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Formulation and Evaluation of Antifungal Gel of *Trigonella foenum graecum* and *Myristica fragrans*

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ABSTRACT

in this project we prepare and evaluate the antifungal gel using Trigonella foenum graecum (fenugreek seed) and Myristica fragrans (nutmeg) firstly we take powder fenugreek seed and nutmeg extraction is started with methanol using the process of maceration. both extraction separately then the extraction is evaluated by various phytochemical test and test shows that fenugreek contain alkaloid, flavonoid, tannin, saponin, carboxylic acid, glycoside. and also nutmeg extraction shows that nutmeg contain alkaloid, flavonoid, tannin, saponin, carboxylic acid and glycoside. after the evaluation prepare the antifungal gel using carbopol 934, triethanolamine, methyl paraben, propyl paraben and water. then the gel is evaluated in that we perform some test like measurement of pH, homoginicity, skin irritation test, spreadability. Then the antifungal activity is perform by using walls method in that we prepare 3 walls one for standard, second wall for sample and last wall for saline, then standard fluconazole is used and fill into wall, sample gel in sample wall and saline solution in saline wall and spread a fungus on plate and closed with another plate and incubate it for 48 hr then the result are shown. both the gel i.e fenugreek gel and nutmeg gel shows antifungal activity.

INTRODUCTION

1. Trigonella greacum foenum

Trigonella foenum gracum(fenugreek) is legume belonging to family Fabaceae. there are various constituent present in fenugreek. fenugreek contain alkaloids, flavonoids, carbohydrates, glycosides, saponin, tannin. It is cultivated in various country. Fenugreek is spice and herbal food. the seeds and leaves of fenugreek are used as medicinal purpose in the extract form. The seed of fenugreek are triangular in shape.

Trigonella is commonly as annual herb. From the ancient ties there are so many uses of fenugreek.it is used as food and spiece in many parts of country. medically it is used to treat wounds, ulcers, and digestive problem, and also act as demulcent.

Fenugreek have various pharmacological uses as anti-inflammatory, antifungal, antibacterial, antidiabetic.

Fig 1- Trigonella greacum foenum (Fenugreek)

2.Myristica fragrans

Nutmeg is spice made by grinding the seed of the nutmeg tree into powder. The spice has distinctive pungent fragrance and a warm slightly sweet taste. It is used as flavor dried nutmeg is brown ovoid with furrowed surfaces. the nutmeg is egg-shaped. nutmeg trees are dioceious plant (individual plant are either male and female). The first harvest of nutmeg trees takes places 7-9 years after planting and the trees reach full production after 20 years.

Nutmeg contain various phytochemical it contain alkaloids, flavonoids, carbohydrates, glycosides, saponin, tannin and nutmeg having various pharmacological activity such as antifungal, antibacterial, antimicrobial, anti cariogenic,, anti obesity.

Fig 2-Myristica fragrans (Nutmeg)

Gels- gels ae transparent to opaque homogeneous semisolid**,** gels are non -greasy, non-sticky, and easily washable with good spreadability therefore it is most accepted semisolid dosage form of recent years.

Fungal infection- fungal infection is caused by hundreds of fungi that exist in everyday environment. It is common infection in skin also it is caused in mouth, urinary tract , any part of body. Symptoms of fungal infection are itching of skin, rashes, inflammation, small pores, redness.

MATERIAL AND METHOD

Collection of plant material

The fenugreek seed and nutmeg are collected from local market then prepared its powder for the use in extraction.

preparation of extraction of fenugreek seed

1) Take 100gm of fenugreek seed powder, sieved through mesh.
2) In close container take 200ml of methanol as solvent, and fenugreek powder in it.
3) Keep the mixture as it is for 3 days after that filter it.
4) Filtered collect in one beaker and keep it for natural evaporation.

5) Make the extraction concentrated.

preparation of extraction of nutmeg

Fig 3– Extraction (maceration) of fenugreek

1. Take 100gm of nutmeg powder, sieve through mesh.

2. In close container take 200ml of methanol as solvent, and fenugreek powder in it.

3. keep the mixture as it is for 3 days after that filter it.

4. filtered collect in one beaker and keep it for natural evaporation and make the extraction concentrated.

Fig no 4- Extraction (maceration) of nutmeg

Evaluation Of Extraction

Evaluation Of Fenugreek Extract:

1) Detection of alkaloid –

Mayer test – few ml of extract and 2,3 drops Mayer reagent, white creamy precipitate indicate test positive.

Fenugreek extraction shows white creamy precipitate.

Fig 5 – Mayer test

2) Hager test – few ml of filtrate and add1to 2 ml Hagers reagent prominent yellow precipitate indicate test positive.

Fenugreek extraction shows yellow precipitate.

Fig 6- Hager test

2)Detection for flavonoid

lead acetate test – extract 50mg dissolve in 3ml of water and add lead acetate solution was added bulky white lead precipitate indicated positive.

Fenugreek extraction shows bulky white precipitate.

Fig 7 - Lead acetate test

3) Detection of tannin –

Ferric chloride test – 0.4 extract and 4ml NaoH form the emulsion

Fenugreek extraction shows emulsion.

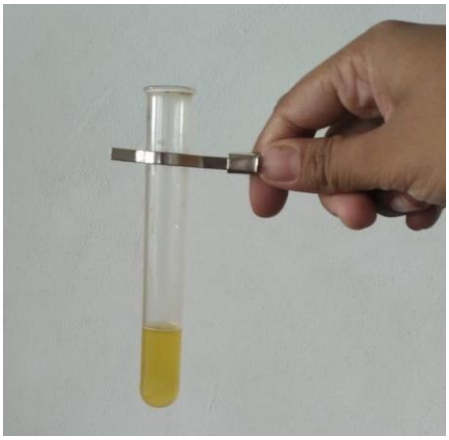

Fig 8– Ferric chloride test

4) detection of tannin –

Ferric chloride test – 0.4 extract and 4ml NaoH form the emulsion

Fenugreek extraction shows emulsion.

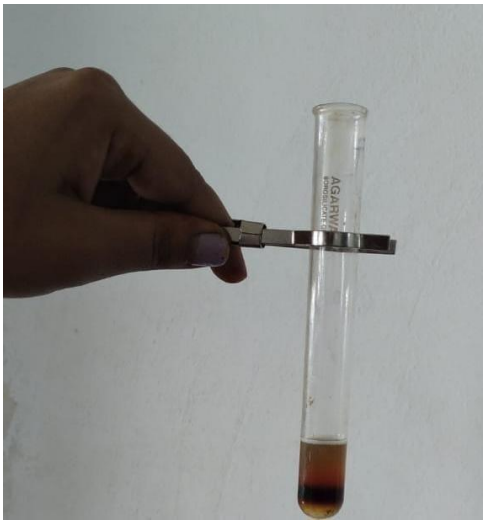

Fig 9 – glycoside test

5)Detection of carboxylic acid –

Extract and sodium bicarbonate mix together appearance of effervescence.

Fenugreek extraction shows appearance of effervescence

6)Detection of saponin

Extract and 20ml of water take in test tube shake for 15 min, a 2cm foam layer indicate the presence of saponin.

Fenugreek extract shows foam layer.

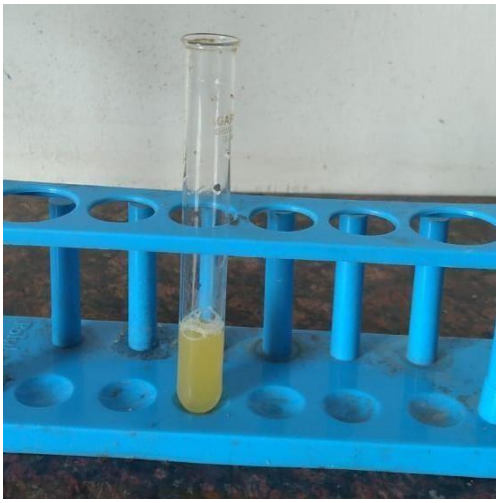

Fig 10- Foam test

Evaluation of nutmeg extract

Detection of alkaloid - Mayer test – few ml of extract and 2,3 drops Mayer reagent, white creamy precipitate indicatetest positive.

Nutmeg extraction shows white creamy precipitate

Fig 11- Mayer test

2) Hager test – few ml of filtrate 1to 2 ml Hagers reagent add, prominent yellow precipitate indicate test positive.

Nutmeg extraction shows yellow precipitate

Fig 12- Hager test

Detection for flavonoid

lead acetate test – extract 50mg dissolve in water 3ml of lead acetate solution was added bulky white lead precipitate indicated positive.

Nutmeg extraction shows bulky white precipitate.

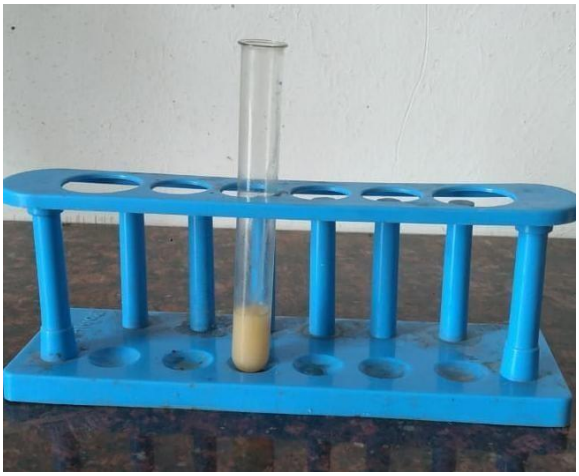

Fig 13- lead acetate test

7) detection of tannin –

Ferric chloride test – 0.4 extract and 4ml NaoH form the emulsion

Nutmeg extraction shows emulsion.

Fig 14- ferric chloride test

8) Detection of glycoside –

Extract and 1.5ml glacial acetic acid ,5 percent ferric chloride, concentrated sulfuric acid formation brown ring shows presence of glycoside.

Nutmeg extraction shows brown ring formation.

Fig 15- glycoside test

9) Detection of carboxylic acid –

Extract and sodium bicarbonate mix together appearance of effervescence.

Fenugreek extraction shows appearance of effervescence

10) Detection of saponin

Extract and 20ml of water take in test tube shake for 15 min, a 2cm foam layer indicate the presence of saponin.

Fenugreek extract shows foam layer

Fig 16- foam test

PREPARTION OF GEL

PREPATION OF FENUGREEK GEL

1)Take Carbopol 934 polymer (gelling agent) weigh individually take in beaker and add water mix it after that it was stirred continuously by mechanical stirrer until it will be mixed properly and form gel.

2)Add of triethanolamine and again stirred it continuously for 2 to 3 min and kept it 24 hours at room temperature.

3)Add methyl paraben and propyl paraben.

4)Finally add fenugreek extract to gel base with continuous stirring till the extract dispersed completely.

Formula for fenugreek gel

Table no.1 Formula for fenugreek gel

Ingredient	**Quantity**	**Role**
Fenugreek extract	10ml	Antifungal agent
Carbopol-934	2gm	Gelling agent
Triethanolamine	2,3 drop	Ph controller
Methyl paraben	3gm	preservative
Propyl paraben	1gm	preservative
Distilled water	100ml	Vehicle

Fig 17- gel base

Fig 18- fenugreek gel

Preparation of nutmeg Gel

1)Take Carbopol 934 polymer (gelling agent) weigh individually take in beaker and add watermix it after that it was stirred continuously by mechanical stirrer until it will be mixed properly and form gel.

2) Add triethanolamine and again stirred it continuously for 2 to 3 min and kept it 24 hour at room temperature.

3) Add methyl paraben and propyl paraben.

4) Finally add nutmeg extract to gel base with continuous stirring till the extract dispersed completely.

Formula for nutmeg gel

Table no.2 Formula for nutmeg gel

Ingredient	Quantity	Role
Nutmeg extract	10ml	Antifungal agent
Carbopol-934	2gm	Gelling agent
Triethanolamine	2,3 drop	Ph controller
Methyl paraben	3gm	preservative
Propyl paraben	1gm	preservative
Distilled water	100ml	Vehicle

Fig 19-Gel base

Fig -20 Nutmeg gel

EVALUTION OF GEL

Evaluation of fenugreek gel

physical evaluation

1) colour-the formulated herbal gel checked for colour, the colour is found to beorange.

2) Odour – odour is checed it is bitter.

3) pH

Take 1gm of gel in 9 ml of distilled water, dissolved it properly and deep into prob of pH meter, and measure the pH using PH meter in triplet form**.**

Take average of three reading and note a pH.

4) Homogenicity

The prepared gel is tested for homogenity by visual inspection after gel set into the container, it is tested for their presence of any aggregates.

5) Spreadability

Spreadability was determine by using glass side.

Take 2 glass slides and take 1gm of gel in lower slide and keep another slide on it apply force. Note down the first slide gel length and after force applying length of gel .and calculate spreadability by using formula.

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

Where, S- Spreadability

M – Weight apply on upper slide L- Length of glass slide

T- time (in sec) taken.

6)Skin irritation test

The gel is applied on skin and observe for any rashes, redness and irritation.

EVALUTION OF NUTMEG GEL

physical evaluation

1)Colour -the formulated herbal gel checked for colour, the colour is found to be light orange.

2)Odour - sweet

3)pH

1gm of gel dissolved 9ml of water and checked the pH in triplet form by using pH meter Take average of three reading and note a pH.

4)Homogenicity

The prepared gel is tested for homogenity by visual inspection after gel set into the container, it is tested for their presence of any aggregates.

5)Spreadability

Spreadability was determine by using glass side.

Take 2 glass slides and take 1gm of gel in lower slide and keep another slide on it apply force. Note down the first slide gel length and after force applying length of gel .and calculate spreadability by using formula:

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

Where ,S- Spreadability

M – Weight apply on upper slide L- Length of glass slide

T- time (in sec) taken.

6)Skin irritation test

The gel is applied on skin and observe for any rashes, redness and irritation.

Antifungal activity

Procedure

1) Take 50ml of potato dextrose agar as nutrient media.

2) Sterilized it with using autoclave and also autoclaves the 2 plates.

3) Pour the nutrients media in 2 plates as 25ml in first plate and 25 ml in another plates.

4) Take fungus and gel, dilute the gels and mix properly

5) Make walls (holes) in plate and add gels in that walls, make three holes 1st for sample,2nd for standard ,3rd for control.

6) Put gel as sample in 1st wall, in 2nd wall put dilution of fluconazole tablet dilution as standard and in 3rd wall put saline solution as control.

7) spread the fugus in plate and close the plate

8) Labelled it properly and keep it in the incubator for incubation at 37 ˚c.

9) after 48 hour check it, zones will be obtain hence it prove antifungal activity.

Result of antifungal activity

Fenugreek. gels show antifungal effect, and nutmeg gel shows antifungal effect when we perform antifungal activity comparing with a standard antifungal drug i.e fluconazole.

Zone of inhibition

Gel name	sample	standard	saline
Fenugreek gel	7-8mm	15-17mm	1mm
Nutmeg gel	4-5mm	15-17mm	1mm

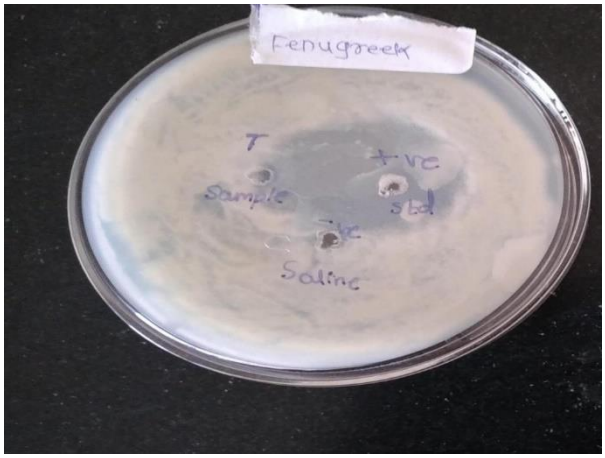

Fig 21Antifungal activity

Of fenugreek gel

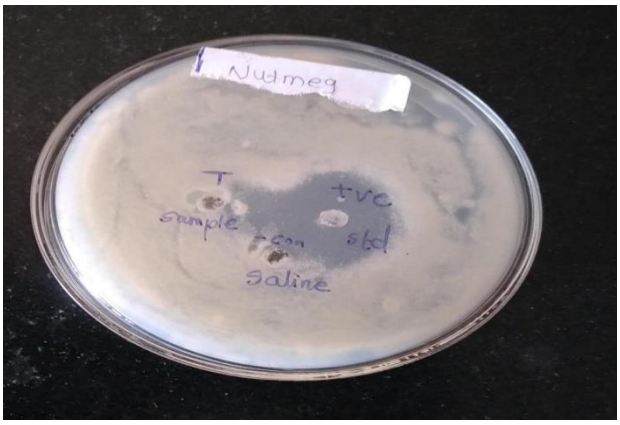

Fig 22– Antifungal activity

of nutmeg gel

RESULT

Result of phytochemical test for fenugreek extraction

Sr no	Phytochemical test	Chemical test	Result
1	Alkaloids	1) Mayer test 2) Hagers test	positive positive
2	Flavonoids	Lead acetate test	positive
3	Tannin	Emulsion test	positive
4	Glycoside	Keller -killani test	positive
5	Carboxylic acid	Effervance test	positive
6	Saponin	Foam test	Positive

Phytochemical test for Nutmeg extraction

Sr no	Phytochemical test	Chemical test	Result
1	Alkaloids	1) Mayer test 2) Hagers test	positive positive
2	Flavonoids	Lead acetate test	positive
3	Tannin	Emulsion test	positive
4	Glycoside	Keller -killani test	positive
5	Carboxylic acid	Effervescence test	positive
6	Saponin	Foam test	positive

Evaluation Of Fenugreek Gel

Sr no	parameter	Result
1	colour	Orange
2	Odour	Bitter
3	pH	6.1
4	Homogenicity	Good
5	spreadability	26
6	Skin irritation test	No irritation rashes,redness is seen on skin.

Evaluation of nutmeg gel

Sr no	parameter	Result
1	colour	Orange
2	Odour	Sweet
3	pH	5.93
4	Homogenicity	Good
5	spreadability	26
6	Skin irritation test	No irritation, rashes, redness is seen on skin

Conclusion

In this work it is conclude that the herbal gel prepared by fenugreek extract and nutmeg extract for fungal infection is formulated successfully. Extracted by the maceration process of extraction and evaluated by phytochemical test it shows various phytochemical is present in extract. Then the gel is formulated and evaluated ,it shows good result of evaluation test then its pharmacological activity checked against the fungus ,it shows the zone of inhibition so fenugreek gel and nutmeg gel both shows antifungal activity.

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