International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Extraction of Fruit & Seed from Black Grape & Fenugreek using Different Solvents for Phytochemical Screening followed by Anti-Microbial Activity Against *E. Coli*

Ankita Priyadarshini Das[1], Dharitri Priyadarsini[2], Rajat Kumar Nayak[3]

[1]MTech, Biotechnology, GIET University, Gunupur, Odisha
[2,3]Senior Research Associate, SBio Science Pvt Ltd, Bhubaneswar, Odisha.

Abstract

The phytochemical components of many plant species are used in herbalism because they have been scientifically proven to have medicinal characteristics and approved by regulatory agencies including the European Food Safety Authority or the United States the Food and Drug Administration (FDA). Alkaloids, tannins, flavonoids, as well as phenolic compounds are examples of the bioactive components found in plants that offer health advantages with a demonstrated physiological effect on people. Around worldwide, the resistance of pathogens to conventional antibiotics is expanding. Since more bacteria are becoming resistant to antibiotics due to their extensive use, current antimicrobial drugs may become ineffective in treating a number of bacterial infections. Medicinal plants have been the most prolific source of medication bio-resources in terms of phytochemicals for conventional medicines, conventional therapies, nutritional supplements, dietary additives, medicinal intermediary substances, and chemical entities for synthesized pharmaceuticals. Spices have been historically used in food as culinary ingredients and food preservatives from the beginning of time. The current study assesses the screening of fenugreek (*Trigonella foenum-graecum*) and grapes' phytochemical and anti-microbial activities. The two specimens had been gathered, extracted using various solvents, and qualitatively checked for the existence of various phytochemical kinds. For assessing the antibacterial property against *E. coli*, the agar diffusion methodology was employed. When employed against *E. coli*, black grape produced the zone of inhibition with the largest diameter, measured 23.5 mm, while fenugreek produced the smallest, measuring 21.25 mm. The goal of the current study is to identify plants that possess different antimicrobial properties that could be used to analyze and isolate novel phytochemicals to prevent the spread of various infectious diseases, particular in light of the sudden emergence of a growing number of drug-resistant microorganisms and the requirement to create more effective antimicrobial agents.

Keywords: Fenugreek, black grapes, flavonoid, tannin, alkaloids, *E. coli*, antimicrobial activity, chromatography.

Introduction:

Fenugreek

Fenugreek, also known as *Trigonella foenum-graecum*, is a perennial plant that belongs to the Leguminosae family. It consists of the renowned spices used in human food. According to a centuries-old tradition in human history, the seeds of fenugreek and green leaves can be used in both food and medicinal. Additionally, it has been applied to alter the flavour, colour, and texture of food items. Fenugreek seeds offer a number of health advantages, such as the capacity to reduce cholesterol, encourage lactation, combat bacteria, energise the stomach, cure anorexia, manage diabetes, safeguard the liver, and combat cancer. These beneficial physiological effects, which include anti-diabetic and hypocholesterolemic effects, are principally brought on by the intrinsic dietary fiber found in fenugreek, which has promising nutraceutical value **[1]**.

It is well known for its gum, fibre, and other chemical substances as well as its volatile compounds. About 25% of dietary fibre is found in fenugreek seeds, which changes the texture of food. It is presently used as a food stabiliser, adhesive, and emulsifying agent due to its high fibre, which means protein, and gum content. Alkaline pH levels make fenugreek's protein more soluble. The use of fenugreek can alter the manner in which food is cooked and has a beneficial effect on digestion **[1].**

The benefits of sprouted fenugreek seeds versus non-germinated seeds are numerous. The essential amino acids tryptophan, lysine, and leucine are especially abundant in them. Germination increases the ability to digest protein and absorb fat in vitro. Germination causes an increase in saturated fatty acids while decreasing total unsaturated fatty acid content, total lipids, which triglycerides, phospholipids, and unsaponifiable matter. They have been shown to help diabetic people with their blood sugar and cholesterol levels. Numerous studies have looked into the medical advantages of fenugreek seeds, but none have looked into how effective they are as antioxidants **[2].**

[Fig.1: Fenugreek seeds]

Black grapes

The black grape (*Vitis vinifera*) harvest is one of the most well-liked fruit harvests worldwide. In the global market as a whole, jams prepared from juice from grapes and raisins are also important products. Numerous studies have looked into the antioxidant and health-promoting properties of grapes. Black grape's high phenol content has increased attention in their health benefits. Most of the phenolic chemicals are found in the seed pods of black grapes. Gallic acid, catechin, and epicatechin are the three primary phenolic substances in black grape seeds, whereas ellagic acid and myricetin are the two main phenolic compounds in the skins **[3].**

Grape seeds are rich in bio-active flavonoid that have different antioxidant properties. The therapeutic advantages of employing extracts from grapes are based on their antioxidant properties, which reduce the creation of free radicals, in addition to their capacity to reduce the replication of cytoskeletal proteins, which limits the development of bacterial cell walls. As a result, it has been suggested that combining various antibiotics with Vitis vinifera's alcoholic extract has a beneficial synergistic effect **[5].**

[Fig.2: Black grapes]

Materials and Methods:

Sample Collection: Black grapes (*Vitis vinifera*) and fenugreek seeds (*Trigonella foenum-graceum*) were procured from the neighbourhood market in Bhubaneswar, Odisha, as good and healthy samples.

Sample Preparation: Fenugreek dry seeds and black grapes were obtained and crushed into powder and liquid respectively by using a mortar and pestle. The powdered sample of 2.5 gm was placed in four separate test tubes with 25 ml each of distilled water, acidified water, hydro-alcohol, and methanol. After 24-48 hours, the extracts were filtered by using filter paper and then the sample extracts were used for further analysis. In the same way, the liquid samples of 2.5 ml were mixed with 22.5 ml of different solvents and then filtered.

Qualitative Phytochemical Screening: The sample extracts were tested for phytochemical through various screening methods.

Test for Alkaloids: To the 0.5 ml extract, 2-4 drops of Mayer's reagent were added and observed the formation of reddish-brown precipitate **[4]**.

Test for Flavonoids: 1.5 ml of extract was treated with 4-6 drops of 20% sodium hydroxide solution shows yellow colouration which disappeared on addition of dilute hydrochloric acid **[4]**.

Test for carbohydrate: The extracts of 2 ml added to 10 drops of Molisch's reagent, along with 1.5 ml of concentrated sulphuric acid down the side of the test tube. Then allow the mixture to stand for 2-3 minutes. Then the formation of red or dull violet colour at the interface of the two layers is a positive result **[7]**.

Test for Proteins: 2 ml of extract and 5 drops of 1% ninhydrin solution were added and placed in a boiling water bath for 2-5 minutes and observe for the formation of purple colour **[4]**.

Test for Cardiac Glycosides: 1.5 ml of extract was treated with glacial acetic acid and 3-5 drops of 5% aqueous ferric chloride solution. To this mixture 0.5 ml of concentrated sulphuric acid was added and observed for a reddish-brown ring at the interface **[7]**.

Test for Phenols: 1 ml of extract were treated with 0.5 ml of aqueous 5% ferric chloride and observed for the formation of deep blue or black colouration **[4]**.

Test for Steroids: To 1 ml of extract, 2 ml of sulfuric acid and 1 ml of chloroform was added. The formation of reddish-brown precipitate indicates the presence of steroids.

Test for Tannins: 5 ml extract and 2.5 ml of 10% ferric chloride solution was mixed, which gives bluish-green or black precipitation **[4]**.

Test for Terpenoids: 2 ml of chloroform was added to 2 ml of extract and few drops of concentrated sulfuric acid were added. A reddish brown precipitate produced immediately after the mixture was shaken well **[7]**.

Test for Saponins: To 1 ml of extract add 10 drops of sodium bicarbonate solution and left to rest for 5 minutes after shaking vigorously. Formation of a honey comb like froth indicates the presence of saponins **[4]**.

Test for Resin: 20 ml of distilled water was added to 5 ml of extract. The presence of resins was recorded when a precipitate occurred.

Test for Coumarin: 1 ml of alkaline solution was added to 1 ml of sample and observed the formation of yellow colour.

Thin Layer Chromatography: Each of the plant extracts were checked by Thin Layer Chromatography (TLC) on analytical plates over silical gel. For each extract, the solvent system Benzene:Chloroform:Acetone = 3:1:1 was used as developing systems. In each case, the spots were visualized by naked eye and UV transilluminator.

Antibacterial Activity Assay: Antibacterial assay of plant extracts was performed against *E. coli* bacteria on the basis of its suitability and functionality. The microorganism used was cultured at SBio Science Pvt. Ltd., Bhubaneswar, Odisha. Inoculation of the culture was carried separately in each nutrient agar. Wells of 8 mm in diameter were prepared on inoculated agar and filled with 100 µl of extract. It was then incubated at 37°C for 24 hours and the clear zone of inhibition was measured after 24 hours.

Results and Discussion:

Qualitative Phytochemical Analysis:

It is clear from the data that major phytochemicals present in fenugreek seed as compare to black grape. Almost all the phytochemicals are identified from hydro-alcohol and acidified water extracts of

fenugreek as compare to methanol and distilled water extracts.

The qualitative phytochemical analysis of fenugreek seed extract and black grape extract is shown in Table 1 and table 2 respectively.

Phytochemicals	Hydro alcohol extract	Acidified water extract	Methanol extract	Distilled water extract
Alkaloid	Less +ve	+ve	+ve	+ve
Flavonoid	-ve	-ve	-ve	-ve
Carbohydrate	+ve	+ve	+ve	+ve
Protein	+ve	Less +ve	+ve	+ve
Cardiac glycoside	+ve	-ve	+ve	+ve
Phenols	+ve	+ve	-ve	-ve
Steroids	+ve	+ve	-ve	-ve
Tannin	+ve	+ve	+ve	+ve
Terpenoid	+ve	+ve	-ve	-ve
Saponin	+ve	+ve	+ve	+ve
Resin	-ve	+ve	-ve	+ve
Coumarin	+ve	+ve	+ve	+ve

[Table.1: Qualitative phytochemical analysis of different solvent extracts of seed of fenugreek]

Plant extract	Hydro alcohol extract	Acidified water extract	Methanol extract	Distilled water extract
Alkaloid	+ve	Less +ve	+ve	-ve
Flavnoid	-ve	-ve	-ve	-ve
Carbohydrate	-ve	-ve	+ve	-ve
Protein	-ve	-ve	+ve	-ve
Cardiac glycoside	-ve	-ve	-ve	-ve
Phenols	-ve	-ve	-ve	-ve
Steroids	+ve	+ve	+ve	+ve
Tanin	-ve	-ve	-ve	-ve
Terpenoid	+ve	+ve	+ve	+ve
Saponin	-ve	Less +ve	-ve	-ve
Resin	-ve	-ve	-ve	+ve
Coumarin	+ve	+ve	+ve	+ve

[Table.2: Qualitative phytochemical analysis of different solvent extracts of black grape]

Thin Layer Chromatography:

The retardation factors (Rf) for each of the eight extracts in the solvent systems are detailed below.

Plant Extracts	Rf Value
Fenugreek (HA)	0.72, 0.92, 0.41
Fenugreek (AW)	0.87, 0.55, 0.92
Fenugreek (M)	0.59, 0.65. 0.92
Fenugreek (DW)	0.92, 0.87, 0.77
Black Grape (HA)	0.91, 0.73, 0.90
Black Grape (AW)	0.80, 0.84, 0.59
Black Grape (M)	0.91, 0.86, 0.47
Black Grape (DW)	0.63, 0.61, 0.90

[Table.3: The retardation factor (Rf) for each of the eight extracts of fenugreek and black grape in different solvent system (HA: Hydro Alcohol, AW: Acidified Water, M: Methanol, DW: Distilled Water]

Antibacterial Activity of Plant Extracts:

Data regarding antibacterial properties of the plant extract is presented in table 4. From the data, it also depicted that acidified water extract of fenugreek and acidified water, hydro alcohol extract of black grape showed higher activity in case of *E. coli* which might be due to the presence of high levels of terpenoids and tannins in these extracts. This antibacterial activity was attributed the ability of tannins to inhibit cell wall synthesis and the ability of terpenoids to weaken the membranous tissues creating dissolution of cell wall of microorganism. The highest antibacterial activities against *E. coli* were found to be acidified water extract of black grape (23.5 mm), followed by acidified water extract of fenugreek (21.25 mm) and hydro alcohol extract of black grape (12.75 mm).

Plant Extracts	Zone of Inhibition (mm)
Fenugreek (HA)	NA
Fenugreek (AW)	21.25
Fenugreek (DW)	NA
Black Grape (HA)	12.75
Black Grape (AW)	23.5
Black Grape (DW)	NA

[Table.4: Antibacterial activity of different solvent extracts of fenugreek and black grape against *E. coli*]

Conclusion:

Because it is often believed that natural products are readily available and have no adverse effects, herbs are used medicinally in a range of nations. An unusual herb with a long history of usage in complementary medicine is fenugreek. Fenugreek offers advantages for decreasing blood sugar levels, raising testosterone, and improving milk production in people who are breastfeeding, according to the research that is currently available. Fenugreek may also help with appetite control, reduce inflammation, and lower cholesterol levels, though more research is needed in these areas. Standardized fenugreek extract is gaining acceptance as a botanical medication, medical food, and nutraceutical in the United States and throughout the world based on pharmacological knowledge and commercial feasibility. Since the late 1980s, it has gained enormous popularity as a nutraceutical supplement both domestically and internationally. Even though fenugreek shows significant promise for the prevention and treatment of many disorders, further research is needed to discover the actual potential of fenugreek products as effective nutraceutical supplements, medical foods, botanical pharmaceuticals, or over-the-counter drugs.

The research reviewed here strongly suggests that fenugreek is a special medicinal plant with a wide range of health advantages. Fenugreek and black grape are discovered to be the best sources of bio-active chemicals for improved health prospects. These substances can also be used to create prospective pharmaceutical molecules that could be developed into drugs for a variety of serious illnesses, including cancer. The development of a potential drug that could treat various types of infections and lead to full utilization by the local community may result from the identification and isolation of the active chemical components held by these traditional plants like fenugreek and fruit like grapes for further study. Research organizations and the pharmaceutical industry may find the findings of this study to be of use in developing new medicines and also can be used in the treatment of different types of diseases.
The findings of this study will help identify some molecules that are highly beneficial for creating new medications. Due to the existence of phytochemical elements, the results of the current investigations and prior phytochemical analyses are almost identical.

Acknowledgment:

We are grateful to SBio Science Pvt. Ltd. for supporting us for well-established laboratory facilities and helping us in paper writing.

Conflict of Interest:

Nil

References

1. Wani, S. A., & Kumar, P. (2018). Fenugreek: A review on its nutraceutical properties and utilization in various food products. *Journal of the Saudi Society of Agricultural Sciences*, *17*(2), 97-106.
2. Dixit, P., Ghaskadbi, S., Mohan, H., & Devasagayam, T. P. (2005). Antioxidant properties of germinated fenugreek seeds. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *19*(11), 977-983.
3. Lakshmi, B. V. S., Sudhakar, M., & Aparna, M. (2013). Protective potential of Black grapes against lead induced oxidative stress in rats. *Environmental toxicology and pharmacology*, *35*(3), 361-368.

4. Tanya, M., Mishra, S. P., Das, A. P., Acharya, B., Priyadarsini, D., & Nayak, R. K. Physiochemical and Antioxidant Evaluation of Moringa Oleifera Leaf Extract Followed by Phytochemical Screening and Antibacterial Activity. *IJFMR-International Journal for Multidisciplinary Research*, *5*(1).
5. Al-Mousawi, A. H., Al-Kaabi, S. J., Albaghdadi, A. J., Almulla, A. F., Raheem, A., & Algon, A. A. A. (2020). Effect of black grape seed extract (Vitis vinifera) on biofilm formation of methicillin-resistant Staphylococcus aureus and Staphylococcus haemolyticus. *Current microbiology*, *77*, 238-245.
6. Tyihák, E., Mincsovics, E., & Kalász, H. (1979). New planar liquid chromatographic technique: overpressured thin-layer chromatography. *Journal of Chromatography A*, *174*(1), 75-81.
7. Tanya, M., Priyadarsini, D., & Nayak, R. K. (2022). Screening of Bioactive Compounds from Madagascar Periwinkle and Their Importance in Drug Discovery. *IJFMR-International Journal for Multidisciplinary Research*, *4*(6).
8. Rios, J. L., Recio, M. C., & Villar, A. (1988). Screening methods for natural products with antimicrobial activity: a review of the literature. *Journal of ethnopharmacology*, *23*(2-3), 127-149.
9. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, *6*(2), 71-79.