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Trigonella foenum-gracum and *Murraya koenigii* Extract Herbal Gel: Formulation and Development

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Abstract : The present research has been undertaken with the aim to study the antimicrobial activity of *Trigonellafoenum-gracum* and *Murraya koeniggi* extract and formulation, development and evaluation of its herbal gel. *Trigonellafoenum-gracum* and *Murraya koeniggi* extract, reported antibacterial action against gram negative and gram positive bacteria. MIC of *Trigonellafoenum-gracum* extract for *E.coli* and *S. aureus* was found to be 333 and 125 ug/ml respectively. Similarly the MIC of *Murraya koeniggi* extract for *E.coli* and *S. aureus* was found to be 750 and 250 ug/ml respectively. Further, the gel formulation was designed by using Carbapol 940p, *Trigonellafoenum-gracum* and *Murraya koeniggi* extract propylene glycol, methyl paraben, polyethylene glycol, polyvinyl pyrrolidone, triethanolamine and required amount of distilled water. The pH was maintained 6.8-7 by drop wise addition of tri-ethanolamine. The physicochemical parameters of formulations were evaluated by pH, spreadability, appearance, viscosity and *in-vitro* drug diffusion study using franz diffusion cell. The *in-vitro* drug diffusion study showed 77.7 % for *Trigonella foenum-gracum* and 75.0 % for *Murraya koeniggi* release from formulation.

Key Words : *Trigonella foenum-gracum* and *Murraya koeniggi* extract, herbal gel, antimicrobial activity, *E.coli, S. aureus,* MIC.

Introduction :

Gels are semisolid systems in which there is interaction (either physical or covalent) between colloidal particles within a liquid vehicle. Herbal gel is consists of one or more herbs or processed herb(s) incorporated within the colloidal gel dispersion. Medicinal plants play a vital role for the development of new drugs. The medicinal plants contribute to cater 80 % of the raw materials used in the preparation of drugs. Herbal medicine is still the mainstay of about 75% of the world population, especially in the under developed and developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects[1].

Fenugreek (*Trigonellafoenum-gracum)* is an annual leguminous bean, and belongs to *Fabaceae* family. Its seeds and green leaves used as food posses medicinal applications, and is an old practice of human history. It has been used for diverse medicinal benefits that include wound healing, aid in digestion, treatment of sinus, lung congestion and diabetes, inflammation and infection, mitigation, hair treatment, breast enhancement and aphrodisiac effects. In India, it is extensively used as Ayurvedic medicine and in China as traditional medicine. Fenugreek to posses immunomodulatory, anti-carcinogenic, anthelmintic, anti-nociceptive, antioxidant, anti-microbial, anti-ulcer, gastro- and hepatoprotective, anti-obesity, anti-hyperglycemic, anti-diabetic and hypocholesterolemic effects[2].

Curry leaves, known as *Murraya Koeniggi*, belonging to *Rutaceae* family are widely used as a medicinal herb and has characteristic aroma. Curry leaf is commonly used as spice due to aromatic nature of leaves. It is an important export commodity from India as it fetches good foreign revenue. leaves are rich in many bioactive compounds like polyphenols, alkaloids and flavonoids which showed multiple bioactive functions like antioxidant, anticancer, antimicrobial antidiabetic and hepatoprotective. In addition to the basic bioactive compounds, they also rich in essential oil compound namely coumarine, bicyclomahanimbicine, mahanimbicine, phebalosin in curry leaves[3].

Materials and Methods

Plant Materials

The seeds of *Trigonella foenum-gracum* and leaves of *Murraya koeniggi* were collected from Nagpur region. The seeds and leaves were dried for the period of six days in a shade and grounded into fine powder and sieved using a laboratory sieve.

Chemicals

Carbopol 934p (Loba Chemical), Polyethylene glycol 400 (Raechem lab), Propylene glycol (Loba Chemical), Isopropyl alcohol (Loba Chemical), Triethanolamine (Loba Chemical), Methyl paraben (Loba Chemical), Polyvinyl pyrrolidone (Raechem lab), Distilled water.

Solvent Extraction

Procedure

50g of dried leaves and seeds powder were taken and subjected to separate soxhlet apparatus by ethanol as a solvent for 24 hrs. About 150 ml of ethanol was poured in a round bottom flask and extraction was allowed for 24 hours between 65°C and 70°C. The extract was then concentrated at 45 °C by evaporating the solvent to obtain solid residue. All the crude extracts were filtered using filter paper to obtained particle free crude extract.

Physico-chemical characteristics of *Trigonella foenum-gracum* and *Murraya koeniggi* extract

The dried extract of *Trigonella foenum-gracum* seeds was found to be solid, yellowish brown, aromatic, slightly bitter, soluble in water, 5% HCL, 5% NaOH, Con. H_2SO_4, methanol, ethanol, DMSO, and isopropyl alcohol. The extract of *Murraya koeniggi* leaves dark greenish, aromatic, bitter and soluble in 5% NaOH, conc.H_2SO_4, ethanol, DMSO, isopropyl alcohol and insoluble in water, 5% HCL, and methanol.

Phytochemical screening of *Trigonella foenum-gracum* and *Murraya koeniggi* extract

As shown in Table No.2; the extract of *Trigonella foenum-gracum* and *Murraya koeniggi* tested for carbohydrates (Molish's test, Fehling's test), protein (Biuret test), amino acid (Ninhydrin test), glycosides (Killer-killiani test), steroids (Salkowski test), alkaloids (Mayer' s test, Wagner's test), flavonoids (Shinoda test)[4].

Table No.2: Formula of *Trigonellafoenum-gracum* and *Murraya koeniggi* extract Herbal Gel

Sr. No.	Ingredients	Quantity Used
1.	*Trigonellafoenum-gracum* extract	100 mg
2.	*Murraya koeniggi* extract	50 mg
3.	Carbopol 934p	1.0 g
4.	Polyethylene glycol 400	10 ml
5.	Propylene glycol	10 ml
6.	Isopropyl alcohol	10 ml
7.	Polyvinyl pyrrolidone	0.1 g
8.	Methyl paraben	0.15 g
9.	Triethanolamine	q.s.
10.	Distilled water	Up to 100 ml

UV analysis of *Trigonella foenum-gracum* and *Murraya koeniggi* extract

10 mg of *Trigonellafoenum-gracum* and *Murraya koeniggi* extract was weighed accurately and transferred to the 100 ml of volumetric flasks separately. It was dissolved in small quantity of ethanol and then volume make upto 100 ml with phosphate buffer (pH 6.8). From this stock solution 3.0 ml aliquots were pipette out and diluted up to 10 ml with phosphate buffer (30*µg/ml).* Further, it was scanned between 400-200 nm and absorbance maxima λ *max* was determined[5,6,7].

For calibration graph, 10 mg of *Trigonella foenum-gracum* and *Murraya koeniggi* extract was weighed accurately and transferred to the 100 ml of volumetric flask. It was dissolved in small quantity of ethanol and then volume make up to 100 ml with phosphate buffer (pH 6.8). From this stock solution (100 µg/ml) 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml aliquots were pipette out and diluted up to 10 ml with phosphate buffer. The absorbance was taken at λ= 265, 266, 223 nm for fenugreek extract and 221, 292, 218 nm for curry leaves extract using UV visible spectrophotometer [8].

Antimicrobial activity

Agar well diffusion method

Nutrient agar medium was prepared and sterilized by autoclaving at 121°C for 15 minutes 15 lbs. The six agar plates were prepared and labeled. The nutrient agar after sterilization was poured into the six plates and allowed to get solidify. After solidification the culture of *E.coli* and *S. aureus* were applied on each three plates. Then the well were prepared using cork borer for *Trigonella foenum-gracum* extract, *Murraya koeniggi* leaves extract, 1:1 ratio of *Trigonella foenum-gracum*:*Murraya koeniggi* extract and control in all plates. After the well preparation the samples were poured in respective wells using micropipette. The plates then allowed incubating at 37°C for 24 hours[9,10].

Minimum inhibitory concentration determination

Broth dilution method

Nutrient broth medium was prepared and sterilized by autoclaving at 121°C for 15 minutes 15 lbs. About 44 nutrient broth tubes were selected, comprises of 11 test tubes for each four groups. 5ml of nutrient broth was added in all the test tubes. Three to four drops of inoculums *E.coli* and *S. aureus* of were added in all the test tubes. The test compound was added in increasing volume (0.5 -5.0 ml) except first test tube which was kept as control. The final volume was adjusted to 10 ml with distilled water. All the test tube incubated at 37°C for two days[9].

Formulation of *Trigonella foenum-gracum* and *Murraya koeniggi* extract herbal gel

As shown in Table no1, measured quantity of carbopol 940p was dissolved in 35 ml of distilled water using magnetic stirrer. In another beaker 100 mg extract of *Trigonella foenum-gracum* and 50 mg extract of *Murraya koeniggi* was dissolved in 10 ml of isopropyl alcohol and filtered. Meanwhile measured quantity of polyvinyl pyrrolidone, polyethylene glycol and propylene glycol was added to the dispersion of carbol and allowed to form a homogenous mixture. After that the solution of extract which is prepared in isopropyl alcohol was added to the homogenous mixture. Finally the triethanolamine was added until the smooth gel is formed and pH adjusted to 6.8.[6-9] .

Table No.1: Phytochemical screening of *Trigonella foenum-graceum* and *Murraya koeniggi* extract

Sr. No.	Phytochemicals	Test	Observation	Inference
1	Carbohydrate	Molisch's test	Voilet ring	Carbohydrate Present
		Fehling's test	Brick red ppt	Carbohydrate Present
2	Protein	Biuret test	violet-colored solution	Protein present

3	Amino acid	Ninhydrin test	Blue colour solution	Amino acid present
4	Steroid	Salkowaski test	Red color appears at lower layer	Steroid present
5	Glycosides	Killer killiani test	-ve	Glycosides absent
6	Flavonoids	Shinoda's test	Pink color appears	Flavonoids present
7	Alkaloids	Mayer's test	Creamy ppt.	Alkaloids present
		Wagner's test	Reddish brown ppt.	Alkaloids present

Evaluation of *Trigonella foenum-gracum* and *Murraya koeniggi* extract herbal gel

Physico-chemical evaluation

Physical parameters of *Trigonella foenum-gracum* and *Murraya koeniggi* extract herbal gel such as color and appearance were checked.

pH Determination

The pH of 1% of *Trigonella foenum-gracum* and *Murraya koeniggi* extract herbal gel was measured by using pH meter (Elico Pvt .Ltd, Mumbai).

Viscosity determination

The viscosity of *Trigonella foenum-gracum* and *Murraya koeniggi* extract herbal gel was determined by Brookfield viscometer (Brookfield Engineering Lobo) using Spindle no. 64. The 100 gm of gel was placed in beaker and spindle of viscometer inserted in it [7,12-14].

Spreadibility Analysis

1 g of *Trigonella foenum-gracum* and *Murraya koeniggi* extract herbal gel was placed on the ground slide. Another glass slide having the dimension of fixed ground slide was placed above it. A 500 g weighted was placed on the top of the two slides for 5 minutes 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 removed and the time required by the top slide to detached from another slide was noted [12,13].

***In-vitro* Gel diffusion study**

Diffusion study for *Trigonella foenum-gracum* and *Murraya koeniggi* extract gel was carried out using franz diffusion cell. It consist of two compartment i.e. Donar compartment and Receptor compartment. Firstly the cellophane membrane was boiled in phosphate buffer. Then it was placed between the compartments of franz diffusion cell. The two compartments were then tied with the help of rubber band. Phosphate buffer filled into the receptor compartment by the side tube. 1 g of gel was applied on the top layer of cellophane membrane. The whole assembly then placed on the magnetic stirrer with the help of clamp. 1ml of aliquots were pipette out at the time interval of 30 minute and diluted upto 10 ml with phosphate buffer and absorbance was measured at λ= 223 and 218nm [7, 15].

Results and Discussions

The Ethanolic extraction of *Trigonella foenum-gracum* seeds and *Murraya koeniggi* leaves was done by using Soxhlet extractor. The characterization of both the extract was carried out which includes physical characteristics and solubility. *Trigonella foenum-gracum* extract was yellowish brown in colour, aromatic in odour, bitter in taste, and soluble in water, 5% HCL, 5% NaOH, Con. H_2SO_4, methanol, ethanol, DMSO, isopropyl alcohol. *Murraya koeniggi* extract was dark greenish in colour, aromatic in odour, bitter in taste, soluble in 5% NaOH, Con. H_2SO_4, ethanol, methanol, DMSO, isopropyl alcohol and insoluble in water, 5%

HCL. The phytochemical screening of *Trigonella foenum-gracum* and *Murraya koeniggi* extract specified in Table No. 1 indicated presence of carbohydrate, protein, flavonoids and alkaloids whereas steroid present in *Trigonella foenum-gracum* extract and glycosides absent in both the extracts. As shown in Figure 1, UV spectrophotometric analysis, the *Trigonella foenum-gracum* extract showed the λ max at 223 nm while *Murraya koeniggi* extract showed λ max at 218 nm. Similarly, calibration curve for *Trigonella foenum-gracum* extract and *Murraya koeniggi* extract was determined using phosphate buffer and shown in Figure 2 and 3, respectively.

UV- spectrum of *Trigonella foenum-gracum* seed extract showing λ max at 223 nm

UV- spectrum of *Murraya Koenigii* extract showing λ max at 218nm

Figure 1: UV Spectrum of *Trigonellafoenum-gracum* and *Murraya koeniggi* extract in Phosphate buffer

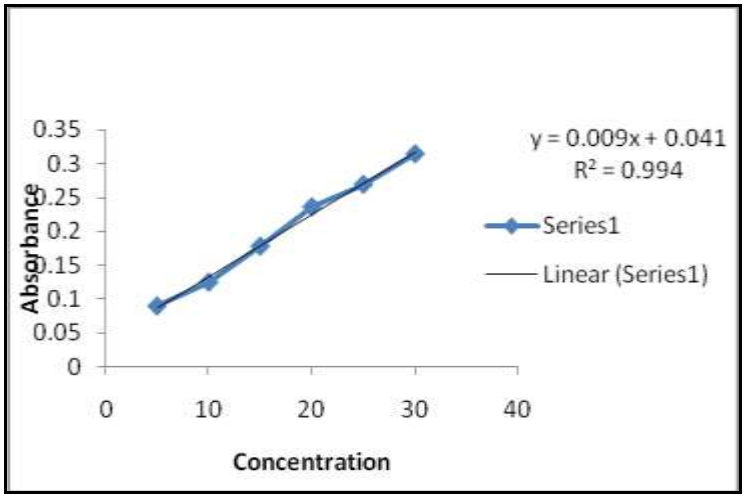

Figure 2: Calibration plot for *Trigonella foenum-gracum* extract in Phosphate buffer

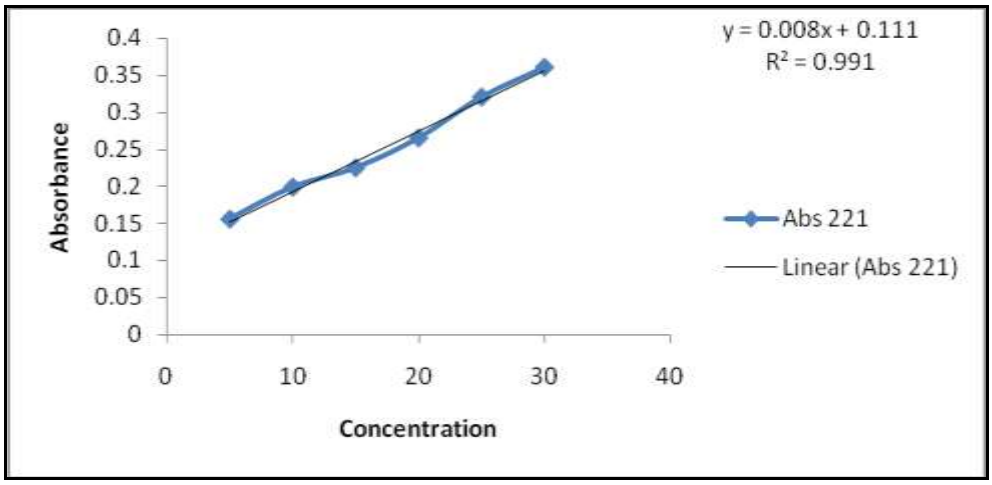

Figure 3: Calibration curve for *Murraya koenigii* extract in phosphate buffer

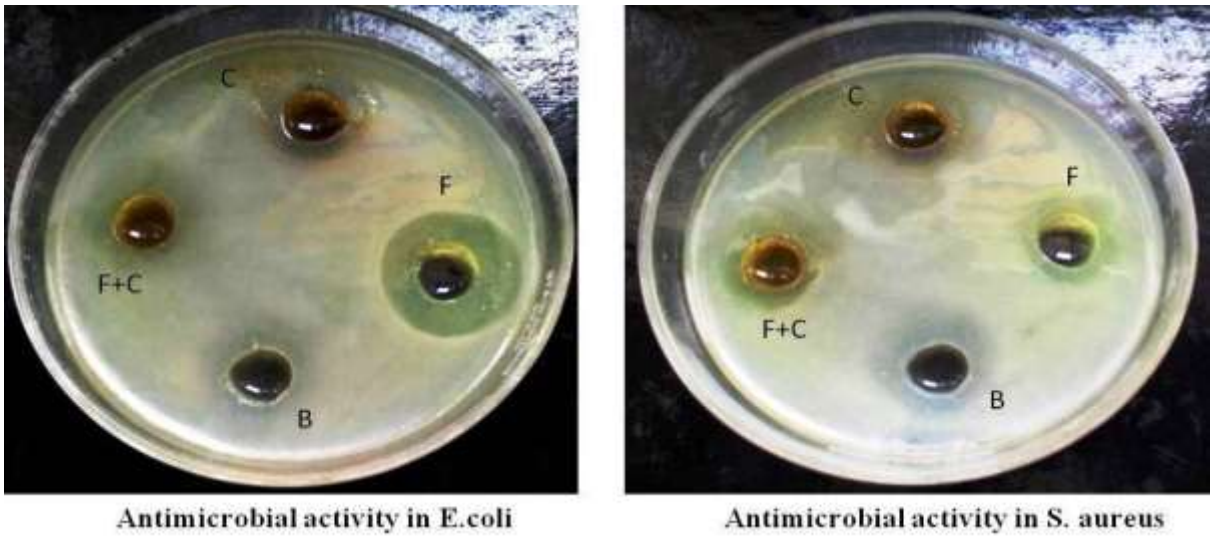

Figure 4: Antimicrobial activity of *Trigonella foenum-gracum* and *Murraya koeniggi* extract

Where; B= DMSO, F= *Trigonella foenum-gracum* extract, C= *Murraya koeniggi* extract, F+C= 1:1 *Trigonella foenum-gracum*: *Murraya koeniggi*

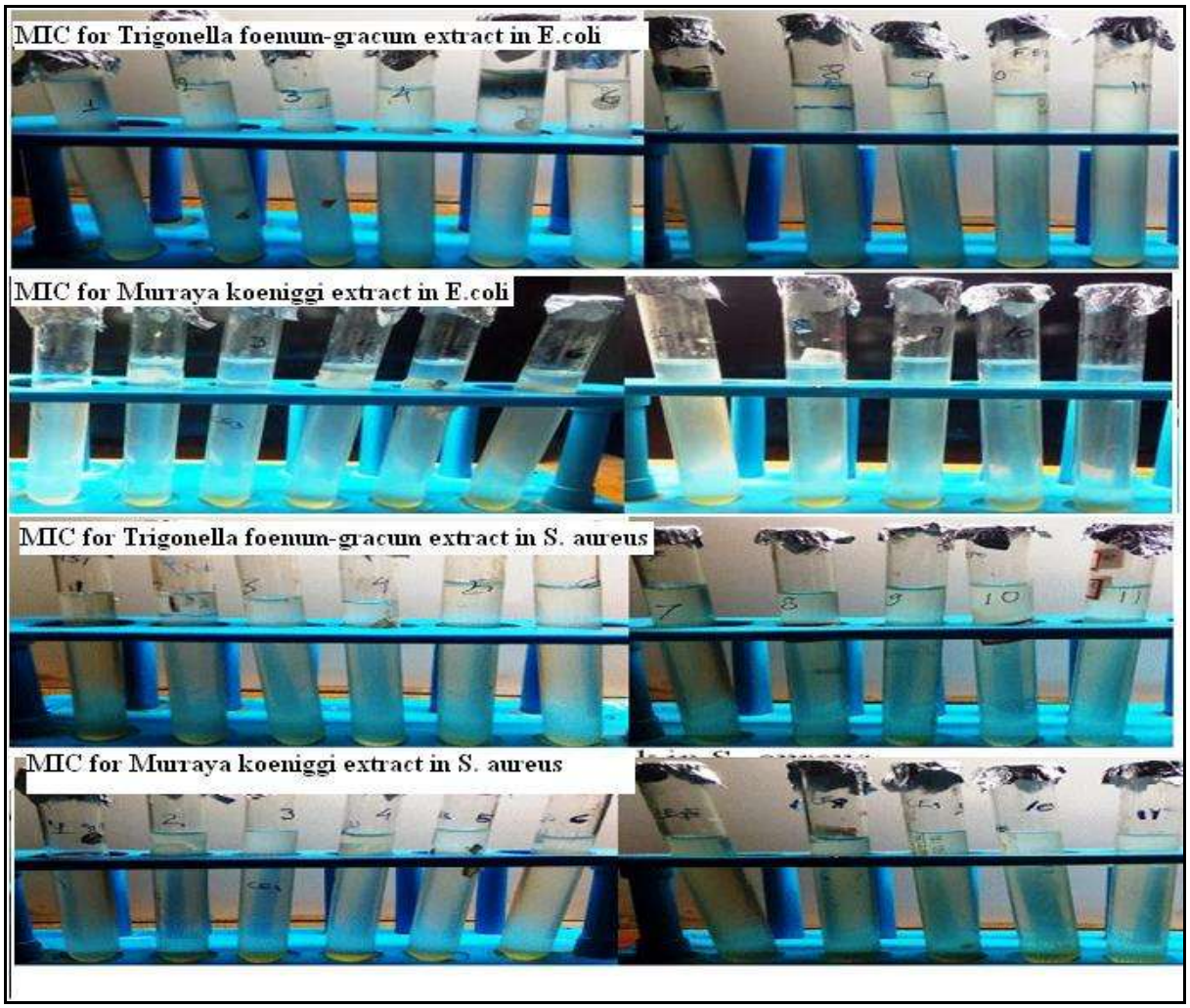

Figure 5: MIC of *Trigonella foenum-gracum* and *Murraya koeniggi* extract in *E.coli and S. aureus*

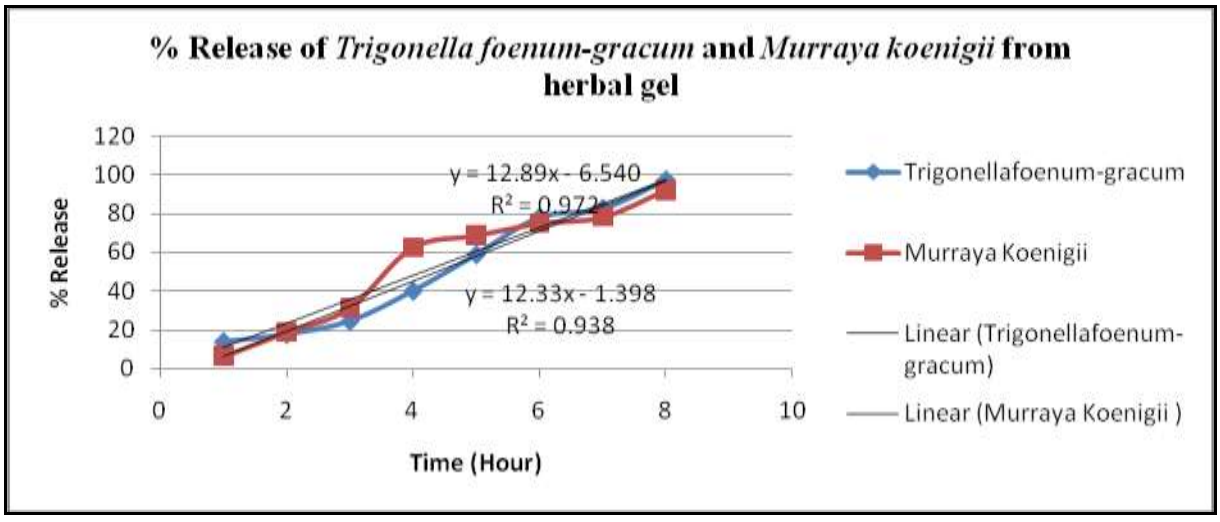

Figure 6: % Release of *Trigonella foenum-gracum* and *Murraya koenigii* from herbal gel

The antimicrobial activity of *Trigonella foenum-gracum* and *Murraya koeniggi* extract, reported zone of inhibition against *E.coli* and *S. aureus* given in Figure 4 and Table No. 3. Moreover, *Trigonella foenum-gracum* extract exhibited clear and larger zone in comparison with *Murraya koeniggi* extract suggested prominent antibacterial activity of *Trigonella foenum-gracum* extract. In addition, Figure 5 and Table no. 4 indicated the MIC of *Trigonellafoenum-gracum* extract for *E.coli* and *S. aureus* was found to be 250 and 50

ug/ml respectively. Similarly the MIC of *Murraya koeniggi* extract for *E.coli* and *S. aureus* was found to be 350 and 100 ug/ml respectively. The antibacterial activity of both the extract suggested more activity against gram positive bacteria *S. aureus.*

Table No.3:- Antimicrobial activity of *Trigonella foenum-gracum* and *Murraya koeniggi* extract in *E. coli* and *S. aureus*

Sr. No.	Samples	Zone of Inhibition (mm)	
		E. coli	***S. aureus***
1.	*Trigonella foenum-gracum* extract	18.66±3.05	18.33±1.52
2.	*Murraya koeniggi* extract	14.66±2.08	16.66±1.15
3.	*Trigonella foenum-gracum* extract: *Murraya koeniggi* extract (1:1)	15.00±1.00	15.33±0.57
4.	Vehicle control	No activity	No activity

Table No.4: MIC of *Trigonella foenum-gracum* and *Murraya koeniggi* extract in E.coli *and S. aureus*

Sr. No.	Tube no.	Volume of double strength medium (ml)	Volume of test sample (ml)	Volume of sterile water (ml)	MIC			
					Trigonella foenum-gracum		Murraya koeniggi	
					E.coli	S. aureus	E.coli	S.aureus
1	Control	5	0.0	5.0	++	++	++	++
2	1	5	0.5	4.5	+	-	+	+
3	2	5	1.0	4.0	+	-	+	+
4	3	5	1.5	3.5	+	-	+	-
5	4	5	2.0	3.0	+	-	+	-
6	5	5	2.5	2.5	-	-	+	-
7	6	5	3.0	2.0	-	-	+	+
8	7	5	3.5	1.5	-	-	-	-
9	8	5	4.0	1.0	-	-	-	-
10	9	5	4.5	0.5	-	-	-	-
11	10	5	5.0	0.0	-	-	-	-
	MIC of extract (ug/ml)				250	50	350	100

Table No.5: % Release of *Trigonella foenum-gracum* and *Murraya koenigii* from herbal gel

TIME (HRS)	% Release from herbal gel	
	Trigonellafoenum-gracum	***Murraya koenigii***
1	13.8889	6.25
2	17.7778	18.75
3	24.4444	31.25
4	40	62.5
5	58.6667	68.75
6	77.7778	75
7	82.33	78.25
8	96.99	92.13

The *Trigonella foenum-gracum* and *Murraya koeniggi* extract herbal gel was pale yellowish in color, translucent in appearance, and smooth in application. pH also maintained constant throughout the study which was found to be 6.7 to 6.9 and the gel was non-irritant upon application on the skin. Likewise, the viscosity of *Trigonella foenum-gracum* and *Murraya koeniggi* extract herbal gel was observed in the range of 5982 to 9580 CPS which is related for topical applications. Spreadibility was found to be 36.76 gm.cm/sec. In vitro diffusion

of *Trigonella foenum-gracum* and *Murraya koeniggi* extract herbal gel for 8 hours showed maximum release of 96.99 % for *Trigonella foenum-gracum* extract while 92.13% at for *Murraya koeniggi extract.*

Conclusion

Herbal gel containing *Trigonella foenum-gracum* and *Murraya Koenigii* extract was found to be stable, neutral pH, suitable viscosity, and good spredability as well as exhibited excellent antibacterial activity. In future, the *Trigonella foenum-gracum* and *Murraya Koenigii* extract herbal gel may be better formulation for topical application in infections.

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