



Phyto-chemical analysis of various mustard seeds in different solvent extracts

Aishwarya^{1*}, Varishth Khanna², Pushpa C Tomar¹, Charu Rajpal¹

¹ Department of Biotechnology, Faculty of Engineering and Technology, Manav Rachna International Institute of Research and Studies, Faridabad, Haryana, India

² MRPC, Janak Puri, New Delhi, India

Abstract

Indian mustard is considered as nutritionally rich oilseed crop. The aim of current research was to study, the phytochemical and nutritional potential of selected cultivars with an intent to provide therapeutic benefits to the human. The phytochemical present in plant parts (seeds) were extracted by overnight dipping with methanol, ethanol, acetone, distilled water. Three types of mustard seed were taken for analyses that are *Brassica juncea*, *Brassica nigra*, *Sinapis alba*. Qualitative phytochemical analysis was done for various phyto-constituents like alkaloids, tannins, flavonoids, steroids, cardiac glycosides and saponins. The result of analysis shows phytochemical constituents of different mustard seeds have therapeutic effect which will be helpful in studying the future pharmacology.

Keywords: antioxidants, *Brassica juncea*, phyto-chemicals, terpenoids, therapeutic

Introduction

Brassica spp. is an explicit source of nutritionally essential components like fibres, vitamins, phenolic compounds and minerals (Crozier A. *et al*, 2009) [1]. Cultivation of oilseed (*Brassica spp.*) has been remarkably improved from last many years. *Brassica spp.* has been placed third worldwide for its specific benefits (Choudhary K. *et al*, 2011) [2]. It produces and supplies nearly 7% of the world's edible oil after soyabean and palm oil. It has been studied that mustard has low glucosinolates and erucic acid content, thus it is being used for oil production in many Asian countries like India, China, and many others (Merah O., 2015) [3]. India maintains first place in production of rapeseed and mustard amongst all the Asian countries (Malan R. *et al*, 2011) [4]. In India, oil obtained from mustard seeds is used as one of the most consumable cooking oils and it is also attributed for its therapeutic properties.

Medicinal plants are beneficial to heal and to cure human diseases as well because of the existence of phytochemical components (Nostro *et al*, 2000) [5]. The diverse genera of plants, fungi, algae, and sponges produces very prominent class of natural compound, that is terpenoids (Luqman *et al*, 2008) [6]. Alkaloids can be used as an anaesthetic agent and can be mostly found in medicinal plants (Gbenou *et al*, 2013) [7]. The therapeutic significance of such plants exists in few chemical constituents that exhibit a certain physiological effect on human body; these substances are called phytochemical, which can be used for therapeutic purpose. Various medicinal plants are majorly consumed for the extraction of edible oils. The seeds are usually about 1-2mm and may be coloured in yellow, brown and black. The *Cruciferae* (*Brassicaceae*) family comprises mustard plant and is used in foods/therapeutics, is commonly a mixture of seeds from two or more species of *Brassicaceae*, for an instance, *Brassica nigra* (black mustard), *Sinapis alba L.* (white or yellow mustard), and *Brassica juncea L.* (oriental or Brown mustard). This is the most significant spice used

in several regional foods (Ildiko SG *et al*, 2006) [8]. Small amount of the seeds used as condiment mix prepared by grinding and mixing with spices, vinegar, water or other liquids, creates the yellow condiment known as prepared mustard and their phytoextracts have shown numerous medicinal properties like antioxidant, anticancer, anti-diabetes, antimicrobial, anti-inflammatory, etc. Therefore, the research is more dedicated towards conventional pharmaceutical procedures in the plants of the family *Brassica juncea* alongside with phytochemical and pharmacological studies of the family.

For the analysis, we have taken three major types of mustard seeds, on account, 1g of all the seeds were overnight dipped in particular solvents (acetone, methanol, ethanol, water). Afterwards the extracts were further used for phytochemical analysis. The ideal purpose of study was to ensure the existence of phyto-chemicals in particular seed extracts.

Pharmacological actions

Mustards are being consumed since approximately 5000 years. Considering the health benefits, establishing the therapeutic potential of the seeds, phenolics and flavonoids is critical; it was observed that the hydroalcoholic extract that showed promising antioxidant activity characteristic of these phytoconstituents. It has been reported that the flavonoid content of *B. juncea* is not too high, but the flavonoids spectrum witnessed for this plant is much wider than that of any other plant in the *Brassicaceae* family (Yang RY *et al*, 2008) [9]. There are well-known to have numerous benefits as they are low in calories and rich in their nutritional value and have various antiseptic and antibacterial properties. Anti-inflammatory effects are seen in nutrient, called selenium mostly present in mustard seeds. Magnesium is found as a rich source in mustard seeds which plays an important role in lowering blood pressure, rheumatoid arthritis symptoms and severe asthma attacks. Seeds of Mustard are enriched with zinc, iron, manganese,

calcium, protein, omega-3 fatty acids and dietary fibre which help in relieving number of different severe diseases. Mustard seeds show tremendous result for digestion because it increases the metabolism rate of the body (Manohar *et al* 2009) ^[10].

Phyto-chemical uses

The plants are suspended completely within various kinds of chemicals. Phytochemicals (from Greek, *phyto*, means "plants") are the chemicals that are synthesized by plants through primary and secondary metabolism. They have organic activity in the host plant and play a very crucial role in the growth of the plant or defence against their competitors, pathogens or predators.

Generally phytochemicals are considered as research compounds than necessary nutrients as an evidence of their probable health effects is not established yet. Several varieties of mustard plants are widely cultivated and are used as a source of spices, edible oils and medicines since ancient times (Mishra A. *et al*, 2012) ^[11]. One of the major functions of phytochemicals is their role as antioxidants. This is important because the more we eat antioxidant rich foods, the greater the health benefit.

Phyto-chemical screening

Certain chemical tests using standard procedures were performed on the aqueous phase of ethanolic and methanolic extracts of different plant parts to determine the phytochemical components present in it qualitatively. The whole phenolic estimation was estimated by using Folin-Ciocalteu reagent and whole content of phenol was expressed in mg/g gallic acid equivalent. Total flavonoid content was determined by the aluminium chloride colorimetric method (Karamian R. *et al*, 2013) ^[12] or by lead acetate test. Total flavonoid content was expressed as quercetin equivalents.

Materials and Methods

Collection of the sample

The seed samples of mustard were gathered from different cities nearby to New Delhi. Seeds were cleaned and screened, naturally dried, homogenised till finely powdered and then kept in the air-tight plastic bags.

Extraction of the sample

Mustard seeds (500g) were dried in hot air oven at temperature of 40-50°C. Dried material was grinded by mortar and pestle until it comes in powdered form and kept in air tight plastic bags for further use. Further, it was dipped overnight in 100ml of methanol and ethanol, acetone. The solvent extract was filtered in separate beakers using whatman filter paper No.1. The filtrate was then subjected to water bath at 80-90°C to evaporate the solvent. These extracts were then subjected to chemicals testing as per the protocols stated below for the exposure of several phytochemicals (Tiwari P *et al*, 2011) ^[13].

Preliminary Qualitative Analysis

For alkaloids

The methanolic and ethanolic extract of the crude dry powder of each seed type was kept into to a hot water bath for complete dryness. Dissolve the residue in 2N HCl. Filter the mixture with the help of whatmann No.1 filter paper and divide filtrate into 2 equal halves. Treat one part of the

filtrate with a few drops of Mayer's reagent, and other part with Wagner's reagent. The precipitates of each halves would be creamish and reddish brown respectively indicating the presence of different alkaloids.

For tannins

The solvent extract of the rough dry powder of each seed type, were treated with some drops of alcoholic ferric chloride reagent. Blue-Black colour specifies the existence of tannins in particular seed type.

For Saponins

The solvent extracted dry powder of different seed type was vigorously shaken with distilled water in a test tube and permitted to stand for 5-6 minutes. Then the saponin content was checked by analysing the formation of froth of more than 1.5cm indicates the presence of saponins in particular seed type. Absence of phytoconstituent is indicated by no froth.

For flavonoids

A few drops of diluted NaOH solution was added in 1ml of extract taken in test tube, it developed an intense yellow colour in the test tube. The presence of flavonoid is checked by adding few drops of dilute acid in the test tube, it becomes colorless. 4ml of 1% NH₃ was supplemented to 0.5ml extract and then conc. H₂SO₄ (1ml) was added. It turns into yellow color which indicates the positive results.

For terpenoids

5ml of sample extract was diluted in 2ml of the chloroform and then 3ml of concentrated H₂SO₄ was added to the solution. Reddish brown colour formed at interface illustrates the existence of respective phytoconstituent. This is known as salkowski test.

For resins

3ml of CuSO₄ was added into 0.5ml extract separately and then these tubes were traumatized dynamically for 1-2 mins, consequential solution was permitted for dispersal. Presence of resins was checked by the green color precipitate.

For cardiac glycosides

According to Keller Killiani Test, 1ml of the sample extract was dissolved in 1ml of the glacial acetic acid (CH₃COOH) and then kept for cooling. Then a few drops of ferric chloride (FeCl₃) were supplemented. 2ml of concentrated H₂SO₄ was added cautiously to this solution alongside the walls of the test tube. Presence of a brownish red coloured ring at the intersection of two layers depicts the occurrence of amino acids.

For phenols

As per Ferric Chloride Test, 2ml of double distilled water was added in 1ml of the extract. A few drops of 10% ferric chloride solution were supplemented to this same test tube. A dark green colour precipitate specifies the occurrence of phenolic content.

For phlobatanins

2ml of the sample extract was mixed with 2-3ml of 10% of the HCl in a test tube and then the contented water was kept for boiling for 5-6 mins. Development of red color precipitates specifies the occurrence of phlobatanins.

For quinones

The extracts were treated distinctly with alcoholic KOH solutions. The presence of quinones is indicated by the appearance of change in colours ranges from red to blue.

For proteins

(To the extract) 25% w/v ninhydrin reagent was added and then kept for boiling for 5-6 min. Blue colour formation depicts the presence of amino acids.

For steroids

10ml of chloroform was mixed in the test tubes with 1ml of sample extracts and then an equivalent volume of concentrated H₂SO₄ was added to the test tube along its sides. The upper layer of the test tube turns red and H₂SO₄ layer exhibits yellowish colour with green fluorescence that depicts the presence of steroids.

Results and Discussion

“Rai” is also famous as Indian mustard (*Brassica juncea*) amongst the consumable oil-seed crops cultivated for commercialization. Since a last few years a remarkable upsurge is observed in oilseed (*Brassica* sp) cultivation (Trivedi *et al*, 2014) [14]. It ranks 3rd worldwide (Choudhary K. *et al*, 2011) [2]. Plants are being used since years because of their antioxidant and antimicrobial properties and due to numerous secondary metabolites that are being biosynthesised in some or all their parts. A huge number of *Brassica* plants are considered for their bioactive phyto-constituent and an antioxidant potential (Uttara B. *et al*, 2009) [15]. The foremost application of mustard is in production of oil as the oil content in the mustard seeds is one of the substantial quantitative attribute in oilseed crops

but, it also contributes in treatment of different diseases (Grover JK *et al*, 2002) [16].

The results of this preliminary phytochemical screening are summarized in Table 1. This examination quotes the presence of several significant phytochemicals in diverse extracts of this plant. These bioactive phytochemicals are the origin of therapeutic potentials of pharmaceutical plants and beneficial in the treatment of numerous diseases. The different phytochemical present in *Brassica* possesses various useful properties. Flavonoids act as an antioxidant and prevent oxidation. Flavonoids have metal ions chelating and free radicals scavenging potential and comprises an inhibitory action on inflammatory cells specially the mast cells (Haq Nawaz *et al*, 2018) [17]. Antioxidant properties of mustard plant have been taken under study that supports to stabilize the symptoms of oxygen free molecules and defends from the effects caused because of oxidative pressure in diabetes (Soengas P *et al*, 2011) [18]. Nutrients such as isothiocyanates naturally occur in mustard or developed with aid of enzymes is a promising cancer preventive agent such as colon, bladder and intestinal cancer (Zhang Y, 2010) [19]. These nutrients support to lower down the peroxide activity and activate the healthy metabolism. Leaves of mustard plant along with its seeds are very helpful for diabetic patients (H.Y. KIM *et al*, 2003) [20]. The bioactive phyto-constituents act as the medical implication of the plant which produces characteristic physiological action on human beings. UV Spectrophotometer analysis for phytochemicals in different extracts of various mustard seeds is summarized in Table 2. The presence of flavones, phenols and anthocyanins was detected by observing the absorbance of different mustard seeds at specific wavelength.

Table 1: Phytochemical screening of various extracts of different mustard seeds

Constituents	Test performed	Black mustard				Brown mustard				Yellow mustard			
		M	W	E	A	M	W	E	A	M	W	E	A
Alkaloids	Mayer's Test	+	-	+	-	+	-	+	-	+	-	+	-
Cardiac Glycosides	Keller Killiani Test	+	-	+	-	+	-	+	-	+	-	+	-
Flavonoids	Lead Acetate Test	+	-	+	+	+	-	+	+	+	-	+	+
Phenols	Ferric Chloride Test	+	-	+	+	+	-	+	+	+	-	+	+
Phlobatannins	Hydrochloric Acid Test	-	-	-	-	-	-	-	-	-	-	-	-
Proteins	Biuret Test	+	-	+	+	+	-	+	+	+	-	+	+
Quinones	Alcoholic KOH Test	+	-	+	-	+	-	+	-	+	-	+	-
Resins	Copper Sulphate Test	+	-	+	-	+	-	+	-	+	-	+	-
Saponins	Froth Test	-	+	-	-	-	+	-	-	-	+	-	-
Steroids	Salkowski's Test	+	-	+	-	+	-	+	-	+	-	+	-
Tannins	Ferric Chloride Test	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	Salkowski's Test	+	+	+	-	+	+	+	-	+	+	+	-

Table 2: UV spectrophotometer analysis for phytochemicals in different extracts of various mustard seeds

Phytochemicals	Sample	Extract	Black Mustard		Brown mustard		Yellow mustard	
			Max. wave length	Absorbance	Max. wave length	Absorbance	Max. wave length	Absorbance
Phenols	50ul	MeOH	280nm	0.398	280nm	0.229	280nm	0.320
Flavones	50ul	MeOH	320nm	1.143	320nm	0.714	320nm	0.962
Anthocyanins	50ul	MeOH	520nm	0.0012	520nm	0.0015	520nm	0.0027

Conclusion

Having diverse range of bioactive molecules, makes medicinal plants a richest source for drugs that determine the therapeutic nature of *Brassica* seeds. Extraction by Soxhlet method involves boiling the solvents and passing this heated solvent through the plant powder and extracting the solutes, which generally results in loss of heat labile

phytoconstituents. The results obtained from qualitative phytochemical analysis revealed the presence of plant biomolecules like fats, proteins, amino acids along with other important secondary metabolites like alkaloids, flavonoid.

Phenolics and tannins are known for their varied pharmaceutical roles. The use of preliminary data obtained

was extended to extract the relevant biomolecules from the seeds.

Research is still on-going to understand its potential in cancer chemo protective activity of isothiocyanates. *Brassica juncea* is a natural source of an antioxidant alpha-tocopherol.

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