



FORMULATION AND EVALUATION OF HERBAL GEL CONTAINING *MURRAYA KOENIGII* (FRUIT) 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* plant fruit extract. The gel formulations were prepared by using Carbapol 934, guar gum and sodium alginate *Murraya koenigii* fruit 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, spreadability, drug content, diffusion study and stability studies were evaluated. The gels were evaluated for antimicrobial efficiency by agar diffusion method against some bacteria. The herbal gels showed that formulations containing *Murraya koenigii* fruit extract have better antibacterial activity.

KEYWORDS: *Murraya koenigii* fruit, herbal gel, anti-microbial activity

INTRODUCTION

Murraya koenigii is a multipurpose plant, native of India. It is found in tropical and sub-tropical region in the world. All parts of plants are useful to treat and cure various diseases and useful for preparation of various pharmaceutical formulations and cosmetic preparations. Different parts of the *murraya koenigii* plant like roots, leaves, stem, bark, fruits and seeds have been used in combating infections and strengthening the immune system. The plants contains many major phytochemical compounds, Vitamins and nutrients. It is also rich source of nutrients and also it contains many pharmacological activity like anti-inflammatory, anti-pyretic, hypoglycemic, anti-ulcer, wound healing, anti-microbial, anti-fungal and memory enhancing properties,etc.¹ The fruits of *Murraya koenigii* plant is shown in figure:1



Figure 1: Fruits of *Murraya koenigii*

Herbal gel in skin

Gels are applied directly to the skin, mucus membrane or the eye to provide local action. They act as long acting forms of drug injected intramuscularly or implanted into the body. Cosmetically gels have been employed in wide variety of products, including shampoos, fragrance products, dentifrice, skin and in hair care preparations. Gels have better potential as a vehicle to administer drug topically in comparison to ointment, because they are non-sticky, requires low energy during formulation, are stable and have aesthetic value².

MATERIALS AND METHODS:

Materials:

The mature fresh fruits of *murraya koenigii* were collected from local area and authenticated by Dr. N. Senthil kumar, Head and Associate professor of Botany, Ayya Nadar Janaki Ammal College, Sivakasi. Xanthan gum was procured from Madhu hydrocolloids Pvt. Ltd., Gujarat, India. Carbopol 934 was procured from Maruti chemicals, Ahmedabad, India. HPMC K 100 M was procured from Sun bulkactive Pvt. Ltd., Mumbai. Triethanolamine was procured from Ultimate chem India Pvt. Ltd., Maharashtra. Propyl paraben was procured from Unicorn Petroleum Industries Pvt. Ltd., Mumbai. Propylene glycol was procured from Meru chem Pvt. Ltd., Mumbai, menthol oil from Everest Flavours Pvt. Ltd., Mumbai, methyl paraben from Akil healthcare Pvt. Ltd., Gujarat and Purified water was procured from Andavar plus drinking water, Chennai. All other chemicals and reagents used were of analytical grade.³

METHODS

EXTRACTION OF PLANT MATERIAL

Soxhlet extraction is a continuous process of extraction with a hot organic solvent typically soxhlet extraction is used when the desired compound has a limited solubility in a solvent and the impurity is insoluble in that solvents⁴. The fruits of *murraya koenigii* were collected from local area, the seeds removed from fruits and dried in the shade and powdered to get coarse powder. About 100gm of dry powder was weighed and packed in soxhlet apparatus. The powder was extracted with ethanol for 72 hours until the drug gets exhausted. A brownish black waxy residue was obtained and the extracts were concentrated by distillation process and dried⁵

The physico-chemical characters of *murraya koenigii* fruit extract such as ash value, acid insoluble ash, water soluble ash and loss on drying were determined.

Phytochemical screening study of such as test for alkaloids, proteins, glycosides, carbohydrates and sugars, tannins and phenolic compounds, flavonoids, steroids and test for fixed oils and fats were determined.

The chemical constituents of the *murraya koenigii* fruit extract was analysed by Gas Chromatography-Mass Spectroscopy.

FT-IR studies of the pure *murraya koenigii* fruit extract, gelling agent and combination of crude drug and gelling agent were carried out to found any interaction between drug and excipients used in the formulation. FT-IR study was performed using IR spectroscopy (SHIMADZU)⁶.

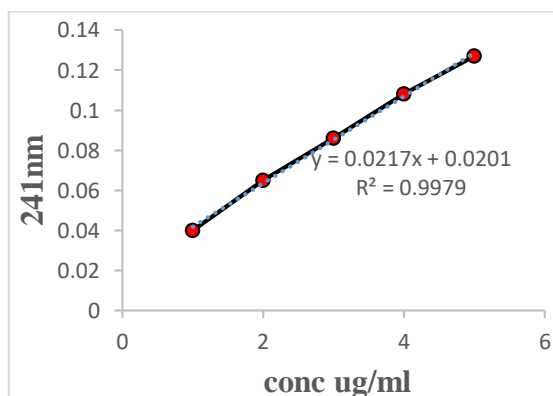
STANDARD CURVE OF MURRAYA KOENIGII FRUIT EXTRACT

Preparation of (various concentrations) working stock solution

Various concentrations of 1,2,3,4 and 5 µg/ml solutions are prepared by diluting 1,2,3,4 and 5ml of (100 µg/ml) to 10ml volumetric flask with phosphate buffer pH 6.8. The absorbance was measured by using UV spectrophotometric method at 241nm against phosphate buffer pH 6.8 as blank. A standard curve was drawn by relating concentration (µg/ml) on X-axis and absorbance of *murraya koenigii* at 241nm on Y-axis. The standard curve was used to estimate the drug content from the *murraya koenigii* herbal gel⁴.

Table 1: Standard Calibration Curve Data of *Murraya koenigii* fruit extract

S.No	Concentration (µg / ml)	Absorbance at 241nm
1	1	0.040
2	2	0.065
3	3	0.086
4	4	0.108
5	5	0.127



Standard Curve of *Murraya koenigii* Fruit Extract

FORMULATION OF *MURRAYA KOENIGII* HERBAL GEL

Preparation of herbal gel containing *murraya koenigii* fruit extract:

Accurately weighed guar gum was taken in a beaker and dispersed in 10 ml of distilled water. Keep the beaker aside to swell guar gum for half an hour and then stirred using blender mixer for 30 min. Take propylene glycol in a separate beaker and add weighed quantity of propyl paraben and methyl paraben to it and stirred properly. Add the dispersed guar gum solution dropwise into extract and stir well. To this add propylene glycol mixture with constant stirring. Finally the volume made up to 50ml by adding remaining distilled water and Triethanolamine was added drop wise to the formulations for adjustment of required skin pH and to obtain the gel at required consistency^{7,8}.

The same above procedure was followed for the preparation of herbal gel prepared by using Carbopol 934 and sodium alginate as gelling agent. Six topical herbal gel formulations were prepared by using *Murraya koenigii* fruit extract. F1 and F2 formulations were made using guar gum. F3 and F4 formulations were made by using Sodium alginate and formulations F5 and F6 were made using Carbopol 934 using as gelling agent⁹.

Table 2: Composition of Herbal Gel containing *murraya koenigii* fruit extract

S.NO	INGREDIENTS	F1	F2	F3	F4	F5	F6
1	<i>Murraya koenigii</i> fruit extract	0.2gm	0.3gm	0.2gm	0.3gm	0.2gm	0.3gm
2	Guar gum	600mg	600mg	-	-	-	-
3	Sodium alginate	-	-	600mg	600mg	-	-
4	Carbopol 934	-	-	-	-	600mg	600mg
5	Methyl paraben	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm
6	Propyl paraben	0.15ml	0.15ml	0.15ml	0.15ml	0.15ml	0.15ml
7	Propylene glycol	3 ml	3 ml	3 ml	3 ml	3 ml	3 ml
8	Menthol oil	2 drops	2 drops	2 drops	2drops	2 drops	2 drops
9	Triethanolamine	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
10	Purified water	Up to 50ml	Up to 50ml	Up to 50ml	Up to 50ml	Up to 50ml	Up to 50ml

EVALUATION OF *MURRAYA KOENIGII* HERBAL GEL

Physical Appearance:

The prepared herbal gel containing *Murraya koenigii* fruit extracts are 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. 1gm 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 are calculated.

Spreadability test:

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. Spreadability was measured on the basis on slip and drag characteristics of gels. An excess of gel (about 2 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 slide and provided with the hook. A one kg weight 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 hook and the time (in sec.) required by the top slide to cover a distance of 7.5cm was noted¹⁰. A shorter interval indicates better Spreadability.

Spreadability was calculated using the, following formula:

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

Where, S= Spreadability, M= Weight in the pan (tied to upper slide), L= Length moved by the slide, T= Time (in sec).

Homogeneity Test

By applying a small quantity of herbal gel on a piece of transparent glass and covered with glass object then it was observed the gel homogenous and their was no visible presence of particles¹¹.

Estimation of Drug content:

100 mg of *Murraya koenigii* gel was dissolved in sufficient quantity of pH 6.8 buffer to get the clear solution and the volume was made up to 100ml with pH 6.8 buffer to get (100 µg/ml) after 1ml (100 µg/ml) of the solution was diluted to 100ml with pH 6.8 buffer to get (10 µg/ml) and the absorbance of the resultant solution was measured at 241nm using UV spectrophotometer. The amount of drug was determined from the standard calibration curve and the percentage drug content was calculated¹².

In Vitro Diffusion Study:

Modified apparatus with cellophane membrane was used to study the *in vitro* release of gel formulation. The cellophane membrane, previously soaked in glycerin was tied to one end of an open end glass cylinder. The dissolution medium used was phosphate buffer (PH 6.8). *Murraya koenigii* gel containing 100mg of drug was placed into this assembly. The cylinder was connected to a stand and suspended in dissolution medium. The dissolution medium was kept at 37±0.5°C temperature and stirred at 100 rpm using magnetic bead. 1 ml aliquots were withdrawn at every 5, 10, 15, 20, 30, 45 and 60 minutes. 1ml dissolution medium was added to maintain equal volume of receptor medium. The sample was diluted and measured using UV spectrophotometer at 241 nm¹³.

Stability Study:

The formulated gel were filled in the collapsible tubes and stored at different temperatures and humidity conditions, viz. 25°C ± 2°C/ 60% ± 5% RH for a period of 30 day and studied for appearance, pH, spreadability, drug content and *in-vitro* diffusion study for initial period and after every 7,15,21 and 30 days¹⁴.

Anti-Microbial Study:

The antimicrobial activity of the *Murraya koenigii* gel was evaluated by agar well diffusion method. Bacteria were grown in Muller Hinton broth. After inoculation, plates were dried for 15 minutes, and the wells were punched using sterile corn borers. Once wells were formed, they were filled with 100 μ L gel and blank water. Commercially available diclofenac gel was used as a positive control in this study. Plates were incubated for 24 h at 37 °C to allow gel to diffuse through the agar media to form zones of inhibition. The diameters of the zone of inhibition for different fruit extracts against different bacteria was measured in millimeter for further analysis. An agar well (6 mm) showing maximum zone of inhibition was considered as good antimicrobial activity. All experiments were done in triplicate and the average values were used for drawing bar diagram¹⁵.

RESULTS AND DISCUSSION

The *murraya koenigii* fruit extraction process, extract and formulated gel was evaluated for physical appearance, pH measurement, spreadability, estimation of drug content, *in-vitro* diffusion study, stability study anti-microbial activity. All the results were presented in appropriate tables and figures. The below figure - 6 shows the *murraya koenigii* fruit extraction process, extract and herbal gel containing the *murraya koenigii* fruit.

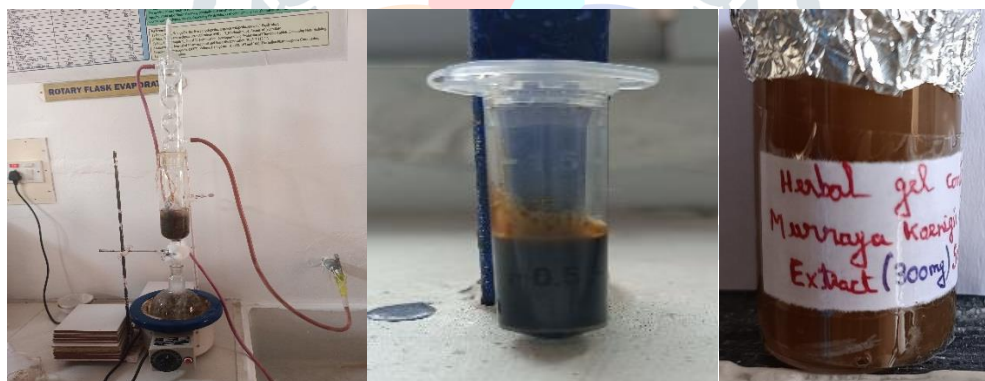


Figure 2: The *Murraya Koenigii* Fruit Extraction Process, Extract and Herbal Gel.

Organoleptic and solubility studies the *murraya koenigii* fruit extract:

The *murraya koenigii* fruit extract was found to be Brownish yellow color, A slightly aromatic odour and bland taste was observed. The solubility studies revealed *murraya koenigii* fruit extract was soluble in ethanol shown in table 4:

Table 3: Organoleptic and solubility studies of *murraya koenigii* fruit extract

Parameters	Characteristics
Color	Brownish yellow
Odour	Slightly aromatic
Taste	Bland

Solvent	Solubility Nature
Water	Insoluble
Ethanol	Soluble
Methanol	Slightly soluble
HCL	Sparingly soluble

Physico - Chemical Characters of *Murraya Koenigii* Fruit Extract

Physico-chemical values are helpful to determine the quality as well as purity of crude drug, especially when the drug is present in powdered form. The ash value, acid insoluble ash, water soluble ash & loss on drying values of *murraya koenigii* fruit extract was found to be 0.27%, 0.04%, 0.01% and 11.8% respectively.

Phytochemical Screening Study of *murraya koenigii* fruit:

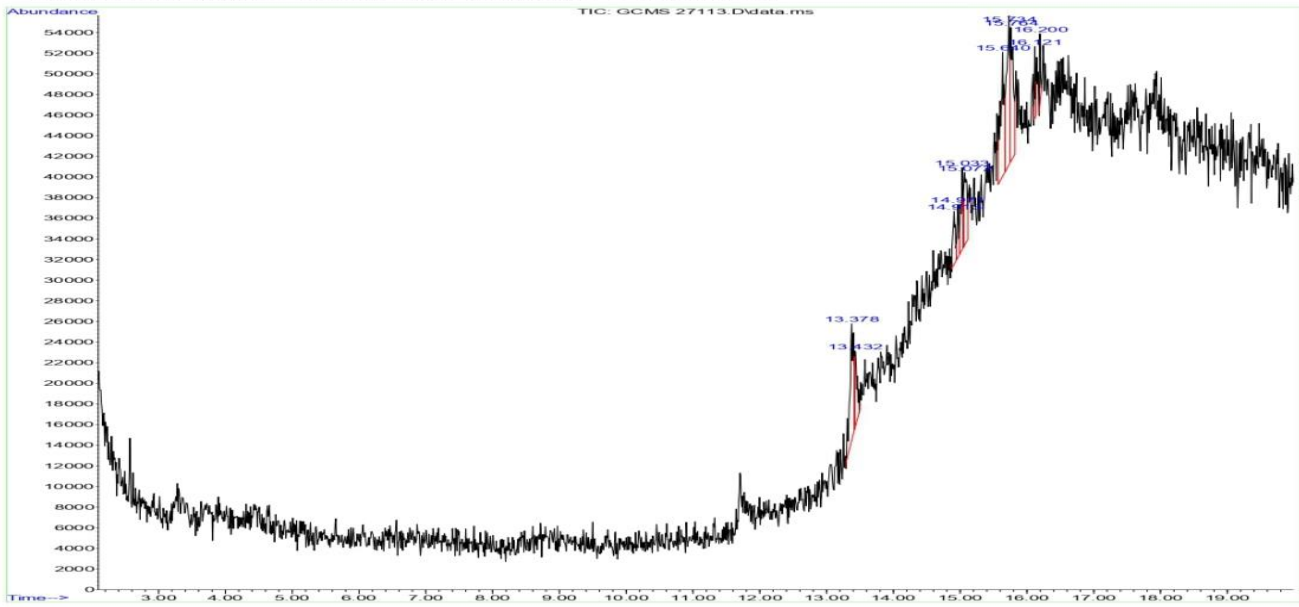
The results of phytochemical screening study of *murraya koenigii* fruit extract demonstrated the presence of glycosides, carbohydrates, flavonoids, tannins and polyphenols in methanol extracts.

GC-MS ANALYSIS:

Murraya koenigii fruit contains several chemical compounds. In GC-MS study, six chemical compounds were separated and identified having antimicrobial activity. They are N-methyl-1-adamantaneacetamide, Pyrido(1,2-a)(1,3) benzimidazole-3-aceticacid,4-cyano-1,5-dihydro-1-oxo, methyl ester, Pyridine,1,2,3,6-tetrahydro-1-methyl -4-(4-chlorophenyl, 2-ethylacridine and 1,4-Bis(trimethylsilyl)benzene shown in below figure:3 and table.

Library Search Report

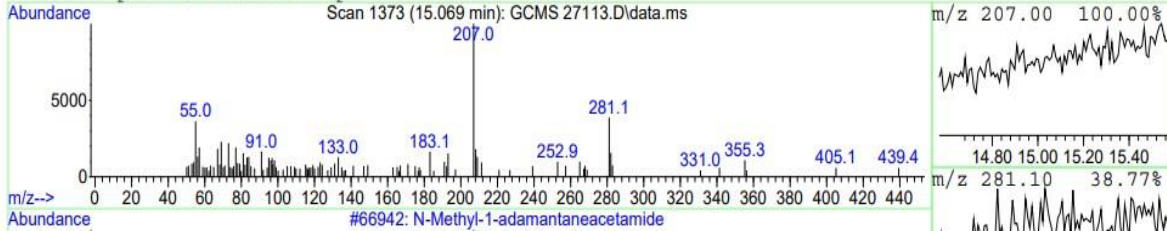
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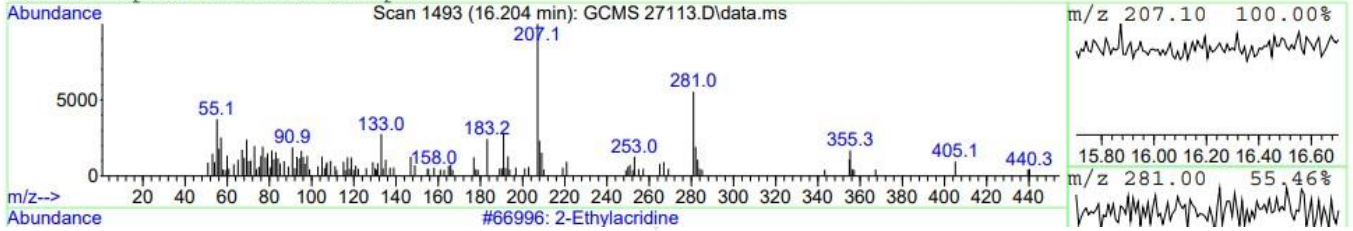
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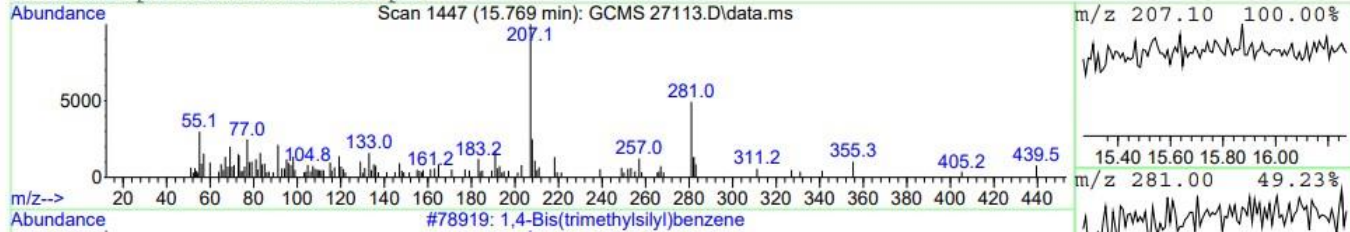
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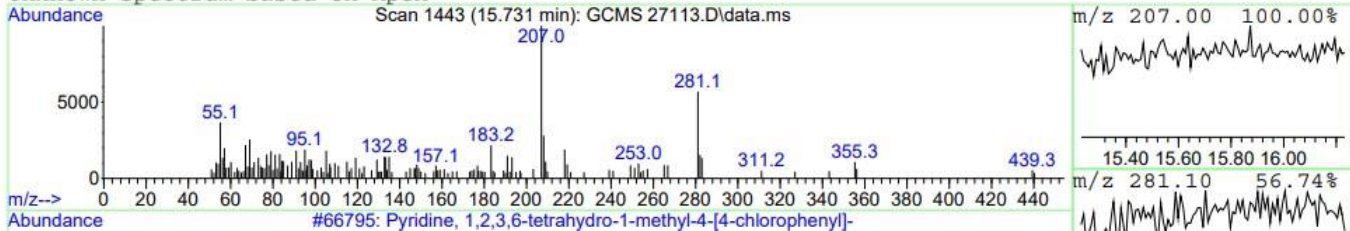
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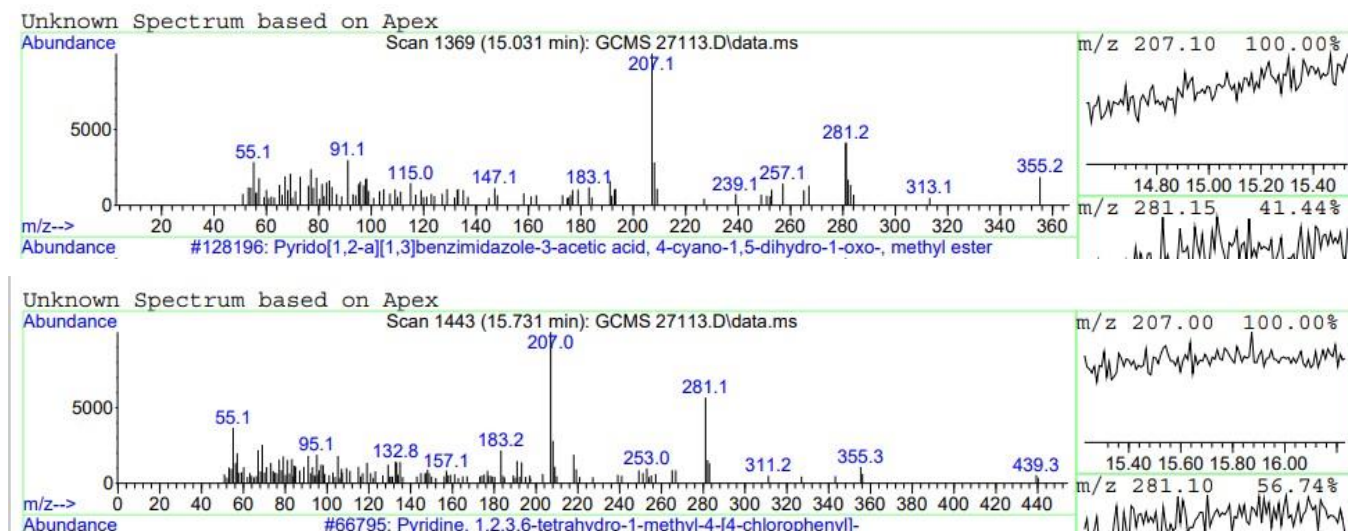


Figure 3 : Mass Spectrum Of N-Methyl-1-Adamantaneacetamide, Pyrido (1,2-A) (1,3) Benzimidazole-3-Aceticacid,4-Cyano-1,5-Dihydro-1-Oxo, Methyl Ester, Pyridine,1,2,3,6-Tetrahydro-1-Methyl -4-(4-Chlorophenyl, 2-Ethylacridine And 1,4-Bis (Trimethylsilyl) Benzene

Table 4: Compounds Identified in the Ethanolic Extract of *Murraya Koenigii* in GC-MS Analysis

S.NO	PEAK NO	RETENRION TIME (MIN)	AREA	PERCENTAGE AREA	COMPOUND IDENTIFICATION	ACTIVITY
1.	1	13.378	423400	16.33	Octadecenoic acid	-
2.	2	13.432	183710	7.09	3,3- diisopropoxy 1,1,1,5,5,5 hexamethyltrisiloxane	-
3.	3	14.913	124218	4.79	Acetamide, N-(4-fluorophenyl)-2,2,2-trifluoro	-
4.	4	14.974	85102	3.28	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	-
5.	5	15.072	223686	8.63	N-methyl-1-adamantaneacetamide	Antimicrobial
6.	6	15.033	156152	6.02	Pyrido(1,2-a) (1,3) benzimidazole-3-aceticacid,4-cyano-1,5-dihydro-1-oxo, methyl ester	Antimicrobial
7.	7	15.640	432471	16.68	Silane, trimethyl (5-methyl -2-(1-methylethyl) phenoxy)	-
8.	8	16.121	60688	2.34	Propiophenone ,2'(trimethylsiloxy)	-
9.	9	15.734	481336	18.56	Pyridine,1,2,3,6-tetrahydro-1-methyl -4-(4-chlorophenyl)	Antimicrobial
10.	10	16.200	44237	1.71	2-ethylacridine	Antimicrobial
11.	11	15.764	377934	14.58	1,4-Bis(trimethylsilyl)benzene	Antimicrobial

FT - IR SPECTRAL STUDIES:

FT-IR spectral studies indicated that the drug is compatible with all the excipients. The FT-IR spectrum of physical mixture showed all the characteristic peaks of *murraya koenigi* fruit extract thus conforming that no interaction of drug occurred to the components of the formulation shown in figure 4.

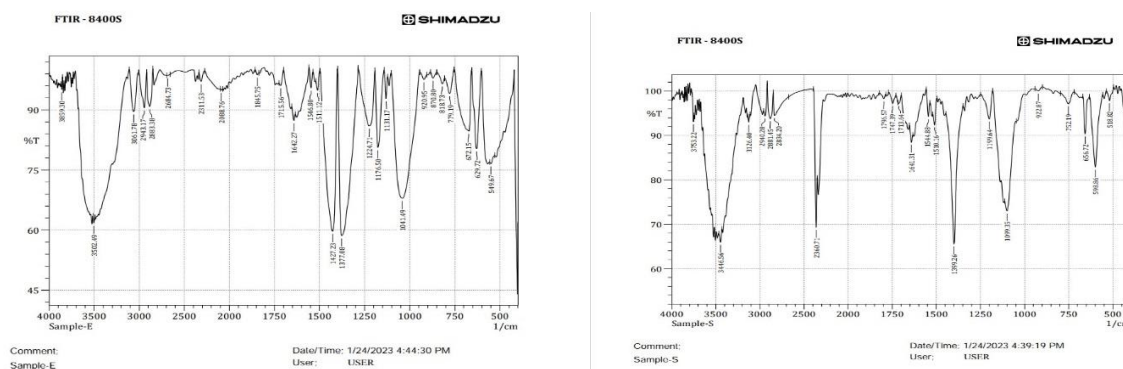


Figure 4: IR Spectrum of *Murraya Koenigii* Fruit Extract and IR Spectrum of Herbal Gel Formulation (F4)

Evaluation parameters of *Murraya Koenigii* herbal gel

S.No	Formulation Code	pH*	Spreadability* (gm.cm/sec)	Drug Content* (%)
1	F1	6.7	22.63	67.81
2	F2	6.8	21.16	75.93
3	F3	6.7	22.50	79.69
4	F4	7.1	23.29	87.50
5	F5	6.1	17.29	77.50
6	F6	6.9	16.25	83.44

pH values of *murraya koenigii* herbal gel formulations are found in the range of 6.1 to 6.9. Formulation F5 showed minimum pH value (6.1) and formulation F4 containing Sodium alginate as gelling agent, exhibited high pH value (7.1).

Formulation F1 and F4 containing Guar gum and Sodium alginate as gelling agent exhibited high spreadability (22.63 and 23.29 gm cm/sec) and formulation F5 and F6 containing Carbopol showed low spreadability (17.29 and 16.25 gm cm/sec).

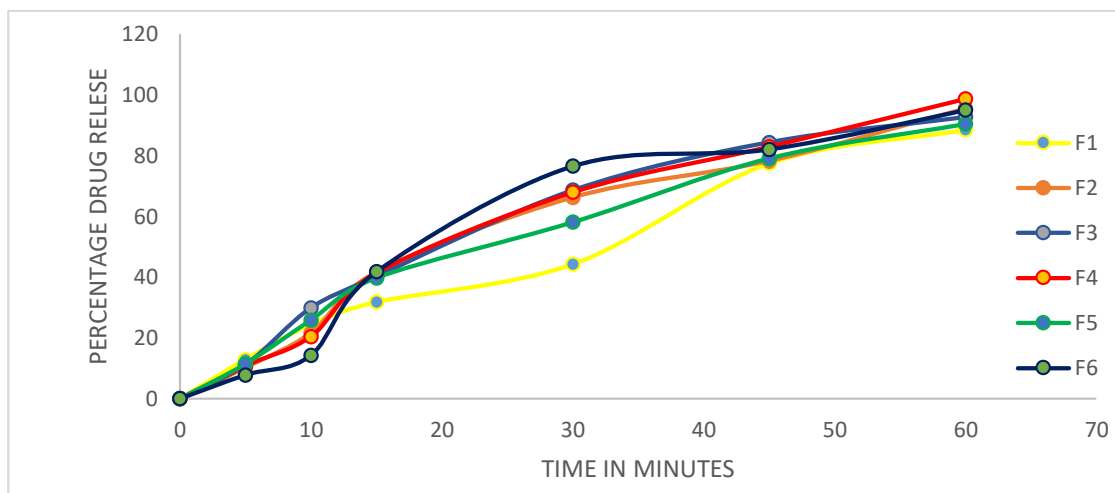
Murraya koenigii fruit gel showed that all of the formulation had similar homogeneity.

The amount and percentage of drug present in *Murraya koenigii* herbal gel formulations were estimated by UV spectrophotometric method. The content of *Murraya koenigii* in all formulations were found in the range of 67.81

% to 87.5 %. The *Murraya koenigii* herbal gel prepared using Sodium alginate (F4) showed maximum drug content (87.5 %) than other formulations.

***In Vitro* Diffusion Study**

The order of enhancement of the drug release with various gelling agent was found to be sodium alginate > guar gum > carbopol. Formulation F4 was observed as optimized formulation based on pH, solubility, spreadability, homogeneity and *in vitro* drug release shown in below figure.



S.no	Time in (min)	Percentage drug release					
		F1	F2	F3	F4	F5	F6
1	5	12.90	10.24	11.21	10.78	11.45	7.80
2	10	24.49	21.68	29.90	20.45	25.90	14.30
3	15	31.79	41.66	40.24	41.37	39.77	41.75
4	30	44.24	66.26	68.57	67.88	58.06	76.49
5	45	77.42	78.25	84.33	82.87	79.06	82.03
6	60	88.48	95.48	92.63	98.62	90.39	94.98

STABILITY STUDIES

Stability studies revealed that there was no significant changes found in physical appearance, pH, and spreadability values of formulations during the period of one month after stored at $25^{\circ}\text{C} \pm 2^{\circ}\text{C} / 60\% \pm 5\% \text{RH}$. The study revealed that the *Murraya koenigii* fruit herbal gel formulations was stable at $25^{\circ}\text{C} \pm 2^{\circ}\text{C} / 60\% \pm 5\% \text{RH}$ even after stored for one month results shown in below table..

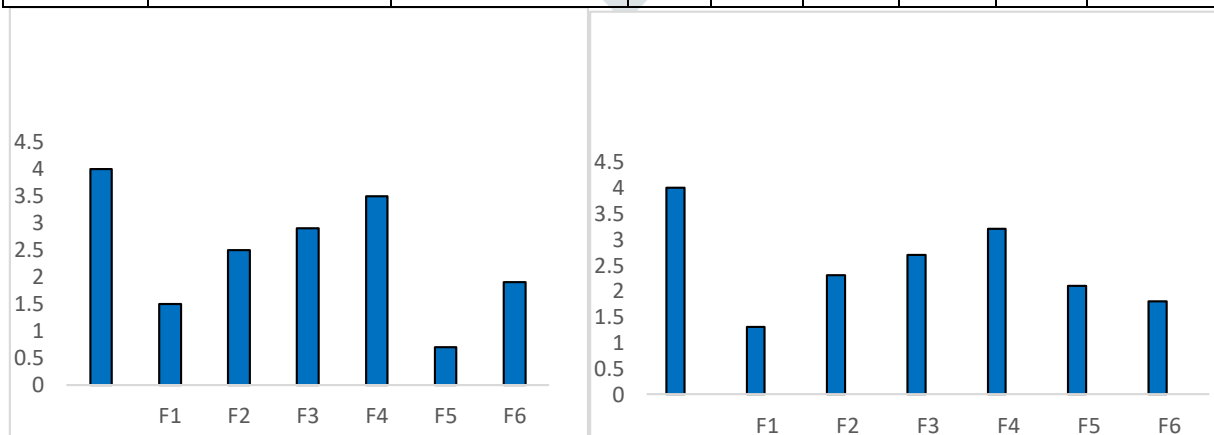
Formulation Code	Physical Appearance	pH			Spreadability			Homogenicity
		7 Days	21 Days	30 Days	7 Days	21 Days	30 Days	
F1	Complies	6.7	6.5	6.7	22.63	22.1	22.25	Good
F2	Complies	6.8	6.8	6.6	21.16	20.56	21.4	Good
F3	Complies	6.7	6.5	6.6	22.5	21.09	22.65	Good
F4	Complies	7.1	6.9	7.0	23.29	24.2	24.91	Good
F5	Complies	6.1	6.5	6.2	17.29	16.24	17.28	Good
F6	Complies	6.9	7.1	6.8	16.25	15.8	16.57	Good

ANTI-MICROBIAL ACTIVITY OF MURRAYA KOENIGII HERBAL GEL FORMULATIONS

In this study gram positive and gram negative microorganism were used in agar well diffusion method. Formulations (F2, F4, F6) containing 0.3 gm of *murraya koenigii* showed maximum zone of inhibition against 2 microorganism. Hence formulation F4 containing sodium alginate as gelling agent exhibited maximum Anti-microbial activity than other formulations shown in below table and figure

Table 5: Anti - microbial Activity of *Murraya koenigii* Herbal Gel Formulations

S.NO	Micro organism	Standard Zone of Inhibition (mm)	Sample Zone of Inhibition(mm)					
			Guar Gum		Sodium Alginate		Carbopol	
			F1	F2	F3	F4	F5	F6
1	Escherichia coli	4	1.5	2.5	2.9	3.5	0.7	1.9
2	Staphylococcus aureus	4	1.3	2.3	2.7	3.2	2.1	1.8





E.COLI

STAPHYLOCOCCUS AUREUS

Figure:5 Zone of inhibition and Anti- microbial Activity of *murraya koenigii* Herbal Gel

CONCLUSION

The study concluded among all gel formulations, formulation F4 containing *murraya koenigii* along with sodium alginate as gelling agent showed better spreadability, maximum drug content (87.50%), faster drug release (98.62%), promising anti-microbial action against gram positive and gram negative microorganisms. The study concludes that the developed formulation F4 consisting 0.3 gm of *Murraya koenigii* with sodium alginate was found to be promising herbal gel for the topical treatment of some microbial infections.

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