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Phytochemical analysis of the ethanolic extracts of different Pearl Millet (*Pennisetum glaucum*) varieties

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ABSTRACT

Extraction of the different pearl millet varieties (Powdered form) was carried out using aqueous ethanol (50%) at moderate temperature (40 °C for 60 minutes). The extract was concentrated using the same temperature for 24-48 hrs. Standard methods were used to analyze the presence of different phytochemicals. The preliminary tests for phytochemicals revealed the presence of Coumarins, Steroid, Flavonoids, Flavonon, Tannins and reducing sugars.

Keywords: Pearl millet, Phytochemicals, Coumarins, Reducing sugar, Tannins

INTRODUCTION

Cereal grains serves as a source of food, fodder, medicine and other valuable nutraceuticals. Allopathic system of medicine already proved the beneficiary functions of cereal grains. In addition to being a source of basic nutrients cereal grains also works as herbal medicines. Although a variety of drugs are available but there is no medicine prepared till date that have no side effects/adverse effects. Efforts are required in research processes that may elaborate our knowledge about nature. These days phytochemical analysis is in demand and attracting the attention of various researchers. Most of the developed and developing countries have adapted the standard methods to detect the presence of bioactive compounds, alkaloids and other important constituents from plants and cereal grains.

Pearl millet is an indigenous cereal that is well adapted to tolerate semi-arid and sub-tropical conditions. Pearl millet can grow in poor soil and drought conditions. The grains of pearl millet are considered as a poor man food and a source of bioactive compounds such as phenolics and flavonoids [1]. Pearl millet is locally known as bajra, the whole grains and flour from the grains are used for the preparation of chapatti's and khichadi. In some countries out of overall production, approximately 75-80% part is represented by pearl millet only [2]. In addition to being a source of food they also serve as a good source of microelements such as iron and zinc [3].

MATERIALS AND METHODS

Grain samples

Pearl millet varieties such as HC-10, HC-20, HHB-197, HHB-223, HHB-226 and HHB-234 were purchased from Bajra Section, Chaudhary Charan Singh Haryana Agriculture University, Hisar. Before using all grains of different varieties were washed with tap water to remove dust particles and other debris etc. They were shade dried and

stored in airtight plastic containers (Figure 1). For further analytical work, grains of each pearl millet variety grinded separately using mixer to obtain fine powder (Figure 2).



Figure-1: Pearl millet varieties stored in airtight containers



Figure-2: Dried powdered form of pearl millet stored in small airtight containers

Chemicals

All chemicals such as Hydrochloric acid, Sodium Hydroxide, Lead Acetate, Chloroform, Sulphuric acid, α -naphthol, Fehling solutions A and B, Benedict reagent, Ferric Chloride etc. were purchased from Himedia Pvt. Ltd. Mumbai and Qualigens. All chemicals used during the present work were of analytical grade. Before using all glassware's were washed properly and dried in an oven at 180 °C for 1 hr.

Preparation of sample

Millet grains of all selected varieties were ground in a mixer-grinder to obtain a fine powder (Figure 2). All samples tested were defatted by blending the ground material with hexane (1:5 w/v, 5 min, thrice) in a waring blender at ambient temperature [4]. Defatted millet samples were air dried for 24 h and stored at -20 °C till further analysis (Figure 3).



Figure-3: Defatted samples (Pearl millet flour) of each variety stored in airtight containers at -20 °C

Preparation of extracts

1 gm defatted samples of each millet varieties were extracted with 50% ethanol (1:20 w/v) in a flask (250 ml capacity). The flasks were sealed with parafilm and wrapped with aluminum foil to prevent solvent loss. After

completing the extraction process, all extracts were filtered through Whatmann No.1 filter paper in order to obtain a clear crude extract solution.

PHYTOCHEMICAL ANALYSIS

Standardized tests were performed to detect the presence of particular phytochemicals in each extracts [5, 6].

- | | | |
|------------------|----------------|--------------|
| 1. Coumarin | 5. Flavonon | 9. Alkaloids |
| 2. Steroid | 6. Flavonoid | |
| 3. Phlabotannin | 7. Tannins | |
| 4. Carbohydrates | 8. Anthocyanin | |

Test for Coumarins

Coumarins were detected in different extracts by following already standardized method [5]. 1 ml of NaOH (10%) was added to a known volume of the extracts (1 ml). Yellow color shows the presence of Coumarins.

Steroid

The qualitative test for steroids in pearl millet extracts was performed [7]. 2 ml of chloroform was added to 2 ml extracts followed by same quantity (2 ml) of concentrated H₂SO₄. Formation of red color in chloroform layer shows the presence of steroids.

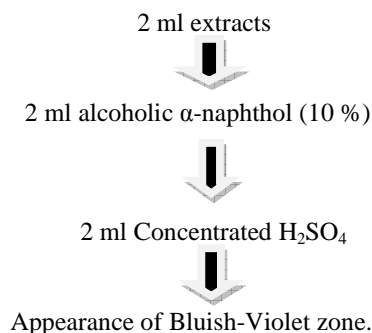
Phlabotannins

The presence of phlabotannins was observed in ethanolic extracts following Krishnaiah et al. [8] method. Equal quantity of extracts and HCl (1%) was added in test tubes and boiled for 5 minutes and cooled. Formation of reddish precipitates indicates the presence in phlabotannins.

Carbohydrates

Presence of carbohydrates in the different extracts of pearl millet was determined by following methods.

i. Molisch`s test



ii. Fehling`s test:

To 5 mL extract, equal volume of Fehling`s solutions A & B was added and heated on Bunsen burner. Appearance of red precipitate showed the presence of reducing sugars.

iii. Benedict`s test:

To 5 mL of extract 1 mL of Benedict`s reagent was added and heated and allowed to cool. Compare the change in colour of extracts with the colour mentioned on the Benedict`s reagent bottle.

Flavonoids

Flavonoids are important bioactive compounds that are ubiquitous in nature and beneficial for living beings. To detect the presence of flavonoids in pearl millet extracts 1ml of aqueous lead acetate (10%) was added to 1 ml of extract. The formation of yellow colored precipitates indicate the presence of flavonoids in the extracts

Tannins

Suitable quantities of various extracts (2 ml) were taken and tested for the presence of tannins with FeCl₃. Formation of greenish-black colour indicates the presence of tannins.

Anthocyanin

About 1 ml of NaOH (10%) was added to the 1 ml extracts. The change in colour to blue indicates the presence of anthocyanins.

Flavanon

To the test sample of each extract (1 ml) equal conc. of H₂SO₄ (Conc.) was added along the wall of test tube. Formation of crimson red color confirms the presence of Flavanon.

Alkaloids

- i. **Wagner's test:** 1.27 g of iodine and 2.0 g of KI (Potassium Iodide) were dissolved in 5.0 ml of water and the solution was diluted to 100 ml with water. When 2 ml of extract was treated with 2 ml of Wagner's reagent, formation of reddish brown precipitate confirms the presence of alkaloids.
- ii. **Mayer's test:** To 1ml of extract, 2 ml of Mayer's reagent was added, presence of dull white precipitates indicates the presence of alkaloids.
- iii. **Hager's test:** when 1 ml of extract was treated with 3 ml of Hager's reagent, formation of yellow precipitate confirmed the presence of alkaloids.

RESULTS AND DISCUSSION

The present study mainly focused on phytochemical profile of different pearl millet varieties. Various tests were performed to detect the phytochemicals in different extracts. Results obtained from different tests revealed the presence of various phytochemicals in pearl millet extracts prepared in 50% ethanol (Table 1).

Table 1: Phytochemicals present in ethanolic extracts of different Pearl millet varieties

Varieties Tests Performed	HC-10	HC-20	HHB-197	HHB-223	HHB-226	HHB-234
Coumarins	+	+	+	+	+	+
Steroid	+	+	+	+	+	+
Phlobotannins	-	-	-	-	-	-
Carbohydrates (Molisch Test)	+	+	+	+	+	+
Reducing sugars (Fehling Test)	+	+	+	+	+	+
Reducing sugars (Benedict Test)	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Anthocyanin	-	-	-	-	-	-
Flavonon	+	+	+	+	+	+
Alkaloid	-	-	-	-	-	-

CONCLUSION

Pearl millet grains are rich source of minerals and bioactive compounds that may help to regain immune power and maintain various metabolic reactions inside the body to dominate over a wide range of stress generated due to free radicals. Results obtained from the above study clearly indicate the presence of various types of phytochemicals in pearl millet extracts. The presence of huge amount of nutrient components in pearl millet might be helpful for the production of various nutraceuticals.

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