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THE STUDY OF PHYTOCHEMICAL CHANGES IN FENUGREEK LEAVES OF TWO DIFFERENT SELECTED AREAS OF GUJARAT

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ABSTRACT

Like the majority of plants, fenugreek has a number of secondary metabolites that have significant promise. This paper's objective is to assess the changes in phytochemicals of Unjha and Adalaj area of Gujarat using standard methods for phytochemical screening and quantitative analysis like TPC, TTC. Tannin, phenol, carbohydrates and diterpens are presented in the phytochemical screening of the both study area's methanolic leaves extract. Unjha area's leaf sample shows the more TPC and TTC. Thin layer chromatography for both the study area's leaf sample was also evaluated.

INTRODUCTION

Fenugreek (Trigonella foenum-graecum L.) is a member of the Fabaceae family, which is found all over the world. Alkaloids, flavonoids, steroids, saponins, and other potent substances are among the plant's active ingredients. The herb is an ancient remedy. Traditional foods and medicines have both frequently included it. Fenugreek is an annual herb that is native to the nations that border the eastern shores of the Mediterranean and is primarily grown in India, Egypt, and Morocco. The herb was formerly used to flavor subpar hay, hence the name fenugreek, which is derived from the Latin foenum graecum, which means Greek hay. The name Trigonella, which means "three-angled," is derived from an ancient Greek term and likely refers to the triangular shape of the flowers. Fenugreek is one of the obscure spices that are added to cuisine as a supplement to improve flavor and color. It also alters the texture of the dish. As an antimicrobial, stomach stimulant, anorexigenic, antidiabetic, and galactogogue activity, fenugreek was used as medicine in many traditional systems. (Snehlata, et al., 2012) (Srinivasan, 2006) (Yadav, et al, 2014)

METHOD

Plant material collection

The leaves of Trigonella foenum-graecum L. were collected from two different regions, Unjha and Adalaj of Gujarat, in the month of January 2023. Then the collected leaves of both area were washed to remove dust. After that, it was dried by shade drying at room temperature for one to two weeks. The dried plant material was crushed with the help of an electronic grinder

Extract preparation:

Extract was prepared using cold extraction method. The powdered leaves of Unjha and Adalaj was respectively weighed with a weighing machine. Both the study area's 10 grams of powder were respectively pour in 100 ml of methanol for 24 hours at room temperature. Followed by the solution was filtered using Whatman filter paper number 1 and allow to solvent becomes evaporate from filtrate.

Phytochemical Screening

Phytochemical screening

Phytochemical examinations were carried out for both the study arae extracts as per the standard method.

1. Alkaloids

a) Mayer's test

Two ml of Mayer's reagent are added along with one ml plant extract. Formation of a yellow coloure and white creamy precipitate indicates the presence of alkaloids.

b) Dragendroff's test

Two ml of Dragendroff's reagent are added along with one ml plant extract. Formation of an orange-red precipitate indicates the presence of alkaloids.

c) Hager's test

One ml of Hager's reagent are added along with one ml plant extract. Formation of a Yellow precipitate indicates the presence of alkaloids.

2.Carbohydrates

a) Molish's test

One ml of Molish's reagent are added along with one ml plant extract. Formation of a violet ring junction indicates the presence of Carbohydrates.

b) Fehling's test

One ml of Fehling solution A and B are added along with one ml plant extract and it should boil in a water bath for two minutes. Formation of red precipitate indicates the presence of Carbohydrates.

c) Benedict's test

One ml of Benedict's reagent are added along with one ml plant extract. Formation of a red-orange precipitate indicates the presence of Carbohydrates.

3. Flavonoids

a) Lead acetate test

One ml extract treated with three ml of 10% lead acetate solution. Formation of a yellow colour and bulky white precipitate indicates the presence of flavonoids.

b) Alkaline reagent test

Few ml extract treated with few drops of sodium hydroxide (NAOH) solution. Formation of a yellow colour turn in colourless indicates the presence of flavonoids.

c) H_2SO_4 test

Few ml extract treated with few drops of sulfuric acid. Formation of an orange precipitate indicates the presence of flavonoids.

4. Phenols

a) Ferric chloride test

Few ml extract treated with few drops of 5% ferric chloride solution. Formation of a Bluish black and blue-green precipitate indicates the presence of phenols.

b) Alkaline reagent test

Few ml extract treated with few drops of sodium hydroxide solution. Formation of a yellowish-red precipitate indicates the presence of phenols.

5. Tannins

a) Gelatin test

Few ml extract treated with few drops of 1% gelatin solution containing sodium chloride. Formation of a white precipitate indicates the presence of tannins.

b) Lead acetate test

Few ml extract treated with 1 ml of 10 % lead acetate solution. Formation of a white precipitate indicates the presence of tannins.

6. Glycosides

a) Borntrager's test

One ml extract treated with few drop of ferric chloride solution and boiling it for 5 minutes. Formation of the rose-pink colour indicates the presence of glycosides.

b) Ferric acid test

One ml extract treated with 1 ml glacial acetic acid and add 1-2 ml of Concentrated H_2SO_4. Formation of a Violet to blue-green indicates the presence of glycosides.

7. Protein

a) Ninhydrin test

Two ml extract treated with two drop of ninhydrin reagent. Formation of a purple colour indicates the presence of protein.

b) Xanthoproteic test

One ml extract treated with few drop of nitric acid. Formation of a yellow colored indicates the presence of protein.

8. Saponins

a) Forth test

50mg extract diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 to 2 cm layer of foam indicates the presence of saponins.

b) Foam test

0.5 gm extract diluted with distilled water to 2ml and this was shaken in graduated cylinder for 10 minutes. Formation of foam indicates the presence of saponins.

9. Diterpens

a) Copper acetate test

One ml extract treated with 3-4 drops of copper acetate solution. Formation of a green colour indicates the presence of diterpens.

10. Steroids

a) Libermann–sterol test

One ml extract treated with one ml acetic acid and add one drop concentrated sulfuric acid. Formation of a red, violet, blue, or green indicates the presence of steroids.

b) Salkowaski's test

Two ml extract shake with chloroform and add sulfuric acid. Formation of a red coloration indicates the presence of steroids.

Total Phenolic content

The total phenolic content was determined using the Folin-Ciocalteu reagent method (Singleton et al., 1965) (Khaled Tawaha and Alali, et al., 2007). In this method, 1 ml of Folin-Ciocalteu reagent (FCR) were added respectively to 1 ml of diluted methanolic extract of both the study area. Then 1 ml of a 20% sodium carbonate solution (Na_2CO_3) was added and the total volume was made up to 10 ml with double distilled water. Absorbances were recorded at 765 nm using a UV spectophotometer after incubation for 30 minutes. The results, determined from the equation of a calibration curve established from the Gallic acid taken as reference, were expressed in mg of Gallic Acid Equivalent per gram of extract (mg GAE/ g).

Total tannin content

Total Tannins were determined using the peri and pompei method. TTC activity is the primary method for determining the amount of tannin content in a sample. 0.3 ml of folin-denis reagent were added respectively to 1 ml of diluted methanolic extract of both the study area. Then 1 ml of a 7.5% sodium carbonate is added and the total volume was made up to 10 ml with double distilled water. Absorbances were recorded at 750 nm was measured using a UV spectophotometer after incubation for 30 minutes. The results, determined from the equation of a calibration curve established from the tannic acid taken as reference, were expressed in mg of Tanic Acid Equivalent per gram of extract (mg GAE/ g).

Thin layer chromatography

Thin layer chromatography is a straightforward technique that is vital for sorting, identifying, and characterizing unidentified chemicals. Both the study area's methanolic extract was analyzed by TLC methods. This technique uses glass or aluminium plates coated with silica gel G- 60 (1-1.5mm thick layers). 50µl aliquot of solution of the leaf extract were applied separately to each plate. Leaves extract was run in 8 different solvent system, which was considered as mobile phase methanol: chloroform (90%:10%), methanol: chloroform (80%:20%), methanol: Acetone (80%:20%) give the best separation the finalization of the solvent system develops the TLC plated and derivatization of P- Anisaldehyde reagent. After spraying the TLC plates observed under the UV short wavelength (245nm) and UV long wavelength (365nm) and the Rf value of spots were calculated and check changes in chromatogram of the selected study area.

RESULT AND DISCUSSION

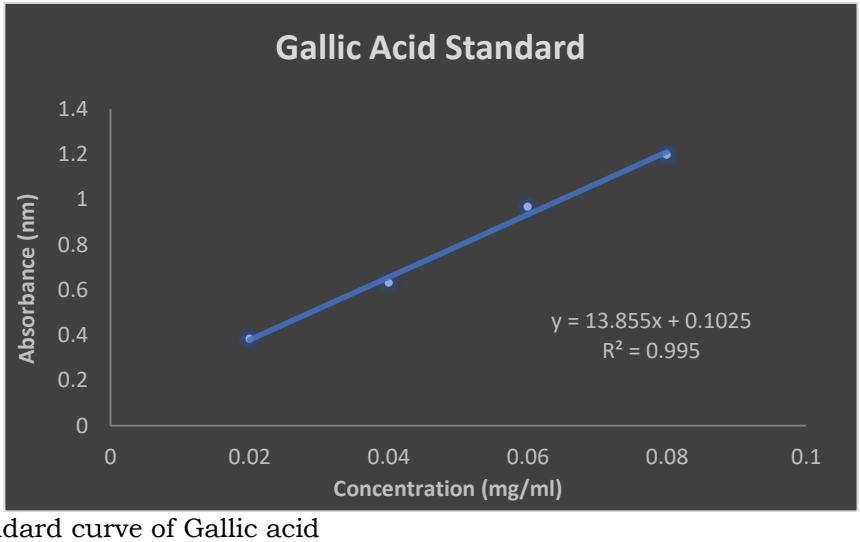
Phytochemical screening

Table 1.The phytochemical analysis of unjha and Adalaj's leaves of fenugreek.

Sr.no.	phytochemicals	Test	Leaves of fenugreek from Adalaj	Leaves of fenugreek from Unjha
1	Alkaloids	mayer's test	-	-
		Dragendorff test	-	-
		Hager's test	-	-
2	carbohydrates	Molisch's test	-	-
		Fehling's test	+	+
		Benedict's test	-	-
3	Flavonoids	lead acetate test	-	-
		Alkaline reagent test	-	-
		H2So4 test	-	-
4	Phenols	Ferric chloride test	+	+
		Alkaline reagent test	-	-
5	Tannins	Gelatine Test	-	-
		lead acetate test	+	+
6	Glycoside	Borntrager's test	-	-
		Ferric chloride test	-	-
7	Protein	Ninhydrin test	-	-
		Xanthoproteic test	-	-
8	Saponins	Forth test	-	-
		Foam test	-	-
9	Diterpenes	Copper acetate test	+	+
10	Steroids	Liebermann sterol test	-	-
		salkowski's test	-	-

Table1 shows the methanolic plant extract of fenugreek leaves of both study area confirmed the presence of carbohydrates, phenols, tannins and diterpenes. There is no changes found in qualitative phytochemical screening of selected area.

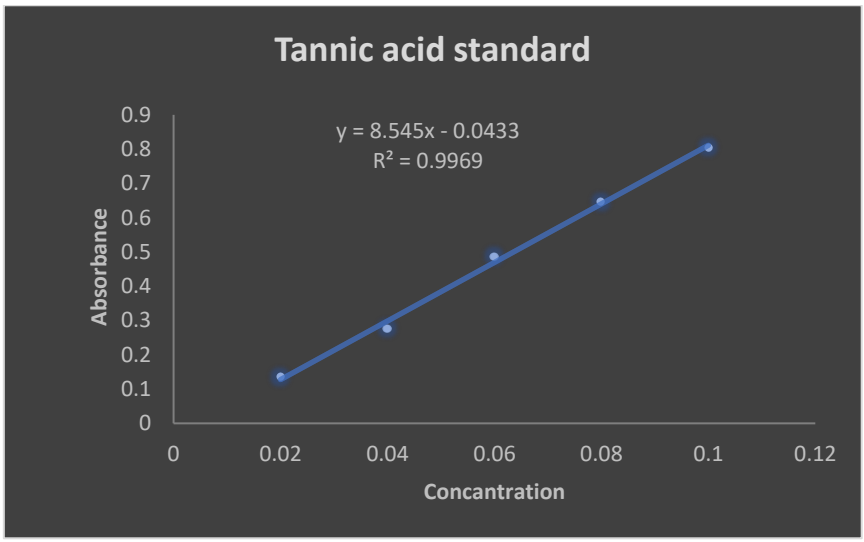
Total phenolic content



Graph1: standard curve of Gallic acid

The Phenolic content of fenugreek leaves of unjha and adalaj area determined using Gallic acid standard. The standard calibration equation for total phenolic concentration was found to be y = 13.855x + 0.1025 (R^2 = 0.995) , using the following calibration equation the phenolic content of fenugreek leaf of unjha and adalaj area respectively 0.0159 ± 0.0005 GAE/g and 0.0158 ± 0.0001 GAE/g. Total phenolic content of fenugreek leaves of unjha , slightly found more in concentration than adalaj area .

Total tannin content



Graph 2: standard curve of Tannic acid

The tannin content of fenugreek leaves of unjha and adalaj area, determined using tannic acid standard. The standard calibration equation for total tannin concentration was found to be, y = 8.545x-0.0433 (R^2 = 0.9969). Using the calibration equation the tannin content of fenugreek leaf of unjha and adalaj area respectively 0.0157 ± 0.0008 TAE/g and 0.0144 ± 0.0002 TAE/g. unjha area's fenugreek shows more total tannin content than adalaj area.

Thin layer chromatography

Table: 2 methanolic fenugreek leaves extract solvent system, total bands and Rf values of Unjha and Adalaj area.

Sr. No.	Solvent system	Total band (Unjha)	Rf value (Unjha)	Total band (Adalaj)	Rf value (Adalaj)
1	Methanol: Choloroform (9:1)	3	0.950,0.900,0.825	2	0.916,0.805
2	Methanol: Choloroform 8:2	4	0.948,0.871, 0.794, 0.743	2	0.941,0.794
3	Methanol: Acetone (8:2)	2	0.871, 0.948	3	0.975,0.925,0.850

Table: 2 shows the, unjha area's number of methanolic leaves extract shows more separation in methanol: chloroform (90%:10%) and methanol: chloroform (80%:20%). Solvent system, adalaj area's methanolic leaves extract shows more number of separation in methanol: acetone (80%:20%) solvent system than unjha area's leaves of fenugreek.

CONCLUSION

Presented study concluded that, both the study area confirmed the presence of carbohydrates, phenols, tannins and diterpens. This study also identify the TPC and TTC found in more quantity of unjha area's fenugreek leaves sample. More number of separation also found in Methanol: Choloroform (9:1) and Methanol: Choloroform (8:2) solvent system of unjha area's fenugreek leaf sample.Based on the study's findings, it can be said that the methanolic extract of fenugreek serves as the source of phytochemicals.

REFERENCES

1) Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. International journal of advanced research in chemical science, 2(4), 25-32.
2) Maria, R., Shirley, M., Xavier, C., Jaime, S., David, V., Rosa, S., & Jodie, D. (2018). Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. Journal of King Saud University-Science, 30(4), 500-505.
3) Mujeeb, F., Bajpai, P., & Pathak, N. (2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. BioMed research international, 2014.
4) Pandey, A., & Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry, 2(5), 115-119.
5) Peri C and Pompei C: Estimation of different phenolic groups in vegetable extracts. Phytochemistry. 1971; 10(9): 2187-2189.
6) Roghini, R., & Vijayalakshmi, K. (2018). Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of Citrus paradisi. International Journal of Pharmaceutical Sciences and Research, 9(11), 4859-4864.
7) Slinkard K and Singleton VL: Total Phenol Analysis: Automation and Comparison with Manual Methods. Am J Enol Vitic. 1977; 28: 49-55.
8) Snehlata, H. S., & Payal, D. R. (2012). Fenugreek (Trigonella foenum-graecum L.): an overview. Int J Curr Pharm Rev Res, 2(4), 169-87.
9) Srinivasan, K. (2006). Fenugreek (Trigonella foenum-graecum): A Review of Health Beneficial Physiological Effects. Food Reviews International, 22, 203 - 224.
10) Yadav, U. C., & Baquer, N. Z. (2014). Pharmacological effects of Trigonella foenum-graecum L. in health and disease. Pharmaceutical biology, 52(2), 243-254.