EVALUATION OF THE PHYTOCHEMICAL POTENTIAL OF *BRASSICA JUNCEA*L. SEEDS

K. S. Sontakke[1] and S. L. Shinde[2]
[1]Department of Botany, Gopikabai Sitaram GawandeMahavidyalaya, Umarkhed Dist. Yavatmal
[2]Department of Botany, Rajiv Gandhi Mahavidyalaya, Mudkhed Dist-Nanded
ikailash442@gmail.com, sahebshinde4@gmail.com

ABSTRACT

The present study was aimed to evaluate the phytochemical potential of ethyl alcohol, acetone, chloroform, ethyl acetate and aqueous extracts of Brassica junceaL. seed. For the experiment, seeds of Brassica junceaL.were collected from the region of Nanded (MS), Indian and authenticated. The seed of Brassica junceaL showed the presence of alkaloids, flavonoids, tannins, phenols, saponins, steroids, terpenoids, proteins and carbohydrates. Alkaloids were found to be absent in chloroform extracts while acetone and aqueous extracts showed the highest presence of alkaloids. These Phytochemical compounds are also known as plant secondary metabolites and are reported to have many biological and medicinal properties. Hence this species is expected to have many therapeutic uses and can be further studied for the production of pharmaceutical drugs.

***Keywords*:** Brassica junceaL, Brassicaceae, extracts, Mustard Seed, Phytochemical.

Introduction

*Brassica juncea*L is an annual herb belonging to family Brassicaceae. The leaves are ovate or obovate, simple and petiolated; the flowers are bisexual and of the raceme inflorescences, with four free sepals and four yellow petals, along with two long and two shorter stamens. Mustard seed is commonly used as a spice. Grinding of the seeds and mixing it with water, vinegar, or other liquids creates the yellow condiment known as prepared mustard. The seeds can also be pressed to make mustard oil, and the edible leaves can be eaten as mustard greens (Nongmaithem and Rebika, 2018).

The seed of *Brassica juncea*L contains many biologically active compounds. These active compounds are produced by plants through primary or secondary metabolism which generally helps the plants in defense mechanisms against diseases or pathogen, and they are also beneficial to human health in various ways (Parikh and Khanna, 2014). They are mostly found in plant parts like fruits, leaves, vegetables, and seeds. Recent studies suggested that the consumption of plants that are rich in polyphenols will help in the prevention against the development of various cardiovascular diseases, neurodegenerative diseases, cancer, and diabetes (Chauhan *et al.,* 2012). The order of polarity is very important in selecting the solvents for sequential solvent extraction.

In this concern, the present study was aimed to evaluate the phytochemical potential of *Brassica juncea*L seed extracts.

Materials and Methods

Selection of plant material

For the experiment, the seed of *Brassica juncea*L.was collected from the region of Nanded (MS), India, and authenticated at School of Life Sciences SRTMUNanded.

Preparation of plant extracts

The selected plant material was laid on a clean baking paper sheet.The oven temperature was set to 150^{O} F and inserted the baking sheet. The material was allowed to dry out in the oven. After two hours onward, the material was dried and brittle. The material grinded in a clean grinder once the blades of grass were dry and brittle. The developed powder was stored in dry airtight container for the experimental use. For the experiment, the aqueous, ethanol, chloroform and hexane extracts were prepared. The 30 gm of dried powder was extracted with 300 ml solvent using Soxhlet apparatus for 24 hrs. Ethyl alcohol, Acetone, Chloroform, Ethyl acetate and Aqueous extracts were lyophilized and stored in 4^{O} C.

Preliminary Qualitative screening of plant extracts

Standard protocols were used for the phytochemical analysis. Phytochemical

screening for the presence of major types of compounds in the extract was done by Harborne (1973) with some modifications.

- **Alkaloids:** 1.36gm of mercuric chloride was dissolved in 60ml distilled water and 5gm of potassium iodide and diluted to 100ml with distilled water. To 1.0ml of an acidic aqueous solution of samples, few drops of reagent were added. Formation of white or pale precipitate showed the presence of alkaloids.
- **Flavonoids:** In a test tube containing 0.5ml of various extracts of the samples, 5-10 drops of dilute HCl and a small piece of Zn or Mg were added and then the solution was boiled for few minutes. In the presence of flavonoids, the reddish-pink or dirty brown colour was produced.
- **Phenols:** To 1.0ml of alcoholic solution of samples, 2.0 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added and the formation of blue or green colour indicates the presence of phenols.
- **Saponins:** In a test tube containing 5ml of various extract of the sample, a few drops of sodium bicarbonate was added. The mixture was shaken vigorously for 3mins. A honey comb like froth was formed and it showed the presence of saponins.
- **Steroids:** To 2.0ml of various extracts of samples, 1.0 ml of concentrated H_2SO_4 was added carefully along the sides of the test tube. Formation of red colour chloroform layer indicates the presence of steroids.
- **Tannins:** In a test tube containing about 5.0 ml of a various extract, a few drops of 1% solution of lead acetate was added. A yellow or red colour precipitate was formed, indicating the presence of tannins.
- **Terpenoids:** 0.5 ml of extract was mixed with 2 ml of chloroform in a test tube. 3 ml of concentrated sulfuric acid was carefully added to the mixture to form a layer. A reddish-brown colouration was formed for the presence of terpenoids.
- **Carbohydrate:** In a test tube containing 2.0 ml of plant sample, 2 drops of freshly prepared 20% alcoholic solution of a naphthol was added and mixed. To this solution 2.0 ml of concentrated sulphuric acid was added so as to form a layer below the mixture, the formation of the red-violet ring at the junction of the solution and its disappearance on the addition of an excess of alkali solution indicates the presence of carbohydrates.
- **Protein:** 1 part of mercury was digested with 2 parts of HNO_3 and the resulting solution was diluted with 2 volumes of water. To a small quantity of decoction, 5-6 drops of Million's reagent was added. A precipitate which turned red on heating was formed and it indicates the presence of proteins.

Quantitative estimation of plant extracts

- **Estimation of total phenolic content:** Antioxidant compounds generally contain phenolic group(s) and hence, the amounts of total phenolic compounds in the extracts of the flowers, leaves and seeds were estimated by using Folin–Ciocalteu reagent with Gallic acid as standard. The total phenolic content was estimated according to the method of Singh *et al.* (2011). The aliquot of the extract was taken and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) was added sequentially to the test tube. Soon after overtaxing the reaction mixture, the tubes were placed in the dark for 40 min. and the absorbance was recorded at 725 nm using UV-Vis Spectrophotometer against the reagent blank. A standard curve was prepared using Gallic acid. The linearity obtained was in the range of 1-10 µg/ml. Using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of extract.
- **Estimation of total flavonoid content:** The antioxidant activity of medicinal plants could be attributed to its flavonoid content. Flavonoids act as scavengers of various oxidizing species i.e. superoxide anion, hydroxyl radical or per-oxy-radicals, they also act as quenchers of singlet oxygen (Ratty and Das, 1988). The Aluminium chloride colorimetric method was used to determine the total flavonoids content in plant extract using Quercetin as standard.

The total flavonoids content was estimated according to the method of Chang *et al.* (2002). Briefly, 0.5 ml solution of extract in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water, and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with the help of a UV-Visible spectrophotometer. The calibration curve was prepared by preparing Quercetin solutions at concentrations 10 to 100 µg/ml in methanol.

Observations and Results

Preliminary phytochemical analysis of seeds of *Brassica juncea* was carried out by using different solvent extracts. The seed of *Brassica juncea* showed the presence of alkaloids, flavonoids, tannins, phenols, saponins, steroids, terpenoids, proteins and carbohydrates. Alkaloids were found to be absent in chloroform extracts whileacetone and aqueous extracts showedthe highest presence of alkaloids. Similar, flavonoids, Terpenoids and proteins were also found to be absent in the chloroform extracts. Most of all phytochemicalsshowed their presence in Ethyl alcohol, acetone and aqueous solvent extracts. (Table1).

The total phenolic content was estimated according to the method of Singh *et al.* (2011). Among the different crude extracts, the ethanol extract showed highest phenolic content of 6.82 mg/g, followed by aqueous extracts of 5.47 mg/g, which is then followed by ethyl acetate extracts of 1.38 mg/g and the one with acetone and chloroform extracts showed almost same content which is 0.59 mg/g and 0.56 mg/g respectively. The total flavonoids content was estimated according to the method of Chang *et al.* (2002). Total flavonoids content were found to be highest in acetone extracts of 6.18 mg/g, followed by ethyl acetate 2.52 mg/g, followed by aqueous extract 1.86 mg/g, which is then followed by ethanol extract of 1.47 mg/g and the least flavonoids content was found in chloroform extract 0.16 mg/g (Table 2).

Table 1: Phytochemical Analysis of Different solvent extracts of *Brassica junceaL.* seed

Phytochemical Test	Acetone	Ethyl acetate	Chloroform	Ethyl alcohol	Aqueous
Alkaloids	++	+	-	+	++
Flavonoids	+	+	-	+++	+
Phenols	-	+	++	+++	+++
Saponins	++	++	++	++	+
Steroids	++	++	++	++	+
Tannins	-	+	+	+	+
Terpenoids	+++	++	-	++	+
Carbohydrate	++	++	++	+	++
Protein	+	+	-	+++	+
Presence: +++ High, ++ Moderate, + Least, - Absent					

Table 2: Total Phenolic and Flavonoid content of Different solvent extract of *Brassica junceaL.* seed

Total Content	Acetone	Ethyl acetate	Chloroform	Ethyl alcohol	Aqueous
Phenolic (mg/g)	0.59±0.08	1.38±0.05	0.56±0.07	6.82±0.17	5.74±0.14
Flavonoids (mg/g)	6.18±0.08	2.52±0.04	0.16±0.02	1.47±0.11	1.86±0.18

Discussion

Preliminary qualitative phytochemical analysis of the different successive crude extracts of seed of *Brassica juncea L.*revealed the presence of alkaloids, flavonoids, proteins, carbohydrates, phenols, saponins, steroids, tannins and terpenoids. These secondary metabolites are reported to have many

biological and therapeutic properties, so this species is expected to have many medicinal uses (Kamal et al., 2015). The phytochemical properties and antioxidant potential of *Brassica* plants make them the preferred candidates for nutritional and pharmaceutical applications. Due to the presence of these compounds, *Brassica* plants show biological activities against various diseases and have been found to be effective in treating various diseases in humans (Lakshmi and Shalini, 2016).

The *Brassica*plant also shows the presence of high carbohydrate and protein. Hence, the plant is very good for consumption as it has a high nutritional quality. These days acquiring a plant-based diet has become very popular for moral reasons yet in addition to ecological supportability and wellbeing reasons (Hossain et al., 2015). Proteins are the building blocks of our bodies. They are used to build and repair tissues. Plant-based proteins are believed to have lower fat and cholesterol and provide fiber and other health-promoting nutrients (Ahongshangbam and Devi, 2017). The main source of energy for our body is carbohydrates and they have fiber. Eating food with fibre can prevent stomach or intestinal problems, such as constipation and additionally helps in bringing down glucose levels and cholesterol levels (Bhattarai et al., 2016). Including this plant in one's balance diet will surely benefit human health in many ways.

The total phenolic content was estimated according to the method of Singh *et al.* (2011). Among the different crude extracts, the ethanol extract showed highest phenolic content of 6.82 mg/g, followed by aqueous extracts of 5.47 mg/g, which is then followed by ethyl acetate extracts of 1.38 mg/g and the one with acetone and chloroform extracts showed almost same content which is 0.59 mg/g and 0.56 mg/g respectively. They have been reported to have many biological effects on the plants as well as other living organisms. They help in the growth and reproduction of plants and are produced as a response for defense against pathogens (Singh et al., 2013; Sameeh et al., 2016). The presence of these phenolic contents can be related to the antioxidant properties. Present-day researchers are now reliably prescribing to take Brassicaceae vegetables specifically for helping patients experiencing or inclined to diabetes and related psychological wellness conditions. However, not much effort has been given in designing and developing a proper well standardized pharmacological products, especially suited for such purposes (Yokozawa et al., 2003; Lutrika andPraveen, 2020). So, with further study and analysis, this plant can be used as modern traditional Ayurvedic medicines.

The total flavonoids content was estimated according to the method of Chang *et al.* (2002). Total flavonoids content were found to be highest in acetone extracts of 6.18 mg/g, followed by ethyl acetate 2.52 mg/g, followed by aqueous extract 1.86 mg/g, which is then followed by ethanol extract of 1.47 mg/g and the least flavonoids content was found in chloroform extract 0.16 mg/g. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties (Bag et al., 2015; Rajesh et al., 2016; Naima et al., 2019).

These Phytochemical compounds are also known as plant secondary metabolites and are reported to have many biological and medicinal properties. So this species can be expected to have many therapeutic uses and can be further studied for the production of pharmaceutical drugs.

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References

1. Ahongshangbam, S.K., & Devi, G.S. (2017). Proximate Analysis and Mineral (Elemental) Composition of Certain Spices of Manipur, India. International research journal of pharmacy. 8, 28-32.
2. Bag, G. C., Devi, P. G., &Bhaigyabati, T. H. (2015). Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three Hedychium species of Manipur valley. International Journal of Pharmaceutical Sciences Review and Research. 30(1), 154-159.

3. Bhattarai BP, Shing KP, Shakya SM, Khatri-Chhetri GB and Khadka YG (2016). Effects of organic and conventional nutrient management on post harvest status of broad leaf mustard (Brassica juncea var. Rugosa). International Journal of Horticulture and Agriculture. 3(2): 1-4.
4. Chang C, Yang M, Wen H, Chern J. (2002) Estimation of total flavonoid content in Propolisby two complementary colorimetric methods. J Food Drug Anal. 10: 178-82.
5. Chauhan PK, Jaryal M, Kumari K and Singh M. (2012). Phytochemical and in-vitro antioxidant potential of aqueous leaf extracts of Brassica juncea and Coriandrumsativum. International Journal of Pharmaceutical Sciences and Research. 3: 2862-65.
6. Harbone AJ. (1973).Phytochemical methods: a guide to modern technique of plant analysis. New York: Chapman Hall.
7. Hossain MD, Ahmed KU, Chowdhury FN, Roksana KM, Islam S and Barman A. (2015). Experimental study on grain weight, moisture, ash, carbohydrates, protein, oil, total energy and minerals content of different varieties of rapeseed and mustard (Brassica sp.) International Journal of Scientific and Res Publications. 5(12): 394-00.
8. Kamal AM, Chowdhury KAA, Shill LK, Hossain MR, Islam N and Anaytulla IA. (2015). Phytochemical screening, cytotoxic and thrombolytic activity of extract of Brassica oleracea flower (cauliflower). Global Journal of Pharmacology. 9:115-20.
9. Lakshmi DS and Shalini JV. (2016). In-vitro antioxidant studies of fresh Brassica oleracea and the characterization of its bioactive compounds using Fourier transform infrared spectroscopy (FTIR). Journal of Chemical and Pharmaceutical Research. 8(5): 900-05.
10. Lutrika M and N. Praveen. (2020). Phytochemical analysis and antioxidant activity studies of leaf of Brassica junceavar. Rugosa through sequential solvent extraction. IJPSR. 11(1): 343-351.
11. Naima J, Islam MR, Proma NM, Afrin SR, Hossain MR and Hossain MK. (2019). Phytochemical screening and antinociceptive activity of Mimosa diplotricha leaves. Int J Pharm Sci& Res. 10(8): 3679-84.
12. Nongmaithem N and Rebika Y. (2018). Report of Turnip mosaic virus occurrence in broad-leaved mustard (Brassica juncea var. Rugosa) from Manipur, India. International Journal of Microbiology. 10(5): 1191-92.
13. Parikh H and Khanna A. (2014).Pharmacognosy and phytochemical analysis of Brassica juncea seeds. Pharmacognosy Journal. 6(5): 47-54
14. Rajesh BR, Potty VP and Sreelekshmy SG (2016). Study of total phenol, flavonoids, tannin contents and phytochemical screening of various crude extracts of Terminaliacatappa leaf, stem bark and fruit. International Journal of Applied and Pure Science and Agriculture. 2(6): 291-96.
15. Ratty AK, Das NP (1988). Effects of flavonoids on nonenzymatic lipid peroxidation: structure-activity relationship. Biochem Med Metab Biol. 39(1): 69-79.
16. Sameeh MY, Mohamed AA and Elazzazy AM (2016). Polyphenolic contents and antimicrobial activity of different extracts of Padinaboryana Thivy and Enteromorphasp marine algae. Journal of Applied Pharmaceutical Science. 6(9):87-92.
17. Singh KL, Singh LR, Devi PG, Devi NR, Singh LS and Bag GC (2013). Comparative study of phytochemical constituents and total phenolic content in the extracts of three different species of genus Hedychium. Int J Pharm Tech Res. 5(2): 601-06
18. Singh T, Kasture SB, Mohanty PK, Jaliwala Y, Karchuli MS. (2011).In -Vitro antioxidative activity of phenolic and flavonoid compounds extracted from fruit of Garciniaindica. Int J pharma life sci.2(3):613-6.
19. Yokozawa T, Kim HY, Cho EJ, Yamabi N and Choi JS (2003). Protective effects of mustard leaf (Brassica juncea) against diabetic oxidative stress. J NutrSciVitaminol. 49(2): 87-93