of : A) Single Phase
   B) Two Phase
2. Based on source of gelling agent: A) Natural
   B) Synthetic
3. Based on nature of gelling agent: A) Organic,
   B) Inorganic
4. Based on type of solvent: A) Hydrophilic
   B) Hydrophobic.

➢ **Advantages of gel:**
- Softens and moisturizes the skin.
- Does not irritate the skin.
- Non toxic
- Avoidance of first pass metabolism.
- Convenient and easy to apply.
- Improve patient compliance.
- Provide suitability for self-medication.

➢ **Disadvantages of gels:**
- Leaves the skin feeling stickiness.
- May dry out, so for bioavailability and stability of gels the glycerol (10%), polyethylene glycol is added.
- Poor permeability of some drugs through the skin.
- Possibility of allergenic reactions.
- Drugs of larger particle size not easy to absorb through the skin.

II. LITERATURE REVIEW:

1) **Mylorappa B. Ningappa, B.L. Dhananjaya, R. Harsha (2009)**: They perform anti-bacterial property of APC protein from curry leaves. In these Protein design as antioxidant protein from curry leaves. By using Streptomycin and gentamycin as standard comparative study of antibacterial activity of curry leaves are done.
2) **Anja klancnik, sasa diskerink (2010):** The aim of this study was evaluation diffusion and diluation method for determining the antibacterial activity of plant extract.MIC are obtain agar dilution browth micro dilution only for gram positive bacteria.
3) **Bipul Biswas, Kimberly Rogers Fredrick Mcelaugnlin and Anand Yadav (2013):** They perform antimicrobial Activity of extract of

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guava leaves (psidium guajava L) on two gram positive and negative bacteria.

By using (Escherichia coli and salmonella enterindis) and gram positive (strophylococus aureus and bacillus cereus) this bacteria are used for antibacterial study of guava leaves.

4) **Naikwade P.V., salvi Gaurav,dalavi sharyu and jadhav Kailash (2014):** the aim of their study is evaluation of antibacterial study of Musa paradisiaca L. leaves. Solvent like petroleum ether, chloroform and ethanol are used to prepare leaf extract by soxhlet apparatus. The antibacterial study was perform on various bacteria like strophylococus aureus and Escherichia coli.
5) **Ajinkya M. Bankar, Manjusha N. Dole (2016):** They perform formulation and evaluation of herbal antimicrobial gel containing musa acuminata leaves extract. The herbal gel showed that formulation containing Musa acuminata leaves extract have better antimicrobial activity.
6) **Sandeep D. S*, Prashant Nayak, Jobin Jose, Rishal Relita M, Sumana D. R.(2017) :** They perform Formulation and Evaluation of Antibacterial Herbal gels of Murraya koenigii Leaves Extract. The gels were evaluated for antibacterial efficiency by agar diffusion method against some bacterial agents. The herbal gels showed that formulations containing Murraya koenigii leaves extract have better antibacterial activity.
7) **PS Shabnashmi and C Cynthia (2017):**In vitro and In silico Studies of Murraya koenigii (L) against Streptococcus mutant. In silico study was carried out to screen the marker compounds from Murraya koenigii. Bismurrayafoline A and murrayazoin showed least binding energy with Streptococcus proteins.
8) **Junaid R Shaikh and MK Patil (2020):** They perform Qualitative tests for preliminary phytochemical screening: An overview.The pharmacological activity of a plant can be predicted by the identification of the phytochemicals. Currently, phytochemicals are determined by various modern techniques, but the conventional qualitative tests are still popular for the preliminary phytochemical screening of plants.
9) **Henry Kwadwo Hackman1*, Reuben Essel Arhin (2020) :** In vitro antibacterial activity of Psidium guajava (Guava) leaves extract on carbapenem-resistant Klebsiella pneumoniae causing multi-drug resistant systemic infections. The outcome of this baseline laboratory studies indicates the possibility of producing efficacious antibiotic to treat carbapenems-resistant systemic infections.The determination of the toxicological effect of the isolated active antimicrobial compounds of guava leaves extract is worth following in subsequent studies.
10) **Mital N. Manavar and samixa R. patel (2022):** they perform development and evaluation of antibacterial poly herbal gel. They used tridox procombens,aloe vera ,murraya koenigii.

The topical gel formulation are more preferred evidence, due to various advantages such as easy to administrate, rapid intraction ,less gracy and cost effective. The prepared polyherbal gel has a good homogeneity, spredability, extrudability stability without any irritation effect on skin.

AIM AND OBJECTIVES

AIM:-Formulation and evaluation of polyherbal gel for anti-bacterial activity containing extract of Murraya koenigii, Psidium guajava, Musa acuminate leaf extract.

OBJECTIVES:-

I. To study the preliminary phytochemical screening of crude extract.
II. To formulate the herbal gel from medicinal plants.
III. To perform evaluation of herbal gel.
IV. To evaluate the anti-bacterial activity of herbal gel against known pathogens by utilizing convenient bio-logical assay.
V. The herbal gel used as anti-bacterial topical application.

III. MATERIALS AND METHODS:

Chemicals

Carbapol-940, methyl paraben, propyl paraben, propylene glycol-400, tri-ethanolamine,
Glycerine, Distilled water.

Collection of plant material:

The plant Murraya koenigii,Psidium guajava, Musa acuminatewas collected from the surrounding agricultural area Kudwa, Gondia. The fresh leaves were separated from the plant and used for extraction.

Drying:

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The collected plant leaves of Murraya koenigii,Psidium guajava, Musa acuminate were washed with water and dried in shade.

Grinding: After drying plant leaves were coarsely powderd with the help of grinder and kept in well closed container.

Soxhlet Extraction:

- The soxhlet extraction is used for the preparation of extract.
- About 10 gm of powdered drug is placed in thimble.
- Soxhlet extractor is placed onto a flask containing the 250ml ethanol extraction solvent. Soxhlet equipped with a condenser.
- The solvent is heated to reflux.
- The solvent vapours travels up a distillation arm and flood into the chamber housing the thimble of solid.
- Powdered drug in chamber slowly fills warm solvent.
- When the soxhlet chamber is almost full, the chamber is emptied by the siphon. The solvent running back down to the distillation flask.

Concentration of crude extract:
After soxhlet extraction the extract was concentrated and used for further formulations.

Preliminary Phytochemical Screening
Test for alkaloids:

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Dragendroff's/ Kraut's	Few mL filtrate + 1-2 mL Dragendorff's reagents	A reddish-brown precipitate	-	+	+
2	Hager's test	Few mL filtratea + 1-2 mL Hager's reagents	A creamy white precipitate	-	+	+
3	Mayer's/ Bertrand's/ Valser's test	Few mL filtratea + 1-2 drops of Mayer's reagent (Along the sides of test tube	A creamy white/yellow precipitate	-	+	+
4	Wagner's test	Few ml filtratea + 1-2 drops of Wagner's reagent (Along the sides of test tube)	A brown/reddish precipitate	-	+	+

Test for Carbohydrate:

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Barfoed's	1mL filtrateb	A reddish-			

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	test	+ 1mL Barfoed's reagent + Heated for 2 min.	brown precipitate	-	+	+
2	Molish's test	2ml filtrateb + 2 drops of alcoholic α–naphthol + 1mL conc. H2SO4 (along the sides of test tube)	A violet ring	-	+	+

Test For detection of reducing sugar:

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Benedict's test	0.5mL filtrateb + 0.5mL Benedict's reagent + Boiled for 2 min	Green/yellow/red colour	+	+	-
2	Fehling's test	1mL each of Fehling's solution A & B + 1mL filtrateb + boiled in water bath	A red precipitate	+	+	-

Test for detection for glycosides:

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Borntrager's test	2mL filtrated hydrolysatec + 3mL Chloroform + shaken well + chloroform layer is separated + 10% Ammonia solution	A pink coloured solution	-	+	-
2	Modified Borntrager's	Plant extract +	A rose-pink to blood red			

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	test	**ferric chloride solution + boil for 5min. + cooled + equal volome of benzene + benzene layer is separated + Ammonia solution**	**coloured solution**	-	+	+

Test for detection of cardiac glycoside :

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Keller-Killani test	1mL filtrate + 1.5mL glacial acetic acid + 1 drop of 5% ferric chloride + conc. H2SO4 (along the side of test tube)	A blue coloured solution (in acetic acid layer)	-	+	-

Test for Detection of Proteins and Amino acids

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Biuret test	2mL filtrate + 1 drop of 2% copper sulphate sol. + 1mL of 95% ethanol + KOH pellet	A pink coloured sol. (in ethanolic layer	+	-	+
2	Ninhydrin test	2mL filtrate + 2 drops of Ninhydrin solution (10mg ninhydrin + 200mL acetone)	A purple coloured sol. {Amino acids}	+	-	+

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Test for detection for Flavonoids

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Lead acetate test	1mL plant extract + few drops of 10% lead acetate solution	A yellow precipitate	+	-	+

Test for detection of phenolic compounds

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Iodine test	1mL extract + few drops of dil. Iodine sol	A transient red colour	+	-	+

Test for detection of tannins:

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Gelatin test	Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl	A white precipitate	+	-	+
2	Bromine water test	10 ml of bromine water + 0.5gm plant extract	Decoloration of bromine	+	-	+

Test for detection of Phlobatannins:

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	HCl test	2mL aq. extract + 2mL 1% HCl (boiled)	A red precipitate	-	-	+

Test for Detection of Saponins :

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Foam test	0.5gm plant extract +	Persistent foam for 10			

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		2mL water (vigorously shaken)	min	+	-	+

Test for detection of Triterpinoides

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Salkowski's test	Filtratef + few drops of conc. H2SO4 (Shaken well and allowed to stand)	Golden yellow layer (at the bottom)	-	+	+

Test for detection of Diterpenes:

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Copper acetate test	Plant extract is dissolved in distilled water + 3-4 drops of copper acetate solution	Emerald green colour	-	+	-

Test for Detection of Resins:

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Acetic anhydride test	1mL plant extract + Acetic anhydride solution + 1mL conc. H2SO4	Orange to yellow	+	-	-

Test for Detection of Fixed Oils and Fat:

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Spot test/ Stain test	Little quantity of plant extract is pressed in between to filter papers	Oil stain on the paper	-	+	-

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Test for Detection of Volatile Oils

Sr no	Test	Procedure	Observation (Indicating positive test)	Guava leaves	Curry leaves	Banana leaves
1	Fluorescence test	l0 mL of extract, filtered till saturation, exposed to UV light	Bright pinkish fluorescence	-	+	-

Detected = (+) ; Not detected = (-)

Formulation of Placebo Gel (Control formulation):

For the preparation of gel formulation, firstly take carbopol 940 which was then dispersed in distilled water along with methyl paraben, propyl paraben and glycerine kept for overnight. Take the leaves extract of murraya koenigii, Psidium guajava, Musa acuminate 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. The control batch formulation is shown in Table 1.

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 Murraya koenigii, Psidium guajava, Musa acuminate 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.

The pictures of formulated gels are shown in below Fig. 1 and the various formulations of herbal gels are shown in Table 2.

Table 1: Control batch formulation of herbal gels

Ingredients	Quantity
Carbapol 940	5.0 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 p^H)	q.s.
Distilled water	100 ml

Fig. 1: Herbal gel formulations of Plant extract

Table 2: Development of Herbal gel formulations

Ingredients	F1	F2	F3	F4
Murraya koenigii	0.50g	1g	1.5g	2g
Psidium guajava	0.50g	1g	1.5g	2g
Musa acuminate	075g	1.5g	2.25g	3g
Carbapol 940	2.5g	2.5g	2.5g	2.5g
Propylene glycol	5ml	5ml	5ml	5ml
Methyl paraben (0.5%)	0.2g	0.2g	0.2g	0.2g
Propyl paraben (0.2%)	0.2g	0.2g	0.2g	0.2g
Glycerine	0.5ml	0.5ml	0.5ml	0.5ml

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Triethanolamine (to maintain p^H)	q.s.	q.s.	q.s.	q.s.
Distilled water	Upto 50 ml	Upto 50ml	Upto 50ml	Upto 50ml

Thin Layer Chromatography

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures.

In this technique inert surface i.e. tlc plate made by glass. And silica gel G is placed on the slide and then dry in oven after drying put a spot of active drug i.e. leaves extract and then kept in mobile phase upto 75% and then observe the result.

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 20 rpm using spindle no.64.10

Spreadability:

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

Skin irritation study:

Skin irritation study of gel formulation was carried out by applying the gel on the hand.

Determination of clarity and color:

It was done with naked eyes against white background.

Determination of Odor: -

It was done by mixing gel in water and taking the smell.

In Vitro diffusion study:

Antibacterial activity:

The antibacterial screening of herbal gels was done by disc diffusion method. The gels were tested against bacterial agents namely 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.

IV. RESULTS AND DISCUSSION:

The results of physical parameters of formulated herbal gels like colour, homogeneity, pH, viscosity and spredibility were shown in below Table 3.

The spredibility of formulated herbal gels is shown in below Fig 2

Fig 2: Spreabability of formulated herbal gels

The results of antibacterial activity of all formulated herbal gels against some pathogenic microorganisms is shown in below Table 4 and the results of zone of inhibition of all formulated herbal gels against the pathogens is represented graphically in below Fig 3

Table 3: Results of physical parameters of all formulated herbal gels

Formulation code	Colour	Homogenicity	p^H	Viscosity(cp)	Spreadibility(mm)
F1	Light green	Good	6.25	14332	17
F2	Greenish	Good	7	13200	18
F3	Dark greenish	Good	6.60	15100	19.8
F4	Dark greenish	Good	6.29	15970	24

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Table 4: Antibacterial activity of herbal gels

Micro-organism culture	Zone of inhibition of herbal gels (mm)					
	Standard drug		F1	F2	F3	F4
E.coli	2512	15	14	13		
S. aureus	28		16	18	17	15

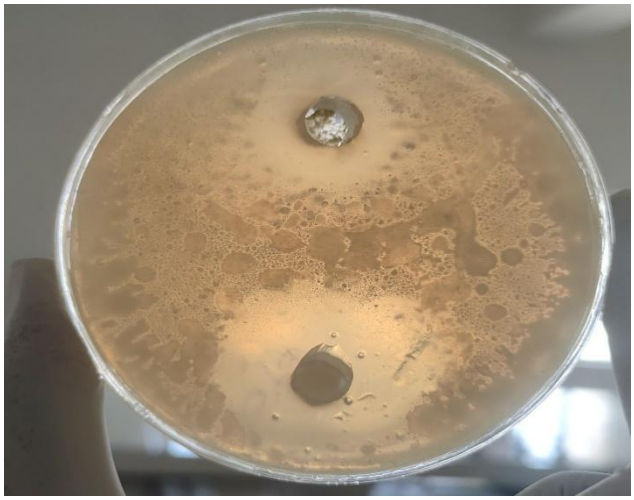

Fig. 3: Antibacterial activity of formulated herbal gels

Discussion:

The colour of all the formulated herbal gels was 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 12000- 16000cp at 20 rpm measured with Brookfield viscometer. The spreadability of all herbal gels was in the range of 36-48 mm. The antibacterial activity of all the formulated herbal gels showed good results of zone of inhibition against skin pathogens.

V. CONCLUSION:

From the present investigation, it has been revealed that herbal gels of plant Murraya koenigii leaves extract can be formulated using carbopol 940 as polymer with other ingredients and the evaluation of physical parameters shown satisfactory results. From the antibacterial activity it was found that prepared herbal gels of Murraya koenigii, Psidium guajava, Musa acuminate leaves extract were significantly active against tested pathogens which was comparable with standard antibiotic. Hence, from the overall results, finally it was concluded that the formulated herbal gels have significant antimicrobial properties and hence will be better, safe and effective than allopathic medications.

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