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Phytochemical analysis of chloroform and hydroalcoholic extracts of *Brassica juncea* seeds

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Abstract
Brassica juncea seeds hold economic significance and have been recognized for centuries in various countries for their medicinal and nutritional benefits. The present research focuses on the qualitative phytochemical analysis of chloroform and hydroalcoholic extracts derived from *Brassica juncea* seeds. In the experiment, *Brassica juncea* seeds were obtained from the local market and underwent authentication. The hydroalcoholic extract of *Brassica juncea* seeds revealed the existence of alkaloids, flavonoids, terpenoids, tannins, saponins, phenols, steroids, carbohydrates and proteins. Phenols, saponins, steroids, tannins, and carbohydrates were found in the chloroform extract of *Brassica juncea* seeds, whereas alkaloids, flavonoids, terpenoids, and proteins were not detected. These plant secondary metabolites, commonly referred to as phytochemical substances, are said to have a wide range of biological and therapeutic uses. So this plant species has a wide range of therapeutic applications and can be further researched to produce pharmaceutical medications.

Keywords: Terpenoids, tannins, saponins, phenols, steroids

Introduction
Brassica juncea (L.) commonly known as Indian mustard or brown mustard is a perennial herb, usually grown as an annually or biannually. It is a plant of economic importance with nutritional and medicinal significance, belongs to genus brassica [1]. It is an herbaceous plant featuring an upright, branched stem that can reach a height of up to 1.0 meter. The plant possesses a taproot extending to a depth of 60-80 cm. Lower leaves have petioles, are green, and occasionally display a whitish bloom, while upper leaves are partially serrated, short, and have petioles. The seeds of *Brassica juncea* contains various chemical constituents, including flavonoids, terpenoids, phenolics, saponins, tannins, proteins, fats, reducing sugars and non-reducing sugars [2]. Additional phytochemical components found in these seeds include alpha-linolenic acid, palmitic acid, erucic acid, nitric oxide and thiamine. The secondary metabolites found in *Brassica juncea*, such as glucosinolates and a variety of polyphenolics, are often recognized as its principal bioactive components relevant to therapeutic applications [3-4]. Dubie *et al.* (2013) [5] have also studied the effect of different extraction procedures on the amount of polyphenols in the extract of *Brassica juncea*. Mustard seeds have unique healing properties due to its high content of vitamins and minerals, such as calcium, magnesium, manganese, iron, selenium, zinc, phosphorus, niacin, and fibre. Additionally, it serves as a beneficial source of protein, omega-3, and fatty acids [6]. Seeds of *Brassica juncea* are also reported for their antioxidant [5], anti-diabetic [7], and hepatoprotective [8], antitumor [9], antimicrobial [10], antifungal [11], anti-inflammatory [12], anthelmintic [13] and anti-genotoxic [14] properties. In this context, the present study aimed to evaluate the initial phytochemical potential of extracts derived from *Brassica juncea* L. seeds, which contributes to its pharmacological activities.

Materials and Methods
Collection and authentication of plant material
The dried ripe seeds of *Brassica juncea* were procured from local market. These seeds were identified and authenticated by the Department of Horticulture, Maharana Pratap University of Agriculture and Technology in Udaipur, Rajasthan.

Macroscopic examination of seeds
The macroscopic features of the seeds were examined concerning the assessment of organoleptic characteristics.

Preparation of extract
Seeds of *Brassica juncea* were cleaned to to eliminate dirt and foreign material, and subsequently crushed to obtain a powdered form. To prepare chloroform and hydroalcoholic extracts, 500 g of powdered seeds were taken in two bottles, and 1.5 litre of chloroform and 70% alcohol were added, respectively. These bottles were placed for seven days, with daily shaking of about five minutes. After the seven days, chloroform and hydroalcoholic extracts were filtered with Whattman filter paper no. 1, and the filtrate was allowed to evaporate by a rotary vacuum evaporator (Macro Scientific Works Pvt. Ltd, Delhi) [12].

Phytochemical analysis
The qualitative phytochemical analysis was conducted to identify the presence of various active constituents in chloroform and hydroalcoholic extracts of *Brassica juncea* seeds by conducting various standard tests for phytochemicals.

1. **Test for alkaloids:** A solution was prepared by dissolving 1.36 g of mercuric chloride in 60 ml of distilled water, to which 5 g of potassium iodide were added. The mixture was then diluted to a total volume of 100 ml with distilled water. In testing, 1.0 ml of an acidic aqueous solution of the samples was taken, and a few drops of the reagent were introduced. The formation of a white or pale precipitate indicated the presence of alkaloids.
2. **Test for flavonoids:** In a test tube, 0.5 ml of sample extracts was combined with 5-10 drops of dilute hydrochloric acid (HCl) and a small piece of either zinc (Zn) or magnesium (Mg). The solution was then boiled for a few minutes. The presence of flavonoids was indicated by the development of a reddish-pink or dirty brown colour.
3. **Test for phenols:** To detect the presence of phenols in a sample, 1.0 ml of an alcoholic solution of the sample is mixed with 2.0 ml of distilled water. A few drops of 10% aqueous ferric chloride solution are then added to the mixture. The presence of phenols was confirmed by the development of a blue or green color.
4. **Test for saponins:** To detect the presence of saponins in a sample, 5 ml of the sample extracts is added to a test tube. A few drops of sodium bicarbonate are then introduced into the test tube. The mixture is vigorously shaken for 3 minutes. The formation of a honeycomb-like froth indicates the presence of saponins.
5. **Test for steroids:** To detect the presence of steroids in a sample, 2.0 mL of extract of the sample is mixed with 1.0 ml of concentrated H_2SO_4. The mixture is carefully added along the sides of the test tube. The development of a red-colored chloroform layer indicates the presence of steroids.
6. **Test for tannins:** To detect the presence of tannins in a sample, 5.0 ml of the sample extract is added to a test tube. A few drops of 1% lead acetate solution are then added to the test tube. The presence of tannins is indicated by the formation of a yellow or red-colored precipitate.
7. **Test for terpenoids:** A test tube containing 0.5 ml of the extract was combined with 2 ml of chloroform. Subsequently, 3 ml of concentrated sulfuric acid was carefully introduced to the mixture, forming distinct layers. The development of a reddish-brown color signified the presence of terpenoids.
8. **Test for carbohydrate:** In a test tube containing 2.0 milliliters of plant sample, 2 drops of freshly prepared 20% alcoholic solution of naphthol were added and mixed. Then, 2.0 milliliters of concentrated sulfuric acid was added to the mixture to form a layer below it. The confirmation of carbohydrates was established through the appearance of a red-violet ring at the solution's junction, which disappeared upon the addition of an excess of alkali solution.
9. **Test for Protein:** Mercury, in a ratio of one part, underwent digestion with two parts of HNO3. The resulting solution was then diluted with two volumes of water. A small portion of the decoction was taken, and 5-6 drops of Million's reagent were added. The appearance of a precipitate, with a subsequent red color upon heating, served as an indication of the presence of proteins

Results and Discussion
Macroscopic examination of seeds
The seeds exhibited a reddish-brown color, possessing a smooth texture and measuring approximately 1-1.2 mm in diameter. When crushed, they presented a bitter taste and emitted a distinctive pungent smell (Figure 1).

Fig 1: Macroscopic examination of mustard seeds

Extraction of seeds
Seeds of *Brassica juncea* were extracted by chloroform and 70% ethanol in water and yield was calculated 7.58% in aqueous extract and 9.81% in hydroalcoholic extract of *Brassica juncea* seeds.

Phytochemical Analysis of *Brassica juncea* seeds
The chloroform and hydroalcoholic extracts of *Brassica juncea* seeds demonstrated the existence of diverse phytochemicals, as detailed in Table 1. The initial phytochemical screening of hydroalcoholic extracts from the investigated plant indicated the presence of bioactive metabolites with medicinal properties, including alkaloids, flavonoids, phenols, saponins, steroids, tannins, terpenoids, carbohydrates, and proteins. In chloroform extracts of *Brassica juncea* seeds revealed the presence of phenols, saponin, steroids, tannins and carbohydrate while alkaloid, flavonoids, terpenoids and proteins were absent. These

findings are in corroboration with Sontakke and Shinde, (2020) [15]. Titan and Yeng, (2020) [16] highlighted that many phytochemicals, including vitamins, minerals, dietary fiber, polyphenols, chlorophylls, and glucosesinolates, are found in mustard. Similar findings were also reported by Aziz *et al.* (2020) [17] who found the presence of alkaloids, tannins, phenolics, flavonoids, glycosides and terpenoids in 30% ethanolic extract of *Brassica juncea* seeds. The variety of biological and therapeutic properties attributed to these secondary metabolites suggests that this species is likely to have multiple medicinal uses [18].

Table 1: Qualitative phytochemical analysis of chloroform and hydroalcoholic extracts of *Brassica juncea* seeds

Test for phytochemicals	Chloroform extract of *Brassica juncea* seeds	Hydroalcoholic extract *Brassica juncea* seeds
Alkaloids	-	+
Flavonoids	-	+
Phenols	+	+
Saponins	+	+
Steroids	+	+
Tannins	+	+
Terpenoids	-	+
Carbohydrates	+	+
Proteins	-	+
Presence: + present, - Absent.		

Conclusion

In this study, the phytochemical analysis of chloroform and hydroalcoholic extracts from *Brassica juncea* seeds revealed the presence of phenols, saponin, steroids, tannins and carbohydrate. Alkaloids, flavonoids, terpenoids and proteins were present in hydroalcoholic extract of *Brassica juncea* seeds only. These plant secondary metabolites, commonly referred to as phytochemical substances, are said to have a wide range of biological and therapeutic uses. So this plant species has a wide range of therapeutic applications and can be further researched to produce pharmaceutical medications.

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