International Journal of Pharmacy and Pharmaceutical Science
www.pharmacyjournal.org
Online ISSN: 2664-7230, Print ISSN: 2664-7222
Received: 05-05-2021, Accepted: 12-06-2021, Published: 13-06-2021
Volume 3, Issue 1, 2021, Page No. 37-43

Formulation and evaluation of anti-bacterial herbal gels of *Psidium guajava, Murraya koenigii*, and *Musa acuminata* leaves extract

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DOI: https://doi.org/10.33545/26647222.2021.v3.i1a.67

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 acuminata* (Banana leaves) plant leaf extract. The gel formulations were prepared by using Carbapol 940, *Murraya koenigii*, Psidium guajava, *Musa acuminata* 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 acuminata* 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 acuminata*, Carbapol 940, Viscosity, Spreadibility. Herbal gel, Antibacterial activity

1. 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 bio friendly 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. [1]

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 dispense their own recipes; hence this requires proper documentation and research. [2]

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 re-emerging infectious diseases and development of resistance to the antibiotics in current clinical use. [3]

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. [4]

Bacterial infection

Bacterial infections are any illness or condition caused by bacterial growth or poisons (toxins). You can get sick from getting harmful

bacteria in your skin, gut (GI tract), lungs, heart, brain, blood or anywhere else in your body.
Harmful bacteria from the environment, an infected person or animal, a bug bite or something contaminated (like food, water or surfaces) can cause infections. Bacteria that's not normally harmful but that gets into a place in your body where it shouldn't be can also cause infections.

Symptoms of bacterial infection
Symptoms of bacterial infections vary depending on where in your body is infected. The main symptom is often fever, except skin infections, which usually cause redness or pain on your skin. Common symptoms of bacterial infections include:
- Fever.
- Chills.
- Fatigue (tiredness).
- Headache

Gel
A gel is a solid or semisolid system of at least two constituents, consisting of a condensed 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 possess 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 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.

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. [5]

Plant profile guava leaves

Fig 1: Guava leaves

Biological source: It consist of dried whole plant of *Psidium guajava.*

Family: Myrtaceae
Synonym: Peru, Amrud, jam, yellow guava, lemon guava, apple guava.
Taxonomy
- Kingdom: Plantae
- Order: Myrtales
- Family:Myrtaceae
- Genus: Psidium Bloom
- Species:P. guajava

General description
- time: June to September (Ambe- bahar) and November to March (Mrig- Bahar)
- Bloom Colour: Flower- white Fruits- Greenish to yellow
- Height: 20 feet (6.1m)

Chemical constituents: The leaves of *P. guajava* contain
- Flavonol morin,
- Morin-3-O-lyxoside,
- Morin-3-O-arabinoside,
- Quercetin and
- Quercetin-3-O-arabinoside

Pharmacological activities of plant
Psidium guajava plant shows pharmacological activity as follows-
- Anti- inflammatory activity
- Antibacterial activity
- Anti-Diabetic activity
- Antihypertensive activity
- Anti-rheumatism activity
- Anti-ulcer activity
- Anti-Diarrheal activity

Uses
- It is used in the treatment of bacterial infection.
- It is used in the treatment of diabetes.
- It is used as anti-diarrheal agent.
- It used in treatment of ulcer.[6]

3.2 Curry leaves

Fig 2: Curry plant

Biological sources: It is consist of whole plant of *Murraya koenigii.*
Family: Rutaceae

Synonyms: Kadipatta, godlimb, sweet neem, Mitha Neem in Hindi, and Karuveppilei in Tamil Nadu and Surabhinimba in Sanskrit.

Taxonomy
- Kingdom: Plantae
- Order: Sapindale
- Family: Rutaceae
- Genus: Murraya
- Species: M. koenigii

General description
- Bloom time: Spring, Summer, Fall
- Bloom Colour: Flower- white
- leaves- Green
- Height: 6-20 feet

Chemical constituents: Compounds found in curry tree leaves, stems, bark, and Seeds include
- Cinnamaldehyde,
- Numerous carbazole alkaloids,
- Mahanimbine,
- Girinimbine,
- Mahanine.

Pharmacological activities of plant
Murraya koenigii plant shows pharmacological activity as follows-
- Antidiarrheal Activity,
- Antifungal Activity,
- blood purifying Agents,
- anti-inflammatory Agents,
- anti-depressant agent

Uses
- It is act as a powerful antioxidant.
- It may reduce the risk of cancer.
- It may reduce the risk of heart diseases.
- It is act as an antibacterial agent.[7]

3.3 Banana plant

Fig 4: Banana plant

Biological source: It consist of dried leaves of *Musa acuminata* and *Musa balbisiana*.
Family: Musaceae
Synonym: kel, robusta, Musa basjoo, herbaceous plant, herb, banana tree, edible banana.

Taxonomy
- Kingdom: Plantae
- Order: Zingiberales
- Family: Musaceae
- Genus: Musa

General description
- Bloom time : July – September
- Bloom Colour: Orange to purple
- Height: 10-20 feet (3-6 meter)

Chemical constituents
- Carotenoids,
- Biogenic amines,
- Phytosterols,
- Cellulose,
- Hemicellulose

Pharmacological activities of plant
Musa acuminata plant shows pharmacological activity as follows-
- Antidiarrheal Activity,
- Antifungal Activity,
- Blood purifying Agents,
- Anti-inflammatory Agents, and
- Anti-depressant agent

Uses
- It is used as an antibacterial agents.
- It is used in the treatment of epilepsy.
- It is used in the treatment of diarrhoea.
- It is used in the treatment of acute dysentery.[8]

Experimental method
Preformulation study
Collection of plant material
The leaves of curry, banana and guava was collected from local house of Gondia region. These leaves was wash with water for remove dust. These plant leaves dry in shade to maintain to chemical qualities of leaves. Dried leaves was grind with the help mixer grinder.

Preparation of plant extract
The dried plant material (leaves) is used for extraction. The fresh part of plant dried by air-cured method which is carried in the shade outdoors, after complete drying of leaves, make finely divided powder by grinding method. Extraction of *Psidium guajava*, *Murraya koenigii*, and *Musa acuminata* done by hot-continuous method or soxhlet extraction method, in which 50gm of finely divided powder take and filled in extractor. Ethanol used as solvent in extraction which is filled in boiling flask, and condensers are connected to it for condensation process. It takes 24hr to complete extraction process and extract collected in boiling flask. Collected extract evaporate on water bath for 2hr and we get concentrated extract. [9]

Preliminary phytochemical screening
A) test for alkaloids [Mayer's test]
1.36gm of mercuric chloride dissolved in 60ml and 5gm of potassium iodide were dissolved in 10 ml of distilled water respectively. These two solvents were mixed and diluted to 100ml using distilled water. To 1ml of acidic aqueous solution of samples

few drops of reagent was added. Formation of white or pale precipitate showed the presence of alkaloids**.**

B) Test for flavonoids [Shinoda test]
In a test tube containing 0.5ml of alcoholic extract of the samples, 5 to 10 drops of diluted HCl and small amount of Zn or Mg were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown colour indicated the presence of flavonoids.
C) Test for glycosides [AQUOUS naoh TEST]
A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.
D) Test for steroids [Salkowski's test]
About 100mg of dried extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface was an indicative of the presence of steroidal ring.
E) Test for cardiac glycosides [Keller killiani's test]
About 100mg of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid was added. A brown ring obtained at the interface indicated the presence of a de oxy sugar characteristic of cardenolides**.**
F) Test for saponins [foam test]
A drop of sodium bicarbonate was added in a test tube containing about 50ml of an aqueous extract of sample. The mixture was shaken vigorously and kept for 3min. A honey comb like froth was formed and it showed the presence of saponins**.**
G) Test for resins [Acetic anhydride test]
To 2ml of chloroform or ethanolic extract 5 to 10ml of acetic anhydrite was added and dissolved by gentle heating. After cooling, 0.5ml of H2SO4 was added. Bright purple colour was produced. It indicated the presence of resins.
H) Test for phenols [ferric chloride test]
To 1ml of alcoholic solution of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols**.**
I) Test for tannins [lead acetate test]
In a test tube containing about 5ml of an aqueous extract, a few drops of 1% solution of lead acetate was added. Formation of a yellow or red precipitate indicated the presence of tannins.
J) Test for terpenoid [Salkowski's test]:
2ml of chloroform and 1ml of conc. H2SO4 was added to 1mg of extract and observed for reddish brown colour that indicated the presence of terpenoid.
K) Test for quinone [Conc. Hcl test]
To 1ml of extract, a few drops of concentrated hydrochloric acid were added. A yellowish brown colour was observed that showed the presence of quinone.
L) Test for proteins [Ninhydrin test]
Ninhydrin was dissolved in acetone. The leaf extract was treated with ninhydrin and observed for the formation of purple colour. [10]

7.1.4 Thin layer chromatography
Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel G as stationary phase. The mobile phases.[11]

7.2 Formulation and evaluation
7.2.1 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 acuminata* 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 3. [12]

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 acuminata* in propylene glycol which was then added in polymer dispersion. Remaining quantity of water was then added and neutralized to pH 7 with trethanolamine 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 4. [12]

7.2.3 Formula

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

Batches prepaired

Table 2: Development of Herbal gel formulations

Ingredients	F1	F2	F3	F4
Murraya koenigii	0.50 g	1 g	1.5 g	2 g
Psidium guajava	0.50 g	1 g	1.5 g	2 g
Musa acuminata	075 g	1.5 g	2.25 g	3 g
Carbapol 940	2.5 g	2.5 g	2.5 g	2.5 g
Propylene glycol	5 ml	5 ml	5 ml	5 ml
Methyl paraben (0.5%)	0.2 g	0.2 g	0.2 g	0.2 g
Propyl paraben (0.2%)	0.2 g	0.2 g	0.2 g	0.2 g
Glycerine	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Triethanolamine (to maintain p^H)	q.s.	q.s.	q.s.	q.s.
Distilled water	Up to 50 ml	Up to 50ml	Up to 50ml	Up to 50ml

Evaluation of herbal gel
1. P^H
The P^H of all the formulated herbal gels was measured by using digital P^H meter. First, P^H meter was calibrated with standard buffers (P^H 4 and 7). P^H of products was measured 20min, 30min, 1 hour after preparation. The test was repeated three times.
2. Appearance and homogeneity
The developed individual and polyherbal gels were evaluated for physical appearance and homogeneity by visual observation.
3. Viscosity
Brookfield DV-III viscometer was used for the determination of

viscosity. At first, viscometer was calibrated by Brookfield Viscal Kit. Gel samples were placed at room temperature for 30 min. Then, they were poured in apparatus container. Number 74 spindle was attached then viscosity was determined at 25°C and 100–250 rpm. The results were reported in average after triplicate experiments.

4. Spreadability

The spreadability of the gel formulations was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm x 20 cm) after one min. The standard weight applied on the upper plate was 125 gm. [12]

5. Skin irritation test

Skin irritation studies were carried out in order to detect irritation and sensitization under conditions of maximal stress which may occur over a prolong contact with the skin surface. Skin irritation test is done by using patch test on the back skin of volunteer. Gel (F2) (2×2 cm2) was applied to the clean skin of the volunteer back. Volunteer was then kept under observation for a period of 4-6 hours to detect any sign of erythema, redness, sensitization or any other allergic reaction.

Antibacterial study

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 [13].

Results and Discussions

8.1 pre-formulation evaluation 3

In this proposed work we developed alternative dosage form for Anti-bacterial activity.

Preparation of plant extracts

The dried leave part of plant powdered and extracted by hot-continuous technique of extraction with ethanol. And the solvent was removed by evaporation method with the help of water bath.

8.1.1 Characterization and identification of plant extract

Physical properties: Colour, odour, Appearance was studied.

Table 3: Physical properties of gel

Colour	Dark Green
Odour	Pleasant
Appearance	Semi-transparent

Solubility studies

Table 4: Solubility studies

Water	Methanol	Ethanol	5%NaOH	Ether
Sparingly soluble	soluble	soluble	Sparingly soluble	insoluble

8.1.2 Preliminary phytochemical screening of plant extract

Preliminary phytochemical test for identification of phytoconstituents in *Psidium guajava*, *Murraya koenigii*, and *Musa acuminata* plant extract.

Table 5: Phytochemical screening

Sr.no.	Plant constituents	Test/Reagent	*Psidium guajava*	*Murraya koenigii*	*Musa acuminata*
1	Alkaloids	Mayer's test	-	+	+
2	Flavonoids	Shinoda test	+	-	+
3	Glycosides	Aquous NaOH test	-	+	-
4	Steroids	Salkowski's test			
5	Cardiac glycoside	Kellar killani's test	-	+	-
6	Saponins		+	-	+
7	Resin	Acetic anhydride test	+	-	-
8	Phenol	Ferric chloride test	+	-	+
9	Tannins	Lead acetate test	+	-	+
10	Terpenoids		-	+	+
11	Quinones	Conc. HCL test			
12	Proteins	Ninhydrin test	+	-	+

8.1.3 Thin layer chromatography

The thin layer chromatography of *Psidium guajava*, *Murraya koenigii*, and *Musa acuminata* plant extract was found to be

Table 6: TLC

Sr.no	Plant Extract	RF value
1	*Psidium guajava*	0.68
2	*Murraya koenigii*	0.51
3	*Musa acuminata*	0.98

8.1.4 Evaluation of herbal gel

8.1.4.1 P^H

P^H of *Psidium guajava*, *Murraya koenigii*, and *Musa acuminata* plant extract was found to be

Table 7: P^H of gel

Sr.no.	Formulation	pH
1	F1	6.25
2	F2	7
3	F3	6.60
4	F4	6.29

8.1.4.2 Appearance and homogeneity

Appearance and homogeneity of *Psidium guajava*, *Murraya koenigii*, and *Musa acuminata* plant extract was found to be

Table 8: Appearance and homogeneity

Sr. no.	Formulation	Colour	Homogeneity	Odour	Appearance
1	F1	Light green	Good	Pleasant	Semi-transparent
2	F2	Greenish	Good	Pleasant	Semi-transparent
3	F3	Dark greenish	Good	Pleasant	Semi-transparent
4	F4	Dark greenish	Good	Pleasant	Semi-transparent

8.1.4.3 Viscosity

The Viscosity of *Psidium guajava, Murraya koenigii*, and *Musa acuminata* plant extract was found to be

Table 9: Viscosity of gel

Sr.no.	Plant extract	Viscosity (cp)
1	F1	14332
2	F2	13200
3	F3	15100
4	F4	15970

8.1.4.4 Spreadability

The Spreadability of *Psidium guajava, Murraya koenigii*, and *Musa acuminata* plant extract was found to be

Table 10: Spreadability of gel

Sr. no.	Plant extract	Spead ability (mm)
1	F1	44
2	F2	36.8
3	F3	37.6
4	F4	45

8.1.4.5 skin irritation test

Table 11: Skin irritation test

Time	Interpretation
10 min.	No reaction
1 hour	No reaction
2 hour	No reaction

Skin irritation studies shows no sign of erythema or any other skin irritation reaction.

8.1.4.6 Determination of *in-vitro* drug release

Table 12: Determination of *in-vitro* release

Time (In Minutes)	Absorbance
10 min	0.168
20 min	0.175
30 min	0.191
40 min	0.220
50 min	0.245
60 min	0.271
120 min	0.476
180 min	0.694

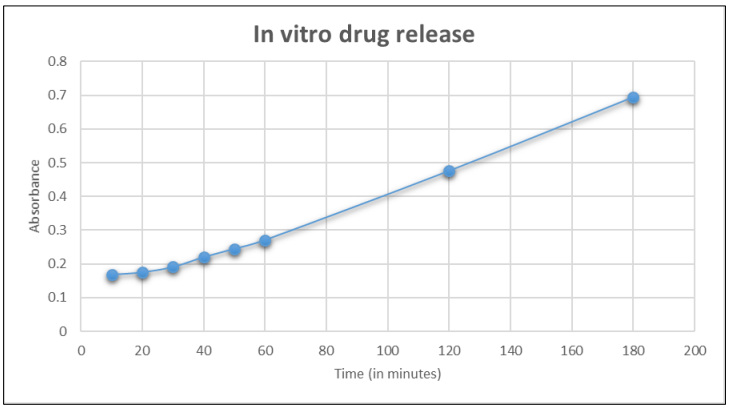

Fig 5: *In-vitro* drug release

8.1.4.8 Antibacterial Study

8.1.4.8.1 Antibacterial Study of Guava, Curry And Banana Leaves Extract

Antibacterial study of guava leaves extract

Table 13: Antibacterial study of guava leaves extract

Micro-organism culture	Zone of inhibition of Guava leaves (mm)			
	A (1%)	B (2%)	C (3%)	D (4%)
E. coli	11	12	15	13.2
S. aureus	14	15.2	14.2	16

Antibacterial study of curry leaves extract

Table 14: Antibacterial study of curry leaves extract

Micro-organism culture	Zone of inhibition of Curry leaves (mm)			
	A (1%)	B (2%)	C (3%)	D (4%)
E. coli	11.2	19	18	16
S. aureus	11..7	18.3	17	15.2

Antibacterial study of banana leaves extract

Table 15: Antibacterial study of banana leaves extract

Micro-organism culture	Zone of inhibition of Banana leaves (mm)			
	A (1%)	B (2%)	C (3%)	D (4%)
E. coli	12.5	13.2	14.4	12.7
S. aureus	12	13.8	12.6	11.4

8.1.4.8.2 Antibacterial study of formulated herbal Gel

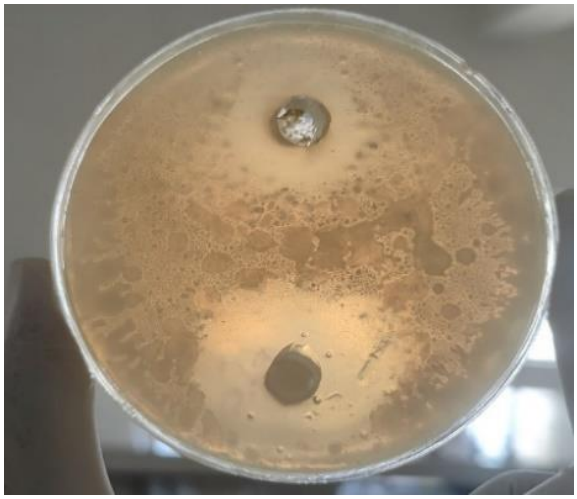

Fig 6: Antibacterial activity of formulated herbal gels

Table 16: Antibacterial activity of formulated herbal gels

Micro-organism culture	Zone of inhibition of herbal gels (mm)				
	Standard drug (Streptomycin)	F1	F2	F3	F4
E. coli	25	22	24	23	25
S. aureus	28	25	27.3	26.5	26

9. Discussions

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.

10. 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 acuminata* 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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