Research Article

Screening of Bio-active Secondary Metabolites for Qualitative Analysis *of Trigonella foenum-graceium* and *Hibiscus sabdariffa*

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ABSTRACT

Trigonella foenum graecum is a yellowish-brown seed containing medicinal annual herb that belongs to the *Fabaceae* family. It is commonly known as fenugreek (methi). It constitutes several phytochemicals and bioactive compounds. Similarly, *Hibiscus sabdariffa* seed is also known as rosella seed. Both fenugreek rosella seed has a brief history of ancient medicinal uses to treat disease, especially in Ayurvedic medicine. A comparative phytochemical chart was prepared in this paper by conducting several qualitative analysis tests on both of the seed samples' methanol extracts. The present investigation found that the methanol extract of fenugreek showed good results in flavonoid detection, alkaloid, and phenolic lignin. However, the methanol extract of rosella seed showed quite different from fenugreek seed, which means it showed good results in detecting resin. This data is quite helpful for future herbal drug formulation based on phytochemicals.

Keywords: *Trigonella foenum gracecum*, *Hibiscus sabdariffa*, phytochemical qualitative analysis.

INTRODUCTION

A compound that seems to be normal but in reality not a normal compound but also a soldier of plant that protects plants against microorganisms, however in ancient medicinal history many more traditional healers and ayurveda explained the dominance of these bioactive compounds in case of resistance against pathogenic microorganism. another main characteristic of these bioactive compounds it considered as a secondary metabolite. not only in ancient but also in the modern era these bioactive compounds are named phytochemicals. according to history, the name comes from the Greek word phyton[1]. Recent scientific studies have established a relationship between the consumption of phytochemicals such as carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibers, polysaccharides, etc., with health benefits such as prevention of diabetes, obesity, cancer, cardiovascular diseases, etc. This has led to the popularization of phytochemicals. Nowadays, foods contain phytochemicals as a constituent (functional foods)[2]. *Hibiscus sabdariffa* is an aromatic, astringent, cooling herb that is used in the tropics. Also, *Trigonella foenum-graecum* is a multipurpose medicinal herb in medicinal plant history. however, both had different local names in different regions of Earth. In India, fenugreek is known as methi, menthol, etc, in the same way, rosella is known as kudrum, khatefuel, gongura, souri, mesta, puli-cheer, Erragomgura. Both rosella and fenugreek plants an edible plant. *Hibiscus sabdariffa* was probably native to West Africa. It was domesticated 600 years ago and spread to Asia and West Indies. now grown all over the world. Fenugreek is native to central Asia. originating around 4000 BC. The Ebers Papyrus, one of the oldest maintained medicinal documents, describes fenugreek and its benefits in 1500 before century Egypt[3]. According to the literature survey of research papers, fenugreek contains several phytochemicals (alkaloids, flavonoids, phenol, steroids, carbohydrates, lipids, amino acids), rosella seed also has phytochemical (flavonoids, tannins, triterpenoids, phenol). both fenugreek and rosella were tremendous medicinal history in ancient modern disease treatment era. Plenty of research papers published on anti-inflammatory, anti-fungal, antibiotic, anti-oxidant, and anti-cancer activity shown by these two medicinal herb species. On February 6, 2024 one of the research articles where on the silico study of a natural compound on wedang Uwuh a rosella plant used as a therapeutic agent for COVID-19. According to the journal promkes rosella tea increases hemoglobin in pregnancy. Another research paper published in 2020 on the action mechanism of rosella used to treat metabolic syndrome in elderly women[4,5]. In the journal of diabetes research, 2019 published the antidiabetic effect of fenugreek seed powder solution on Hyperlipidemia in Diabetic patients. Similarly, another research paper was published on fenugreek-derived disogenin as an emerging source for diabetic therapy, and then another research paper on ethnopharmacological, phytochemical, and clinical studies on fenugreek[6].

MATERALS AND METHODS

Sample Collection

The seeds of *Trigonella foenum-graecum* were one of the self-pollinating annual herbarium aromatic crops collected from Noida, Uttar Pradesh. another one is *Hibiscus sabdariffa* commonly known as rosella. The seed sample was collected from Odisha Ganjam district.

Preparation of sample

The seed sample was crushed with the help of a motor pestle then the whole powdered sample was weighed by using an analytical weighing balance.

Preparation of extract

The seed extract was prepared by employing the Maceration method. For these 5 grams of dry powdered sample was weighed into a conical flask. Then 20 ml of 80 % methanol was added to it. The sample was taken in a conical flask and placed on a shaker at 100 rpm for 48 hours. After completion of 48 hours (methanol + sample), the solution was filtered. The filtrate was decanted into an airtight container and the residue was discarded.

Phytochemical screening by qualitative analysis test:

DETECTION OF FLAVONOIDS

a. Alkaline reagent test[7]

Pipette out 200µl of samples into a test tube. add 2ml of 2% NaOH solution into it then at last add 200µl of dilute HCl. The yellow color turned colorless after adding dilute hydrochloric acid this result indicates presence.

b. Alkaline reagent test with 10 % liquor ammonia[8]

Take 2 ml of sample mixed with 800µl liquor ammonia in a test tube. The appearance of yellow fluorescence indicates the presence of flavonoids in the sample.

c. Lead Acetate test[9]

1 ml of the sample was taken in a test tube. 200 lead acetate solution was prepared and dissolved with the sample. A yellow appearance indicates a positive result.

d. Mg – HCl reduction test[10]

200µl of concentrated HCl dissolved with 1ml sample then 2 to 3 pieces of Mg metal added into it. the appearance of pink to crimson color indicates the presence of flavonal glycosides in the sample.

DETECTION OF PHENOLIC COMPOUND

a. Potassium dichromate test[11]

1ml of sample dissolved with 200µl of 1N potassium dichromate solution. A dark color appearance indicates the presence of the phenolic compound in a sample.

b. Iodine test[9]

1 ml of sample dissolved with dilute iodine solution (200µl) appearance of transient red color indicates the presence of the phenolic compound in a sample.

c. Ferric chloride test[12]

1ml of aqueous extract dissolved with a few drops of 5% ferric chloride solution. Dark green / bluish-black color indicates the presence of the phenolic compound in a sample.

d. Cartenoid test[13]

1ml of plant extract pipette out into test tube. 2ml of chloroform was added to the sample (shaken vigorously) then 1ml of concentrated sulphuric acid to the sample. A blue color at the interface confirms the presence of phenolic in a sample.

SCREENING OF ALKALOID

a. Dragendoffs / krauts test[14]

1ml of the sample treated with 1ml of Dragendoff's reagent. The formation of a reddish-brown precipitate indicates the presence of an alkaloid in the sample.

b. Mayers / bertrands / valsers test[15]

1ml of each plant extract treated with 1ml of Mayers reagent. Formation of a creamy white or yellow precipitate indicates a positive result.

c. Wagner's reagent test[7]

1ml of filtrate treated with 1ml of Wagner's reagent. appearance of brown/reddish precipitate indicates the presence of alkaloids in the sample.

DETECTION OF LIGNIN

Labat test[16]

1 ml of each plant sample was taken in a separate test tube. dissolve it with 2ml gallic acid solution. The formation of an olive-green solution indicates the presence of lignin in the sample.

DETECTION OF DITERPENES

Copper acetate test[17]

1ml of sample was diluted with 1ml distilled water also treated with 200µl Copper Acetate solution. emerald green color indicates a positive result.

DETECTION OF PHLOBATANNINS

HCl test[18]

1 ml of sample was treated with 1 ml dilute HCl. A red precipitate indicates the presence of phlorotannins in the sample.

DETECTION OF TRITERPENOIDS

Salkowski's test[7]

The extract, 1ml was treated with 200µl concentrated sulphuric acid. then shaken vigorously and allowed to stand well. formation of a golden yellow color at the bottom indicates the presence of triterpenoids in the sample.

DETECTION OF TERPINOIDS[19]

500µl of concentrated chloroform was added to 1ml of plant extract and then set for evaporation in a water bath. Keep an eye concentration of the solution because in this step solution doesn't need to be reduced the amount of solution this step was only for the evaporation of

chloroform. after that solution was treated with 1ml of concentrated sulphuric acid (boiled in a water bath). the grey color solution indicates the presence of terpenoids in the sample.

DETECTION OF PHYTOSTEROL

Hesses response[20]

1 ml of plant extract was treated with 1.5 ml chloroform and 500µl sulphuric acid. formation of a pink ring / red color (in the lower chloroform layer) indicates a positive response.

DETECTION OF ANTHOCYANIN

HCl test [21]

1 ml of sample pipette out into test tube. 1 ml of 2N HCl was added to the sample. Also, add 1ml of liquor ammonia. If the pink-red solution turned into a blue-violet-colored solution after the addition of liquor ammonia then it is considered to be positive.

SCREENING OF ANTHRAQUINONE

Borntrager's test [22]

2ml of sample was treated with 5ml of 10 % ammonia solution. A pink, violet, or red color solution indicates the presence of anthraquinone in the sample.

DETECTION OF RESINS

a. Turbidity test [23]

1ml of plant extract dissolved with 1ml acetone in a test tube. then add 2 ml of distilled water to it. Turbidity indicates the presence of resin in the sample.

b. Turbidity test with 4% HCl[13]

1ml sample was taken in a test tube Then add 2ml of 4% HCL to the wall of the test tube. the appearance of turbidity indicates the presence of resin in the sample.

DETECTION OF COUMARINS

NaOH paper test[24]

The filter paper was soaked in 1N NaOH solution for 5 to 10 minutes. After completing 5 to 10 minutes take out the filter paper from NaOH solution. then allow the filter paper to dry. During filter paper drying, pipette out 1ml of the sample into the test tube. then covered the mouth of the test tube with dry NaOH-soaked filter paper. After that, NaOH soaked paper-wrapped test tube was heated for a few minutes in a water bath. after heating take out the tube from the water bath. leave a few minutes to cool down the test tube. then remove the filter paper carefully without getting damaged. leave the filter paper for some time. then observe the result by placing filter paper under UV light. Observance of yellow fluorescence under UV light indicates the presence of coumarins in a sample.

DETECTION OF QUINONE

a. Alcoholic KOH test[7]

1ml plant extract was treated with 1ml of alcoholic KOH solution. Color changes from red to blue color indicate the presence of quinone in a sample.

b. Concentrated HCl test [17]

1 ml of sample dissolved with 200 µl of concentrated HCl. The formation of a green color in solution indicates the presence of quinone.

DETECTION OF CARDIAC GLYCOSIDES

Keller–killani test[16]

1 ml of the sample (plant extract) dissolved with 1.5 ml glacial acetic acid and 200µl of 5% ferric chloride solution then add 500µl concentrated sulphuric acid to the solution. A blue-colored solution in the acid layer indicates the presence of cardiac glycosides in a sample.

SCREENING OF SAPONIN

Foam test [12]

1 ml of plant extract was mixed with 8 ml of distilled water. shaken vigorously until foam starts appearing. after starts appearing foam leave it for a while. After some time if the foam is there in the solution then it is considered a positive result.

DETECTION OF TANIN

Braymers test [22]

1ml extract was diluted with 1ml distilled water then add 100µl 5% ferric chloride to the previously mixed solution. Black/green/blue / color form and precipitation occurs in the lower layer, indicating the presence of tannin in the sample.

RESULTS

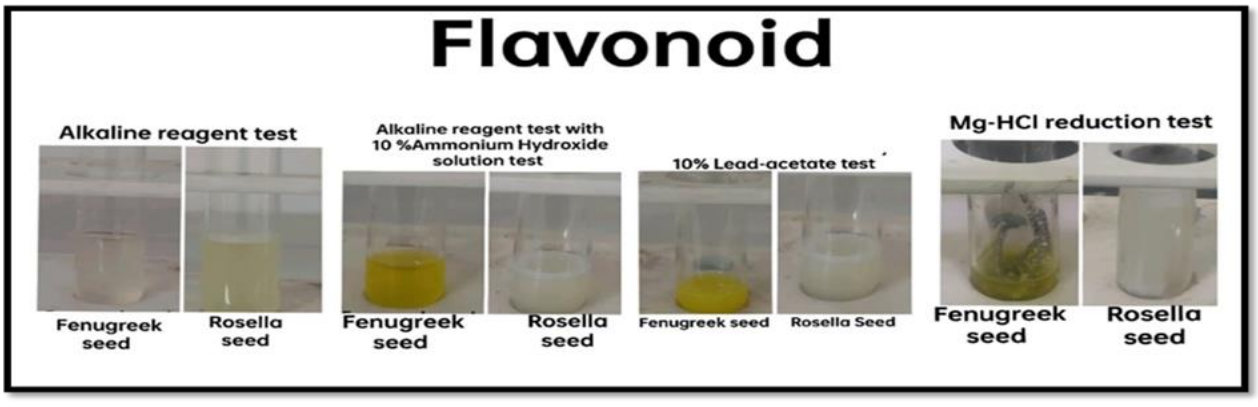
The present research was conducted on comparative phytochemical qualitative analysis of *Trigonella foenum graecium* and *Hibiscus sabdariffa* (Table 1 and Figure 1). through which we found primary secondary metabolites that will help further therapeutic agent-related research in disease resistance. nowadays several research conducted on ancient medicinal herb and their effect on human beings. In the comparative analysis of fenugreek and rosella seed flavonoids were present in fenugreek and absent in rosella seed. however, a minimal amount of alkaloids presence found in rosella seed. We found both fenugreek and rosella seeds show antifungal activity in the study. The concentration of phytochemicals in the methanol extract of fenugreek and rosella seed was quite different which means fenugreek contains several types of phytochemicals however rosella seeds contain only a few types of phytochemicals found. Through qualitative analysis, the results showed the presence of flavonoids, alkaloids, phenol, saponin, tannin, cardiac glycosides, resin, phenolic compound, and diterpenes, in fenugreek

methanol extract. However, a small amount of resin was found in rosella seed methanol extract, cardiac glycosides, and alkaloids. Also flavonoid, phenolic compound, lignin, quinone, coumarin, anthroquinone, anthocyanin, phobatannin, triterpene, saponin, and tannin are absent in the methanolic extract of rosella seed. in methanol extract of fenugreek seed coumarin, anthroquinone, anthocyanin, terpenoids, phobatanin, and quinone, are absent. Different types of solvent play an important role in extractability in different phytochemicals in the present investigation methanol solvent extraction showed good results in fenugreek however in the case of rosella seed it showed a mixed result.

Table 1: Qualitative phytochemical test results

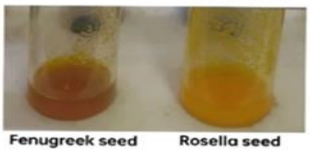
Phytochemical Name	Test name	Fenugreek	Rosella
Flavonoid	Alkaline reagent test	+++	-
	Alkaline reagent with 10% ammonium Hydroxide solution	++	-
	Lead Acetate test	++	-
	Mg-HCl reduction test	+/-	-
Phenolic compound	Potassium dichromate test	++	-
	Iodine test	+	-
	Ferric chloride test	++	-
	Carotenoids test	+++	-
Alkaloids	Dragendroffs test	+++	++
	Mayers test	++	-
	Wagnors test	++	+/-
Lignin	Labat test	+++	-
Diterpenes	Copper acetate test	+/-	+
Phobatannin	HCl test	-	-
Triterpenoids	Salkowski's test	+/-	-
Phytosterols	Hesses response test	++	++
Anthocyanin	HCl test	-	-
Anthraquinone	Borntragers test	-	-
Resins	Turbidity test	+++	+++
	Turbidity test with 4% HCl	+++	+++
Coumarin	NaOH paper test	-	-
Quinone	Alcoholic KOH test	-	-
	Concentrated HCl test	-	-
Cardiac - Glycosides	keller kilani test	+/-	+/-
Saponin	Foam appears test	++	-
Tannin	Braymer' s test	++	-

Figure 1: Different phytochemical test results.



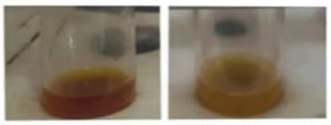
Phenolic compound

Potassium Dichromate test



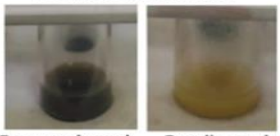
Fenugreek seed Rosella seed

Iodine test



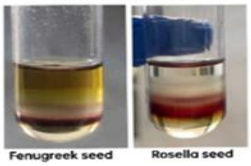
Fenugreek seed Rosella seed

Ferric Chloride test



Fenugreek seed Rosella seed

Cartenoids test



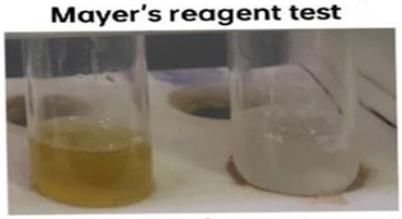
Fenugreek seed Rosella seed

Alkaloid

Dragendroff's reagent test

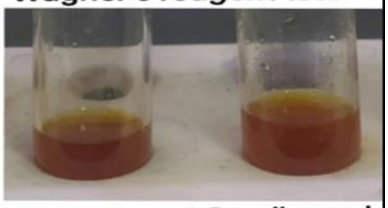
Fenugreek seed Rosella seed

Mayer's reagent test



Fenugreek seed Rosella seed

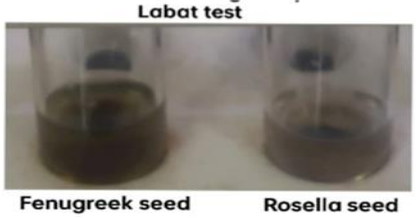
Wagner's reagent test



Fenugreek seed Rosella seed

Lignin

Labat test



Fenugreek seed Rosella seed

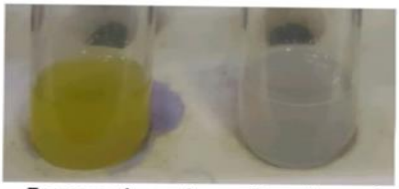
Diterpenes

Copper Acetate test

Fenugreek seed Rosella seed

Phlobatannin

HCl test



Fenugreek seed Rosella seed

Triterpinoid

Salkowski's test

Fenugreek seed Rosella seed

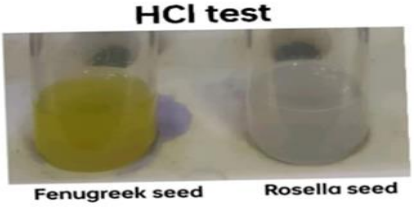
Diterpenes

Hesse's response test

Fenugreek seed Rosella seed

Anthocyanin

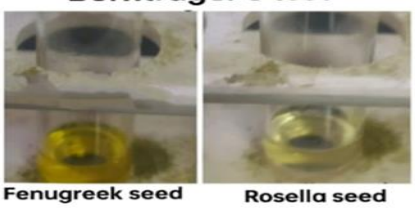
HCl test



Fenugreek seed Rosella seed

Anthraquinone

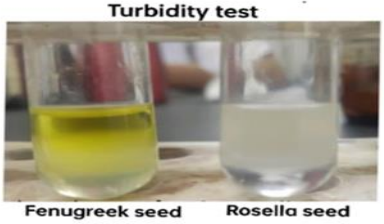
Borntrager's test



Fenugreek seed Rosella seed

Resin

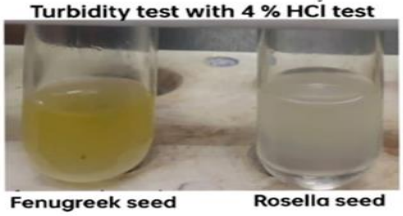
Turbidity test



Fenugreek seed Rosella seed

Resin

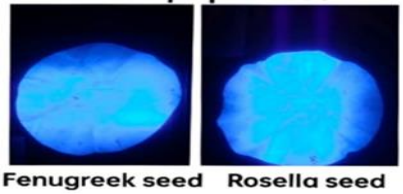
Turbidity test with 4 % HCl test



Fenugreek seed Rosella seed

Coumarin

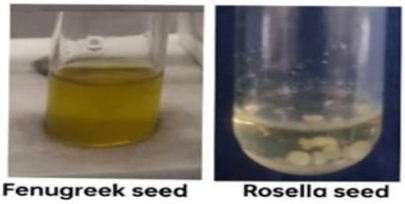
NaOH paper test



Fenugreek seed Rosella seed

Quinone

Alcoholic KOH test

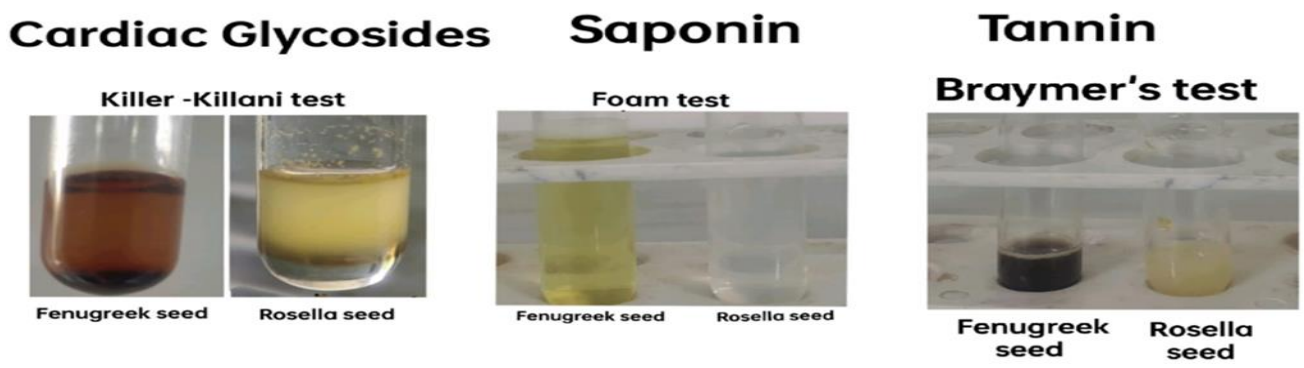


Fenugreek seed Rosella seed

Quinone

Concentrated Hydrochloric acid test

Fenugreek seed Rosella seed



DISCUSSION

Results indicate that the fenugreek seed will be used as a therapeutic agent. it also indicates having anti-inflammatory, antioxidant, and viral properties. In the present study, the qualitative investigation of fenugreek and rosella seed through methanol solvent showed presence of primary and secondary metabolites. This indicates that in this multidrug resistance era, these results will show a positive indication towards use as a therapeutic agent against several diseases that was Alzheimer's, epilepsy, stroke, schizophrenia, and hungington disease. However, in alkaloid test also showed positive results in case of the methanol extract of Rrosella seed. This result also indicates that rosella seed has Antioxidant and anti-inflammatory properties: Rosella seeds are rich in phenolic compounds, flavonoids, and anthocyanins that have potent antioxidant and anti-inflammatory effects[25,26]. Hypolipidemic and antiatherogenic effects: Studies in animal models show that rosella seed extract can decrease lipid oxidation, LDL cholesterol, total cholesterol, and triglycerides while increasing HDL cholesterol. This suggests rosella seeds have cardioprotective and anti-atherogenic properties. Antidiabetic activity: Rosella seed extract has been found to inhibit α-glucosidase and α-amylase enzymes, which can help control blood glucose levels and manage diabetes[27,28,29]. Antibacterial effects: Rosella seed extract exhibits antibacterial activity against various pathogens, including multidrug-resistant strains like MRSA. Potential benefits for kidney health.in India, a decoction of rosella seeds is used to relieve pain during urination, indicating potential benefits for kidney disorders. Nutritional profile[30,31]. Rosella seeds are a good source of carbohydrates, protein, fat, fiber, and various minerals like potassium, phosphorus, magnesium, zinc, iron, manganese, and copper. However, more research is needed to fully establish the efficacy and safety of using rosella seed extract for treating diseases in humans. Clinical trials and further pharmacological studies are necessary to determine appropriate dosages and applications[32].

CONCLUSION

It can be concluded that fenugreek seeds are well-known for having a wide variety of phytochemicals, such as flavonoids, alkaloids, and antioxidants. Conversely, roselle seeds are highly valued for their pectin content, high fibre content, and health benefits like anti-diabetic and antioxidant qualities. Both seeds have significant health advantages and may be used in a variety of culinary and therapeutic contexts.

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