Pharmacognosy and Phytochemical Analysis of *Brassica juncea* Seeds

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ABSTRACT

Introduction: *Brassica juncea* is an economically important plant that has been well-known in India for centuries for its medicinal and nutritive values. The broad spectrum of beneficial effects of the seeds perceived with this plant warrants further exploration of *B. juncea* seeds as a potential source for obtaining pharmacologically standardized phytotherapeutics, which could be potentially useful. The objective of the present study was to perform the pharmacognosy of mustards seeds inclusive of qualitative and quantitative phytochemical analysis, fingerprinting by infrared spectroscopy and high performance thin layer chromatography analysis and toxicity assessment *in vitro*. **Methods:** Different sections of seeds were taken and stained with 0.1% phloroglucinol for microscopic examination. The seeds were extracted by 80% alcohol on a rotary shaker to perform phytochemical analysis and fingerprinting. The toxicity assessment of this extract was performed on human dermal fibroblast cells. **Results:** Microscopic examination of seeds showed characteristic features of mustard seeds. The extraction of these seeds by 80% alcohol resulted in IC_{50} value of 103 ± 3 μg/mL for 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl radical scavenging assay. The fingerprinting analysis of this extract indicated probable presence of sinigrin, quercetin, vanillin, catechin, vitamin E and sulfur-containing compounds. This extract exhibited 50% toxicity (IC_{50}) at 1.79 mg/mL. **Conclusion:** The result achieved will be used to assess the therapeutic efficacy of seed extracts for future pharmacological evaluations.

Keywords: Antioxidant, cytotoxicity, Fourier transform infrared spectroscopy, high performance thin layer chromatography, microscopy, phenolics

INTRODUCTION

Brassica juncea Czern. and Coss., also known as Indian mustard, Chinese mustard, oriental mustard, leaf mustard, or mustard green, is a species of mustard family of *Brassicaceae* (cruciferous) plants.[1] *B. juncea* is an economically important plant that has been well known in India for centuries for its medicinal and nutritive values. Various parts of the plants are edible and used in a range of folk medicines and spices. The mustard seeds have been used traditionally for the treatment of muscular rheumatism, inflammatory neuralgic affections, vomiting and dengue.[2] The seeds have also used as folkloric medicine against jaundice in the Jalgoan district of Maharashtra and by the Sugali tribes of the Yerramalais forest of the Eastern Ghats of Andhra Pradesh, India.[3,4] There are various polyherbal formulations of mustard discussed in ayurveda medicine. A decoction of *Moringa oleifera* root (1 in 20) with the addition of bruised mustard seed is useful in doses of 1-2 ounces in ascites due to liver and spleen diseases.[5] A fresh root of *M. oleifera* mixed with mustard seeds and green ginger is used as a counter-irritant and blistering agent.[5] A paste of equal parts mustard, horseradish seeds, hemp seeds and barley mixed with sore buttermilk is a useful application to the scrofulous glands of the neck. According to ayurveda medicine, mustard preparations are mild laxatives, diuretics, and liver-bile stimulators[6] and have been also documented to purge the toxins out of the body.[7] The leaves and seeds of these plants are edible and diverse medicinal uses of seeds are also well-known in other countries. In China, mustard seed is a folk remedy for arthritis, foot ache, lumbago and rheumatism. It is also used in the treatment of tumors; leaves are used in soups for bladder infections, inflammation or haemorrhage. In Korea, the seeds are used for abscesses, colds, lumbago, rheumatism and stomach disorders. The seeds are also reported to be hypoglycemic,[8] antioxidant,[9] anti-diabetic,[10] hyperglycemic,[11] anxiolytic,[12] goiterogenic,[13] and hepatoprotective.[2] The seed paste is used to treat

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backache, arthritis, paralysis, styes, edema of the lungs and liver, aperient, stimulant and emmenagogue.[7,14] The leaves, seeds, and stems have been shown to reduce the severity of asthma and high blood pressure, restore normal sleep attacks and prevent heart attack in patients suffering from atherosclerosis or patterns in women experiencing symptoms of menopause, and reduce the frequency of migraine diabetic heart disease.[15] The hepatoprotective activity of aqueous extract of mustard leaves have been evaluated against carbon tetrachloride-induced hepatic damage in albino rats.[16]

Taken together, these reports strongly suggest the therapeutic potential of seeds against various disorders. However, no definitive evaluation of nature of phytoconstituents involved in their observed effects has yet been made. This inability arises not only from the diverse types of extracts and experimental design used in different studies but also due to variations in the agronomic conditions used in cultivation and harvest. The broad spectrum of beneficial effects of the seeds observed in these studies warrants further exploration of *B. juncea* seeds as a potential source for obtaining pharmacologically standardized phytotherapeutics, that could be potentially useful. To address the lack of literature on standardization, our work focuses on the pharmacognosy of these seeds inclusive of fingerprinting by infrared (IR) spectroscopy and high performance thin layer chromatography (HPTLC) analysis and toxicity assessment *in vitro*.

MATERIALS AND METHODS

Identification and authentication

B. juncea seeds were identified and authenticated at Agharkar Research Institute, Pune, India (Voucher specimen number: S-158).

Macroscopic examination of seeds

The macroscopic characters of the seeds were studied with reference to evaluating organoleptic characteristics.

Microscopic examination of seeds

For microscopic examination, the seeds were taken, and thin sections were cut with a sharp blade. The specimens were stained with pholorglucinol (1% w/v in ethanol) and mounted with glycerol. The photographs of the seeds and its morphology are presented in results.

Determination of ash content and extractive value

Total, water-soluble and acid-insoluble ash contents and water, alcohol and ether soluble extractive values of the powdered seeds was determined as per the standard procedure.[17]

Extraction of phytoconstituents

The extraction of *B. juncea* seeds was carried out by 80% methanol using a rotary shaker for 6 h and the antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was measured.

DPPH radical scavenging activity

The free radical scavenging activity of the extract was measured with stable DPPH in terms of hydrogen donating or radical scavenging activity. 100 μL of DPPH solution (0.36 mm DPPH in methanol) was added to 1 mL extract (100-1000 μg/mL in methanol), vortexed thoroughly and kept in the dark at room temperature for 30 min. Next, the absorbance was measured at 517 nm using ultraviolet-visible (UV-VIS) spectrometer (Perkin Elmer Lambda 25, Perkin Elmer India, Thane, India). Ascorbic acid was used as the positive control. The percentage of inhibition was given by the formula: Percent inhibition (%) = $([A_0 - A_1]/A_0) \times 100$, where A_0 is the absorbance of the control solution and A_1 is the absorbance in the presence of the sample and standards.

Qualitative evaluation of phytoextract

Phytochemical analysis of crude extracts were carried out to determine the presence of the various biomolecules using standard procedures.[18] Qualitative tests for tannins, flavonoids, steroids, alkaloids, sugars, proteins, and fats were performed.

Quantitative estimation

Determination of the total flavonoid content

The total flavonoid content was determined by the aluminum trichloride method.[19] Briefly, 1 mL of extract (100-1000 μg/mL) or quercetin standard solution (5-30 μg/mL) was mixed with 1.5 mL distilled water in the test tube, followed by 100 μL aluminum chloride (10%, w/v) and 100 μL potassium acetate (1 M). The reaction mixture was then incubated at room temperature for 45 min and the absorbance was measured at 415 nm by UV-VIS spectrometer. The results of the plant sample were expressed as μg quercetin equivalents/mg extract.

Total phenolic content

The total phenolic content of the extract was determined by the Folin–Ciocalteu reagent method.[19] Briefly, 1 mL of extract or gallic acid (2-10 μg/mL in methanol) was added to 5 mL Folin–Ciocalteu reagent (1:20) and incubated for 5 min at room temperature. Next, 4 mL of sodium carbonate (10% w/v) was added and further incubated for 15 min at room temperature for color development. The absorbance was measured at 765 nm by UV-VIS spectrometer. The amount of total phenolic content was expressed as μg gallic acid equivalent/mg extract.

Determination of sugar and protein content

The sugar content was measured by the 3,5-dinitrosalicylic acid method and the estimation of proteins was carried out by the Lowry method.[15,16]

Fourier transform infrared (FTIR) spectroscopic analysis of plant extract

Approximately, 1 mg of dried extract was pressed into a pellet with 200 mg of potassium bromide and IR spectra were recorded with an accumulation of 45 scans and a resolution of 4/cm on IRPrestige-21 (Shimadzu Corporation, Kyoto, Japan).

Qualitative profiling of extract by TLC

The seed extract was checked by TLC on analytical plates over silica gel 60F254 (Merck and Co., New Jersey, USA). The qualitative analysis for different class of phytoconstituents was carried out by spotting the bands of extract using capillaries and using the mobile phase *n*-butanol:*n*-propanol: water:glacial acetic acid (3:1:1:1) using different spray reagents.

HPTLC fingerprinting

DPPH HPTLC autographic assay

The HPTLC method was used to qualitatively determine the antioxidant activity of extract by DPPH scavenging assay using 0.2% DPPH as a color developer. DPPH is a paramagnetic purple colored compound with an odd electron. The color of the DPPH reagent changes from purple to yellow due to the scavenging of free radicals by antioxidants through donation of hydrogen to form the stable DPPH-H molecule, visible on TLC plates.[18] The method was used for the mobile phase system – toluene:ethyl acetate:glacial acetic acid (4:4:1). Ascorbic acid-the water soluble vitamin and α-tocopherol-the fat soluble vitamin were used as the positive control.

HPTLC marker significant fingerprinting

The HPTLC study was carried out for detecting the presence of vanillin, quercetin and catechin sinigrin. HPTLC fingerprinting was performed at room temperature on aluminum plates pre-coated with silica gel 60F254 (Merck and Co., New Jersey, USA). Solutions of standards and sample were applied to the plates as bands 8.0 mm wide, 10.0 mm apart, and 10.0 mm from the bottom edge of the chromatographic plate using a Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100 μL Hamilton (India) syringe. Ascending development to a distance of 80 mm was performed using a suitable mobile phase (Table 1) in a Camag glass twin-trough chamber previously saturated with mobile phase vapor for 20 min. After development, the plates were dried and then scanned with a Camag TLC scanner with WINCAT software for quantification.

Cytotoxicity assessment on human dermal fibroblast (HDF) cells

HDF cells were obtained from Scientific Research Center, V. G. Vaze College, Mumbai, India, were grown in 10% fetal bovine serum in Dulbecco's minimal essential medium (DMEM) containing 100 U/mL penicillin and 100 mg/mL streptomycin at 37°C in a humidified CO_2 incubator. (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide assays of the seed extract on HDF cells were performed. Toxicity toward HDF was assessed with cells

Table 1: HPTLC marker significant fingerprinting of mustard seed extracts

Chemical constituents	Mobile phase	R_f[a]	Quantification (mg/g%)[b]
Vanillin	Toluene:ethyl acetate:glacial acetic acid (4:4:1)	0.62	2.57±0.1
Sinigrin	*n*-butanol: *n*-propanol:glacial acetic acid (3:1:1:1)	0.47	0.7±0.001
Catechin	Toluene:ethyl acetate:glacial acetic acid (4:4:1)	0.15	0.06±0.01
Quercetin	Toluene:ethyl acetate:glacial acetic acid (4:4:1)	0.58	0.13±0.02

[a]Results based on densitometry-HPTLC, [b]values expressed as the mean±standard error. HPTLC: High performance thin layer chromatography

plated in 96-unit well plates at a density of 5×10^4 cells/well. After adherence, the medium was removed and replaced by serum-free media containing seed extract (0.1-6.4 mg/mL) and incubated for 24 h at 37°C in a humidified CO_2 incubator. Doxorubicin (0.001-10 μM) was used as a positive control. Control cells were incubated with DMEM. Cell viability was determined by measuring the absorbance at 570 and 655 nm. Results were expressed as percentage cellular viability.

RESULTS

Macroscopic and microscopic examination of seeds

The seeds were reddish brown with a smooth texture and approximately 0.9-1 mm in diameter; they had a bitter taste and characteristic pungent smell when crushed. The testa was dark reddish-brown to yellow and minutely pitted. The cells of the outer epidermis of the testa contained mucilage. The embryo was oily and yellow in color, containing two cotyledons folded against their midribs to enclose the radicals (Figure 1).

Physicochemical characterization of seeds

The results obtained for the ash values and extractive values determined by methods described in ayurvedic pharmacopeia, can be used for the quality control purposes for mustard seeds, in various pharmacological interventions. The mean, range and standard error values of ash contents and extractive values of *B. juncea* seeds that resulted from analyses, are summarized in Table 2. The moisture content of the seeds was also determined and found to be <2.1%, which is an important quality control parameter indicating the stability and the susceptibility to bacterial and fungal contamination.

Preliminary characterization of seeds

The preliminary phytochemical study of *B. juncea* seeds was carried out to characterize the chemical constituents present in the extracts following standard procedures. The results indicated the presence of polyphenols such as phenolic acids, flavonoids, alkaloids, and tannins in methanolic extract. The qualitative analysis also indicated the presence of fixed oil content in n-hexane extract of seeds. The calculated values of oils were 30%, similar to reported quantities of 24-35%. Preliminary data were extended to isolate phytoconstituents enriched with antioxidant properties from the seeds.

Extraction of seeds

Mustard seeds were extracted by 80% methanol in water to obtain an antioxidant-rich extract. The yield was calculated

Table 2: Ash and extractive values of seeds of *B. juncea*

Constituent	Content (%)	
	Mean±SE	Range
Total ash	4.56±0.14	4.42-4.7
Water-soluble ash	1.05±0.01	1.04-1.06
Acid-insoluble ash	3.61±0.11	3.5-3.72
Water-soluble extractives	5.21±0.15	5.06-5.36
Alcohol-soluble extractives	9.43±1.2	8.23-10.63
Ether-soluble extractives	25.7±3.2	22.5-27.9

The data is represented as mean±SE, where n=3. *B. juncea: Brassica juncea*, SE: Standard error

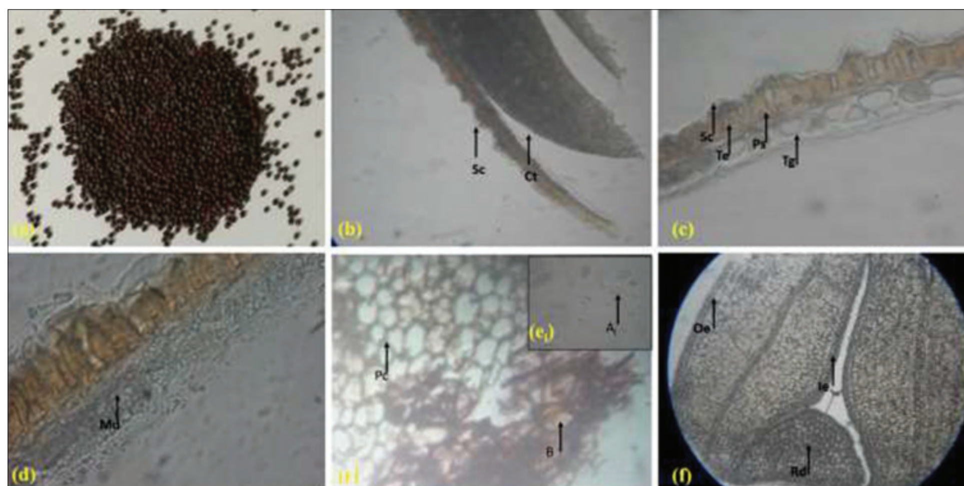

Figure 1: Microscopic examination of mustard seeds stained with phloroglucinol (×100) (full page width). (a) Mustard seeds, (b) seed coat (Sc) and cotyledon (Ct), (c) transverse section of seeds passing through Sc; testa (Te); palisade cells (Ps); and tegmen (Tg), (d) release of mucilage (Mu), (e) parenchyma cells (Pc) and oil globules (B) with the inset (e_i) showing isolated oil globules (A_i), (f) T. S. of seeds showing Ct; outer epidermis (Oe); inner epidermis (Ie) and radical (Rd).

to be of 7.24 ± 0.45% and the IC_{50} value for DPPH radical scavenging capacity was determined to be 103 ± 3 μg/mL.

Quantitative estimations of biomolecules

The flavonoid content of the extract was found to be 4 ± 0.02 μg quercetin equivalent/mg extract ($R^2 = 0.9744$) and the phenolic content was 107 ± 0.03 μg gallic acid equivalent/mg extract ($R^2 = 0.9914$). The glucose concentration in the extract was calculated using the equation $y = 0.0002x - 0.0033$ ($R^2 = 0.9695$) and was found to be 78.95 ± 6.71 μg N-acetyl glucosamine equivalent/mg extract, whereas the protein content was estimated from the equation $y = 0.0028x + 0.013$ ($R^2 = 0.9961$), to be 377.77 ± 00.68 μg bovine serum albumin equivalent/mg extract.

FTIR detected the presence of several functional groups

In FTIR spectroscopy, IR radiation is passed through the extract, from which part of the IR radiation is absorbed by the extract and part of it is transmitted. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the extract representing absorption peaks that correspond to the frequencies of vibrations between the bonds of the atoms present in it. The IR fingerprint of extract also showed presence of multiple peaks, with relatively few however very diagnostic peaks in the region above 2000/cm in contrast the other half contains many peaks with varying shapes and intensities. With the absorption peaks of stretching at 2930, 1665 and 2123.72/cm and bending vibrations at 795.67/cm, BJHAE shows the presence of alkanes, alkenes C=C and alkynes C≡C. BJHAE also showed a broad peak at 3333.14/cm for O-H stretching and 795.67/cm for O-H bending and ring puckering, indicating the presence of alcohols and phenols. Peaks for amines and carboxylic acid O-H bond stretching, C-O-H bending were observed at 1053.18, 2930 and 1426/cm respectively. The sharp peak of 2123.72/cm is a probable indication of presence of isocyanates, isothiocyanates, diimides, azides and ketenes. The spectra also indicated the presence of nitroso and nitro compounds with the peaks at 1514.19, 1514.19 and 1334.8/cm. The peaks at 795.67 and 879.58/cm indicated the presence of sulfane esters, whereas 1053.18/cm an indicative of thiocarbonyl were also present. The extract also showed the peaks for sulfoxide and sulfate at 1053.18 and 1334.8/cm, phosphorous containing compounds phosphine, esters and phosphoramide with the peaks at 1052.18, 926.84, 1053.18 and 1272.11/cm. Oxidized nitrogen is present in the form of oxime and aromatic amine oxides with absorption peaks at 1665.6, 926.84 and 1272.11/cm. Thus FTIR spectroscopy indicated the presence of numerous compounds such as alcohols, phenols, sulfur containing compounds, nitrogen-containing compounds, which are present in plant in abundance and are known to exert various pharmacological effects.

TLC qualitative profile showed the presence of several classes of phytoconstituents

The fingerprinting of extract by TLC was carried out to detect the presence of various class of phytoconstutients that could be present in the extract that are reported to be antioxidants and hepatoprotective. Using different spray reagents, large classes of compounds were detected and observed. A total of seven distinct bands were observed under different spraying conditions with R_f 0.19, 0.26, 0.28, 0.4, 0.43, 0.5 and 0.57. Based on these observations of TLC profile, various classes of phytoconstituents were identified (Table 3).

Fingerprinting analysis of the seed extract

In the study of identification of bioactives, silica plates were used to establish the TLC fingerprint. The characteristic of the extract was observed under UV (254 and 366 nm) and VIS light was compared with the spectra of each component (Table 1). Sinigrin, vanillin, catechin and quercetin were found to be present and were quantified. Of these, vanillin, catechin and quercetin were phenolic acids and reported to be antioxidants. The evidence of antioxidant efficacy was acquired by DPPH HPTLC autographic analysis. The extract showed multiple yellow bands against a purple background when sprayed with DPPH reagent, an indication of antioxidant activity similar to the positive controls: Vitamins C and E (Figure 2).

Non-toxic nature of seed extract on HDF cells

The cytotoxic effect of the seed extract of *B. juncea* was evaluated on HDF cells and it was observed that the extract showed a dose-dependent cytotoxicity (Figure 3) with IC_{50} at 1.79 mg/mL *in vitro*.

DISCUSSION

Brassicaceae vegetables represent an important part of the human diet worldwide and are considered important food crops in China, Japan, India and European countries. The macroscopic and microscopic examination identified the seeds of *B. juncea* by showing typical morphological

Table 3: TLC profile of seed extract indicating the presence of various classes of phytoconstituents

Reagent	Observation			Inference
	Visible	UV short	UV long	
Anisaldehyde-H_2SO_4	Red brown coloration, blue-violet, blue, red	Quenching	Blue, violet, green fluorescence	Essential oils, pigments, triterpenes, saponins
DPPH	Yellow band against purple background	-	-	Antioxidant activity
Dragendorff	Orange-brown	-	Blue	Alkaloids
Ethanolic-H_2SO_4	Brown-black	-	-	Total number of bands
NP-PEG	-	-	Orange, green, blue, blue-green	Bitter drugs, flavonoids, anthracene
Vanillin-H_2SO_4	Lemon yellow, blue, blue-violet, red, yellow brown	-	-	Pungent principle, saponins
Without spraying	-	Quenching	Dark yellow, green, blue, red, dark-blue	Bitter drugs, flavonoids, pigments, pungent principle

DPPH: 2, 2-diphenyl-1-picrylhydrazyl, UV: Ultra violet, TLC: Thin layer chromatography

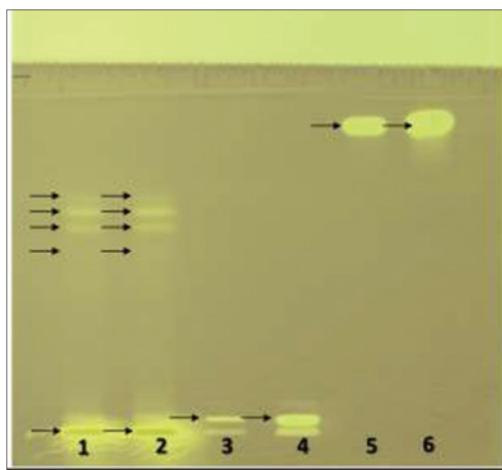

Figure 2: 2,2-diphenyl-1-picrylhydrazyl (DPPH) autographic analysis of mustard seed extract (column width). Key: lane 1 = seed extract (200 μg), lane 2 = seed extract (250 μg/mL), lane 3 = vitamin C (2 μg/mL), lane 4 = vitamin C (5 μg/mL), lane 5 = vitamin E (4 μg/mL), lane 6 = vitamin E (10 μg/mL) when the mobile phase of toluene: ethyl acetate: glacial acetic acid (4:4:1) was used. Antioxidant compounds scavenge the free purple-colored DPPH radicals to the yellow-colored compound. The appearance of yellow color in the and high performance thin layer chromatography plate after developing with 0.2% DPPH reagent indicates antioxidant activity.

characteristics. Furthermore, the total, water soluble and acid-insoluble ash contents of the seeds of *B. juncea* are important indices to illustrate the quality as well as purity of herbal drug. Total ash includes physiological ash, which is derived from the plant tissue itself and non-physiological ash, which is often from environmental contaminations such as sand and soil. *B. juncea*, like other herbal materials, show a variation in the variety and contents of compounds according to differences in growing conditions, such as soil type, climate which may change the ash content depending upon presence or absence of various contaminants thus becoming an important parameter of quality assessment.

B. juncea are known to produce several classes of bioactive phytochemicals including glycosides, flavonoids, phenolic compounds, sterols, triterpene alcohols, glucosinolates (GLSs), proteins and carbohydrates. The available pre-clinical information on this easily cultivable and edible plant strongly suggests that it could be a sustainable source of affordable nutraceuticals or drugs. The beneficial effects of *Brassica* vegetables on health improvement have been partly attributed to their complex mixture of phytochemicals possessing antioxidant activity.[20] Various classes of phytoconstituents from seeds of *B. juncea* were detected via qualitative analysis. The extraction procedure was standardized based on evaluations of DPPH activity as a preliminary tool. Recent reports suggest that cruciferous vegetables act as a good source of natural antioxidants due to their high levels of carotenoids, tocopherols and ascorbic acid.[20] For optimal extraction of antioxidants, 80% methanol was used employing rotary shaker for 6 h, since hydroalcoholic mixtures are the most versatile and widely employed solvent system. Alcohol is at in penetrating cell walls and seed degradation and causes polyphenols to be released from cells; water is a non-toxic solvent with higher polarity. Thus it can solubilize polar compounds to the highest degree, acting as an agent most suited to the extraction of the active principles from plant drugs.[21] This technique translated into a good DPPH scavenging activity (IC_{50} = 103 ± 3 μg/mL) of the extract.

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 very high, but the spectrum of flavonoids observed for this plant is wider than that of any other plant in the *Brassicaceae* family.[22] Mustard meal has been reported to be a good source of phenolic compounds.

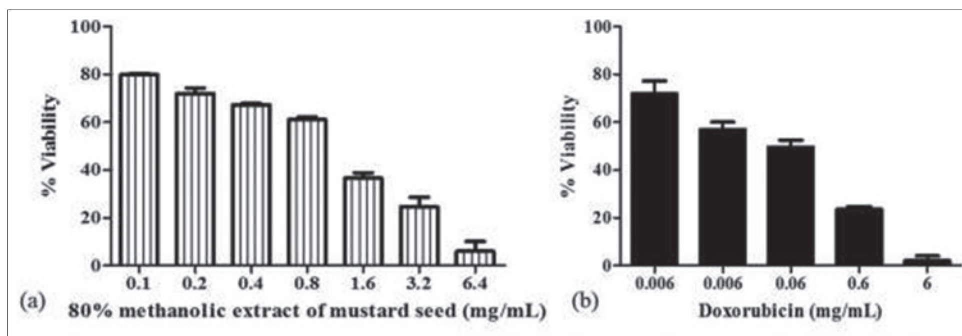

Figure 3: Cytotoxic effect of mustard seed extract on human dermal fibroblast cells (full page width). The cytotoxicity of mustard seeds was assessed by (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide assay using doxorubicin as a positive control. The dose-dependent reduction in the viability of cells was observed with seed extract and doxorubicin.

More than a dozen phenolic acid conjugates have been reported, and the spectrum of phenolics is also unique and broad.[20] The antioxidant capacity of *Brassica* species has been related to their phenolic profile and content, particularly flavonoids, since phenolic compounds have demonstrated a higher antioxidant activity than vitamins and carotenoids.[20]

Glucosinolates (GLSs) are found in *Brassica* vegetables. Among many such vegetables, the GLS content of *B. juncea* has been reported to be the highest.[23] In fact, the majority of cultivated plants that contain GLSs belong to the family of *Brassicaceae*. In mustard seeds, sinigrin (a GLS) gets converted to allyl isothiocyanate (an organo sulfur compound), releasing glucose under the influence of water by the action of the enzyme myrosinase.[24] The IR spectrum indicated the probable presence of sulfur-containing compounds. Further, the extract also showed the presence of many such functional groups that can result in conjugation reactions within the compounds that can aid in reduction of reactive oxygen species and antioxidant activity of the extract.

Fingerprinting and quantification of phenolics and flavonoids such as quercetin, vanillin, vitamin E and catechin in the hydralcoholic extract was carried out since these phenolics and flavonoids have been reported to be present in mustard seeds. The antioxidant activity of these phytoconstituents has also been well documented.[25-30] Vitamin E competes for scavenging peroxyl radicals much faster than polyunsaturated fatty acids and almost 200 times faster than commercial antioxidant butylated hydroxytoluene[31] since only a small amount of vitamin E can protect a large amount of polyunsaturated fat in the membranes. Quercetin detected in mustard seeds stops propagation of lipid peroxidation, increases glutathione (GSH) levels, antioxidant enzyme function and prevents Ca^{2+} - dependent cell death.[32,33] Catechin is known to reduce lipid peroxidation and increase GSH production.[34] Mustard seeds reported to contain a high content of cysteine residues,[35] may even bind with oxygen electrophiles, further assisting the antioxidant potency. Altogether, we postulate that these phyconstituents contribute to the protective efficacy of extract in the suppression of the elevation of reactive oxygen species generation.

The cytotoxicity assessment of hydroalcoholic extract of *B. juncea* seeds was carried out *in vitro*, since toxicity forms a crucial part in pre-clinical studies resulting in drug failure. In this work, the toxicity assessment was carried out *in vitro* on the cells derived from HDF cells that are most abundant cells in humans; the results revealed that the extract exhibit minimal toxicity *in vitro* that can be further explored for *in vivo* study. Also, it has been reported in a recent work that mustard seeds fed to rats at doses equal to normal human intake do not cause any adverse effects on histopathological parameters.[36] These result can be used to assess the therapeutic efficacy of seed extracts for future pharmacological evaluations.

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