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Total Phenolic, Flavonoid, Saponin Content and Anti-oxidant Potential of *Trigonella foenum- graecum* and Raw *Musa paradisiaca*

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KEYWORDS

Fenugreek, Raw Banana, total phenolic content, total flavonoid content, total saponin content, DPPH assay

ABSTRACT:

Objective: The current study was intended to quantify phytochemical content and assess antioxidant potential in extracts of *Musa paradisiaca* (raw banana) fruits with peels and *Trigonella foenum-graecum* (fenugreek) seeds. **Material and methods:** Both plants were extracted by the soxhlet extraction method, and phytochemical tests were carried out. The Folin-Ciocalteau method, the aluminum chloride method, and the vanillin sulfuric acid method were used to determine their total phenolic content, flavonoid content, and saponin content, respectively. Using the DPPH assay, their antioxidant potential was ascertained. Results and discussions: Both plant extracts contained steroids, alkaloids, tannins, phenols, flavonoids, and saponins, according to phytochemical tests. Phenols, flavonoids, and saponins were measured and represented milligrams of gallic acid equivalent (GAE), milligrams of quercetin equivalent (QE), and milligrams of diosgenin equivalent (DE) per gram of dry weight, respectively. Conclusion: Raw banana fenugreek extract contains phenols, flavonoids, and saponins. Both medications have strong antioxidant potential and can be further studied for synergistic effects in treating diabetes mellitus, hyperlipidemia, and hyperthyroidism.

1. Introduction

In Ayurveda, one or more herbs, or polyherbs, are used to treat a wide range of illnesses. The Sarangdhar Samhita, an Ayurvedic literature, emphasized the idea of polyherbalism to increase therapeutic effect. [1] The idea is also present in other traditional medical systems, where illnesses can be treated by combining several herbs in a specific ratio. [2] Many phytochemicals, including ascorbic acid, carotenoids, flavonoids, phenols, and chlorophyll derivatives, are found in medicinal plants and act as natural antioxidants. Phenolic substances have been demonstrated to have antioxidant power in vitro and are more potent than vitamins C and E. [3] According to estimates from the World Health Organization (WHO), 80% of people worldwide still primarily receive their medical care from traditional practitioners. [4] Nutraceuticals and healthful foods contain saponins, [2] and have several qualities, including antioxidant, immunostimulant, hypocholesterolemic, antimicrobial, anti-inflammatory, and anticarcinogenic.[5] Natural antioxidants like saponins and phenolics can scavenge free radicals produced by the body as a result of normal metabolism or environmental pollution.[6] The fenugreek (*Trigonella foenum graecum*) seeds, also known as methi, are members of the Fabaceae family and contain a variety of phytochemicals, including steroids, flavonoids, phenols, saponins, and alkaloids.[7] It can be used to treat diabetes mellitus, gastric lesions, Parkinson's disease, rheumatoid arthritis, cancer, and asthma. Banana, or Musa paradisiaca, belongs to the family Musaeae and contains a variety of phytoconstituents, including tannins, alkaloids, and flavonoids (gallocatechin, quercetin),[8,9] which have therapeutic value and can be used to treat diabetes mellitus, menorrhagia, bronchitis, dysentery, diarrhea, and ulcers [8].

2. Materials And Methods

Plant selection, procurement and authentication:

Trigonella foenum graecum and Raw Musa paradisiaca have been selected for study, and they were bought from Bidkin, Aurangabad's Banni Tanda. Further authenticated by the head of the department of botany, Dr. BAMU, Aurangabad (431001), with accession number 00756

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Extraction of Plants:

500 g of Fenugreek seeds were extracted with a hydro-alcoholic solvent, and 500g of raw banana fruits with peel powder were extracted with 90% ethanol using a Soxhlet apparatus for 48 hours. The extracts were dried at 60°C with reduced pressure and kept in storage at 4°C until needed. Phytochemical tests were performed according to the book by Dr. K.R. Khandelwal. [10]

Quantitative Estimation of Phytoconstitu ents:

Total Phenolic Content Determination:

Using the oxidation-reduction reaction-based Folin-Ciocalteu colorimetric method, the total phenolic content of plant extracts was ascertained. Gallic acid solutions in methanol were prepared in different concentrations (10, 20, 30, 40, 50, and 60 µg/ml). 1 ml of gallic acid of each concentration was added in different test tubes, and 5 ml of Folin-Ciocalteu reagent (10%) and 4 ml of 7% Na2CO3 were added to get a total volume of 10 ml.The blue-colored mixture was shaken well and incubated for 30 minutes at 40° C in a water bath. Then, the absorbance was measured at 760 nm against the blank.Every experiment was run in triplicate. The calibration curve was plotted using the average absorbance values obtained at various gallic acid concentrations. For fenugreek and banana extracts, the same process was repeated. The milligrams of gallic acid equivalents (GAE) per gram of dry extract (mg/g) were used to report the extracts' total phenolic content.

The equation C = cV/m was used to determine the entire phenolic content in every sample. Here, C stands for the entire phenolic compound in mg of gallic acid equivalent/g dry extract, c for gallic acid concentration measured in mg/ml from the curve used for calibration, V for extract volume in milliliters, and m for extract mass in grams [11].

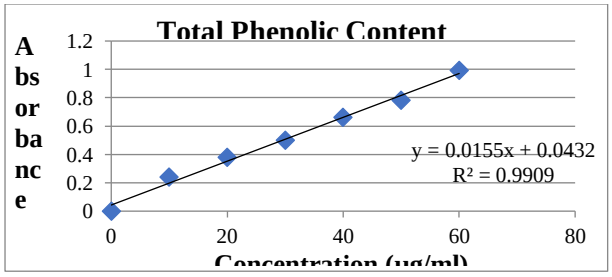

Figure 1: Gallic acid standard curve for estimating total phenolic content

Total Flavonoid Content Determination:

The total flavonoid content was ascertained using the colorimetric assay with aluminum chloride. Standard quercetin was prepared in methanol at different concentrations (20, 40, 60, 80, and 100 µg/ml). An aliquot of 1 ml of quercetin of each concentration was added to a 10 ml volumetric flask containing 4 ml of distilled water. 0.3 ml of 5% sodium nitrite was added at zero time, followed by 0.3 ml of 10% AlCl3 and 2 ml of 1 M sodium hydroxide at six minutes. Distilled water was then added to bring the mixture's total volume to 10 ml, and the mixture turned pink. The mixture's absorbance was measured at 510 nm against a blank that contained all of the reagents except quercetin. The calibration curve was plotted using the average absorbance values obtained at various quercetin concentrations.

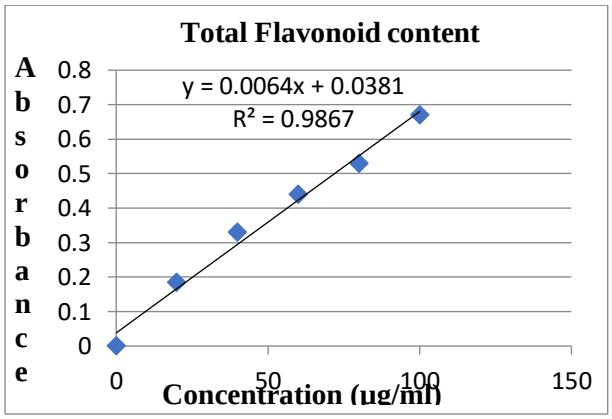

Figure 2: Quercetin Standard Curve for Estimating Total Flavonoid Content

Samples were prepared, and the same methodology was used to measure absorbance as the standard. The extracts' overall flavonoid content was reported in mg of equivalents of quercetin (QE) in one gram of dry extract (mg/g). Every experiment was run in triplicate. C = cV/m is the formula used to calculate total flavonoid content. Here, C stands for total flavonoid content mg QE/g dry extract, c is the quercetin concentration measured in mg/ml from the calibration curve, V is the extract volume in milliliters, and m is the extract mass in grams.[12].

Total Saponin Content

To make a standard saponin solution, 110 mg of diosgenin was dissolved in 16 milliliters of methanol and 4 milliliters of purified water. This was then further

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diluted to create various concentrations (100–600 µg/ml) to estimate the total saponin content. Sulphuric acid (72% v/v, 2.5 ml) and vanillin reagent (8%, 0.25 ml) were gradually added to each concentration. After thoroughly combining the solutions, the tubes were placed in a water bath set at 60°C. After incubating for ten minutes, the tubes were cooled in an ice-cold water bath for three to four minutes. The absorbance was measured at 544 nm with respect to the reagent blank. The same procedure as the standard was followed to prepare the samples, and absorbance was recorded.

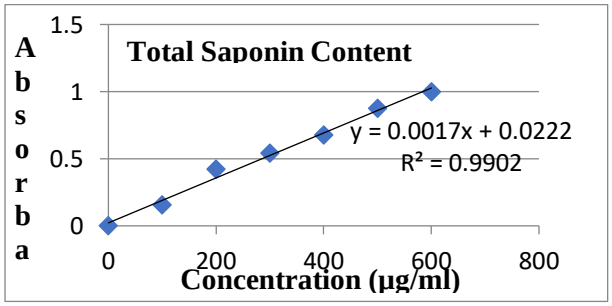

Figure: 3 Diosgenin standard curve for estimating total saponin content.

All the experiments were carried out in triplicate. Total saponin content is calculated by using the formula: C = cV/m, where C = total saponin content mg DE/g dry extract, c = concentration of diosgenin obtained from the calibration curve in mg/ml, V = volume of extract in ml, and m = mass of extract in grams. [13, 14]

Antioxidant activity by the DPPH assay

The antioxidant activity of raw banana and fenugreek was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay with some modifications. Different concentrations of both extracts (10 to 50 µg/ml) were prepared. 0.2 ml of each concentration was combined with 3.8 ml of DPPH solution, and the mixture was left to sit at room temperature for one hour in the dark.

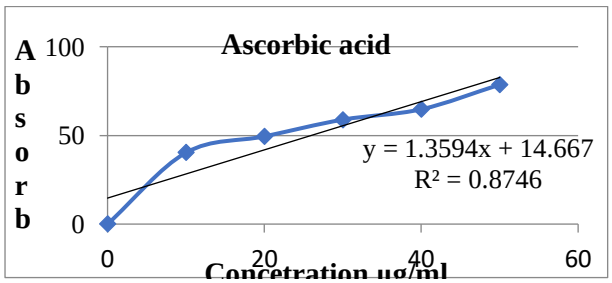

Figure 4: Ascorbic acid standard calibration curve

The mixture's absorbance was then measured at 517 nm. The same procedure was followed for standard ascorbic acid and blank (without a sample), which were used as positive and negative controls, respectively. IC50 was computed using the percent inhibition versus concentration plot. [15, 16] The following equation was used to calculate the sample's percentage of DPPH radical scavenging activity:

(Control absorbance – sample absorbance) Control absorbance

X 100

Statistical analysis:

All values are presented in the form of mean ± S.E., and statistical data is analyzed using a one-way ANOVA test using Graph Pad in-state software.

3. Result And Discussion

Qualitative and quantitative analyses of raw banana and fenugreek were performed by spectrophoto- metric assay. Both drug extracts showed the presence of phytoconstituents like alkaloids, phenols, flavonoids, saponins, tannins, and steroids after several chemical tests. Quantitative estimation of phytochemicals is shown in Table 1. Total phenolic content The TPC of raw banana was found to be 89.46±0.64 mg gallic acid equivalent/g dry weight of the sample, and in fenugreek 57.23±0.21 mg gallic acid equivalent/g dry weight, it was calculated using a calibration curve of standard gallic acid depicted in Figure 1.

Total flavonoid content:

The flavonoid content of both drug extracts was estimated by the aluminum chloride spectrophotometric method. The total flavonoid content of raw banana was found to be 126± 0.155 mg quercetin equivalent/g dry weight of the sample, and in fenugreek extract, it was 66.85 ± 0.56 mg quercetin equivalent/g dry weight of the sample. As seen in Figure 2, it was computed using a calibration curve of standard quercetin.

Table:1. Quantitative Estimation of phyto-constituents.

Sr. No	Phytochemicals	Fenugreek	Raw Banana

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1	Total phenolic content (mg of gallic acid equivalent/g of dry weight)	57.23± 0.21	89.46 ± 0.64
2	Total flavonoid content (mg of quercetin equivalent/g of dry weight)	66.85± 0.561	126.38 ± 0.155
3	Total saponin content (mg of diosgenin equivalent/g of dry weight)	265.66 ± 0.577	46.93 ± 0.585

Mean ± Standard Deviation (n=3)

Total saponin content

The total saponin content was determined by utilizing a standard diosgenin calibration curve as shown in Figure 3. Total saponin content in raw banana was found to be 46.93±0.585 mg diosgenin equivalent/g dry weight and in fenugreek 265.66 ± 0.577 mg diosgenin equivalent/g dry weight. Phenols, flavonoids, saponins, and tannins are responsible for the antioxidant activity of plants and have anti-inflammatory, antibacterial, and anticancer activities. [17]

Antioxidant activity by DPPH assay

The DPPH assay was used to determine the percentage of DPPH radical scavenging activity of the raw banana and fenugreek extracts, as shown in Figure 5. The IC50 values for the two extracts were 62.67 µg/ml and 81.48 µg/ml, respectively.

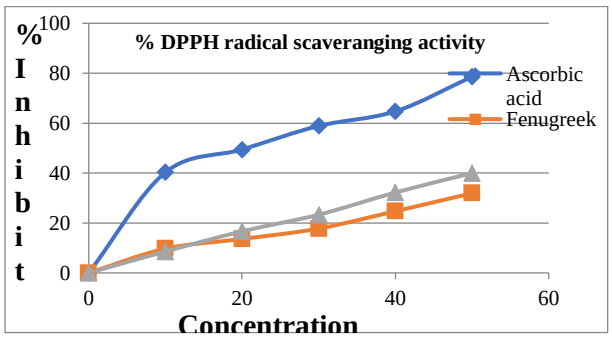

Figure 5: DPPH radical scavenging activity.

Among the various suggested mechanisms, the ability of an antioxidant to neutralize free radicals is the most common one [18]. Antioxidant substances Free radicals are scavenged by antioxidants like flavonoids, polyphenols, and phenolic acids. It has been described and revealed that flavonoids and numerous other phenolic components have potent antioxidant properties. [19]

4. Conclusion

Results reveal that *Musa paradisiaca* (raw banana) fruits with peels and *Trigonella foenum-graecum* (fenugreek) seedss have phytoconstituents like phenols, flavonoids, and saponins, which have been determined qualitatively and quantitatively. Both drugs showed good antioxidant potential and can be further studied for synergistic effects in the treatment of diabetes mellitus, cancer, bronchitis, hypercholesterolemia, and cancer.

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