Journal of Chemical Health Risks

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JCHR (2025) 15(2), 249-254 | ISSN:2251-6727

# HPLC Analysis, Isolation and Characterization of Sinapic Acid from Brassica Juncea Seed Extract

**R. Nithya[1*], R. Surekha[2], G Sriram Prasath[3], S. Iyyampillai [4] and S Subramanian[5]**

1.Department of Biochemistry, St Peter's Institute of Higher Education and Research, Chennai.

2. SRM Institute of Dental college Ramapuram campus, Chennai-600 089, India

3. Department of Biochemistry, Dwaraka Doss Goverdhan Doss Vaishnav College, Arumbakkam, Chennai-600 106, India

4. Pachaiyappa's College, Shenoy Nagar, Chennai - 600 030.

5. University of Madras, Guindy Campus, Chennai-600 025.

***(Received: 16 January 2024 Revised: 20 February 2024 Accepted: 20 March 2025)***

**KEYWORDS**

*Brassica juncea; HPLC, Sinapic acid; FTIR, NMR spectral studies*

**ABSTRACT:**

Introduction: Phenolic compounds are widely recognized for their antioxidant properties and potential health benefits. Among them, hydroxycinnamic acids such as sinapic acid, ferulic acid, p-coumaric acid, and caffeic acid play a crucial role in plant metabolism and human health. Brassica juncea (brown mustard), an important medicinal and culinary plant, contains significant amounts of these bioactive compounds, yet scientific scrutiny regarding its phenolic content remains limited.

Objectives: This study aims to analyze, isolate, and characterize sinapic acid from Brassica juncea seed extract using HPLC and various spectroscopic techniques, including FT-IR, Mass Spectrometry, and NMR.

Materials and Methods: Matured Brassica juncea seeds were procured, authenticated, dried, and extracted using ethanol. The extract was subjected to HPLC analysis to identify phenolic compounds. The isolated compound was characterized using FT-IR, Mass Spectrometry, and NMR spectroscopy.

Results and Discussion: HPLC analysis confirmed the presence of five major hydroxycinnamic acids, including sinapic acid. FT-IR spectral studies indicated the presence of characteristic functional groups, while Mass Spectrometry validated the molecular structure with a molecular ion peak at 224 amu. NMR spectral data further confirmed the presence of methoxy, hydroxyl, and carboxyl functional groups. These results substantiate the presence of sinapic acid in Brassica juncea seeds, emphasizing its potential antioxidant and therapeutic applications.

Conclusion: The study successfully identified and characterized sinapic acid in Brassica juncea seed extract, reinforcing its pharmacological importance. The findings provide a scientific basis for the potential utilization of mustard seeds as a natural source of bioactive compounds in nutraceutical and pharmaceutical industries.

## INTRODUCTION

Phenolic compounds have received substantial attention for being potentially protective against several chronic diseases which in turn due to their significant antioxidant properties and their content in a wide range of consumed foods of plant origin [1]. The biosynthesis and the content of phenolic compounds depend on genetic and environmental factors. Several reports demonstrated that there is a considerable and significant variation for the antioxidant phytochemicals both within and among species thus, the potential health benefits provided by the genotype [2].

Phenolic acids are the major class of phenolic compounds, found widely in foods of plant origin [3].

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These phenolic acid derivatives are abundantly present in the water-soluble phenolic fraction of the plant extracts and differ in the distribution patterns of the hydroxylations and methoxylations of their aromatic rings. Among the phenolic acids, the four most common hydroxycinnamic acids are sinapic acid, ferulic acid, *p*-coumaric acid and caffeic acid which are characterized by the $C_6$-$C_3$ structure [4]. The hydroxycinnamic acid derivatives possess a wide range of pharmaceutical as well as health beneficial effects. Most of the medicinal plants widely used in the traditional system of medicine for the treatment of various human ailments are known to contain a significant amount of these phenolic acid derivatives. However, the reports on the content and the role of hydroxycinnamic acid in the medicinal plants are scanty. *Brassica juncea* commonly known as brown mustard is one such medicinal plant which lacks scientific scrutiny for its medicinal value.

### *Brassica juncea* or Indian Mustard

Mustard is one of the oldest (3000 BC) recorded spices recognized both for its therapeutic as well as condiment value [5]. The term 'mustard' was derived from the Latin word *mustum*, the expressed juice of grapes or other fruits mixed with ground mustard seeds to form *mustum ardens*, a favorite Roman's condiment. Romans use mustard for flavoring food to disguise the taste of degraded perishables. Mustards are a versatile group of plants widely used as an oil seed crop and as a salad crop for its green leaves [6]. Mustards are members of the *Cruciferaceae* or *Brassicaceae* family. The genus *Brassica* consist more than 150 species either annuals or biennial herbs mainly cultivated as oil seed crops or as vegetables or as fodder. However, three major types of mustard seeds are used as condiments globally: Pale yellow/White mustard (*Sinapis alba* syn. *Brassica hirta* or *Brassica alba*): brown or oriental mustard (*Brassica juncea*) and black or dark brown mustard (*Brassica nigra*). Today, there are countless mustard varieties available throughout the world each reflecting local, regional and national cuisine. Of the total world mustard sale, about 60% accounts for seeds of *Sinapis alba* and the rest by *Brassica juncea*.

In the traditional medicine system mustards are used for skin diseases because of its high sulfur content. It induces appetite through the stimulation of salivary and gastric juice secretion. It is used as a laxative and as a treatment for asthma to relive cough. Mustard oil is used as liniment or massage in many paralytic diseases of the nervous system [7]. In leather industry, the mustard oil is used to make the leather soft and pliable. It is also used in the soap manufacture and as a lubricant and illuminant [8]. While mustard seeds have been used as a food since ancient times, it has also been used as a medicine in various traditional medicinal practices to alleviate joint pain in arthritis, fever cough, colds and gastrointestinal tract disorders. Mustard oil has been widely used for the treatment of various skin diseases and wound healing [9,10]

Fang et al. (2008) estimated the contents of free as well as total phenolic acids and evaluated its antioxidant properties in mustard leaves and found that the effects of pickling methods of these compounds. They concluded that the presence of several hydroxycinnamic acids as caffeic acid, *p*-coumaric acid, ferulic and sinapic acid along with benzoic acid derivatives which accounts for the observed antioxidant properties. Oil extracted from the mustard seeds is high in omega-3-fatty acids. Mustard seed meal, a by-product of mustard oil refining possesses unique for food applications. However, the mustard seeds are the good source of sinapic acid. The sinapic acid content in mustard seeds may vary depending upon the variety and processing methods [12].

### SINAPIC ACID

Sinapic acid (3, 5-dimethoxy 4-hydroxy cinnamic acid) is a naturally occurring phenolic acid widely present in high concentrations in crops belonging to the family *Brassicaceae* [13]. The genus is categorized into oilseed, forage, condiment and vegetable crops by using their buds, inflorescence, leaves, roots, seeds and stems. *Brassicaceae* vegetables represent an important part of the human diet worldwide and are consumed by people all over the

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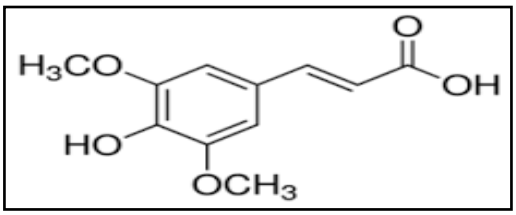
JCHR (2025) 15(2), 249-254 | ISSN:2251-6727

world. They are considered as an important food crop in China, Japan and India and European countries [14, 15].

**STRUCTURE OF SINAPIC ACID**




Sinapic acid extracted from the mustard seeds is used as a natural antioxidant in foods, beverages and cosmetics and its effectiveness is considered to be superior to that of ferulic acid [15,16,17]. Although SA is a frequent phytochemical in the human diet, it has not been received as much attention from the scientific community as other hydroxycinnamic acids such as caffeic acid or ferulic acid [18]. It is usually found in free form, but like other hydroxycinnamic acids it is also found in the form of esters. Sinapic acid can form dimers with itself as well as with ferulic acid present in the cereal cell walls. The maximum plasma concentration of sinapic acid after consumption of the meal has been around 40nM, indicating the absorption occurs mostly through small intestine [19]. The concentration of sinapic acid after consumption of cranberry juice was found to be 1.5µg/ml [20]. The amount of sinapic acid in plants has a wide range such as with 0.07 to 0.145µg/g for cereal grains, 72.15µg/g for lemon, 4505µg/g for strawberries and 12105µg/g for Dill [21, 22, 23,24].

Iin the present study, an attempt has been made to identify the presence of sinapic acid in the Indian mustard (*Brassica juncea*).

**MATERIALS AND METHODS**

**PLANT MATERIAL**

Matured mustard seeds (*Brassica juncea* commonly known as brown mustard) were procured from the traditional medical shop in Chennai, Tamil Nadu. The seeds were packaged in vacuum tight polythene bags. The seeds were identified and authenticated by a qualified taxonomist at Centre for Advanced studies in Botany, University of Madras, Guindy Campus and a voucher specimen was deposited.

**PREPARATION OF SEEDS EXTRACT**

*Brassica juncea* seeds were dried in an air oven at 37˚C then powdered in an electrical grinder, which was then stored in an airtight brown container at 5° C until further use. The powdered seeds were delipidated with petroleum ether (60 - 80° C) for overnight. It was then filtered and soxhalation was performed with Ethanol (95%). Ethanol was evaporated in a rotary evaporator at 40 – 50° C under reduced pressure. The 100gm of dried powder of *Brassica juncea* seeds yield around 32g.

**HPLC–DAD SYSTEM FOR THE ANALYSIS OF PHENOLIC COMPOUNDS**

HPLC analysis was performed using Shimadzu HPLC system equipped with a diode array detector (DAD). The solvents used for the HPLC analysis were of HPLC grade and purchased from Thermo Fisher Scientific India Pvt.Ltd. Mumbai, India and all other chemicals including the reference standards were purchased from Sigma Aldrich Corporation, St. Louis, USA. The chromatographic separations were performed on an Inertsil C18 analytical column (4.6 × 250 mm i.d., 5 µm). The composition of solvents and the gradient elution conditions used in the present study were based on the methods described previously by Bengoechea et al., [26,27,28], with some minor modifications. The mobile phase consisted of purified water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B) and the flow rate was 0.8 ml/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5% to 9% solvent B; from 5 to 15 min, 9% solvent B; from 15 to 22 min, linear gradient from 9% to 11% solvent B; from 22 to 38 min, linear gradient from 11% to 18% solvent B; from 38 to 43 min, from 18% to 23% solvent B; from 43 to 44 min, from 23% to 90% solvent B; from 44 to 45 min, linear gradient from 90% to 80% solvent B; from 45 to 55 min, isocratic at 80% solvent B;

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from 55 to 60 min, linear gradient from 80% to 5% solvent B and a re-equilibration period of 5 min with 5% solvent B used between individual runs. Operating conditions were as follows: column temperature-30°C, injection volume, 10 µl, and UV-diode array detection at 280 nm (hydroxycinnamic acids) at a flow-rate of 0.8 ml/min. Spectra were recorded from 200 to 600 nm. Phenolic acids in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds and were detected using an external standard method.

## IDENTIFICATION OF THE ISOLATED COMPOUNDS

The IR spectral studies employed to characterize the isolated compounds were performed in the solid state as pressed KBr pellets using Perkin Elmer FT-IR spectrophotometer in the range of 400-4000 cm-$^{1}$. The mass spectrum of the *Brassica juncea* seeds extract was studied using Jeol Gcmate. The $^{1}$H NMR as well as $^{13}$C NMR spectral studies were carried out at 500.13 and 125.758 MHZ, respectively. The spectra were recorded without any correction for instrumental characteristics.

## RESULTS

### HPLC ANALYSIS

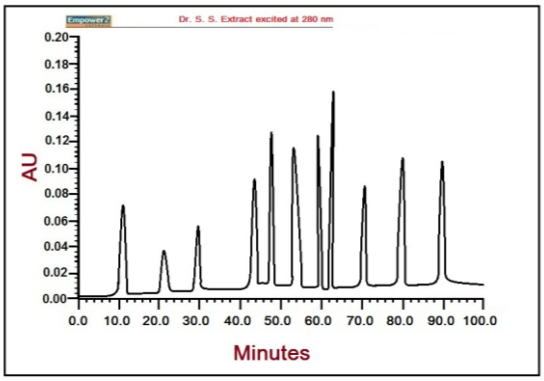


**Figure 1: HPLC analysis of *Brassica juncea* seeds extract**

Five compounds were identified in the ethanolic extract of *Brassica juncea* seeds by HPLC **(Figure 1).** HPLC analysis of the purified fraction showed that the isolated components have retention times similar to that of Caffeic acid, p-Coumaric acid, ferulic acid, chlorogenic acid (**Data not shown) and sinapic acid (Figure** 2) when compared to their respective authentic standards

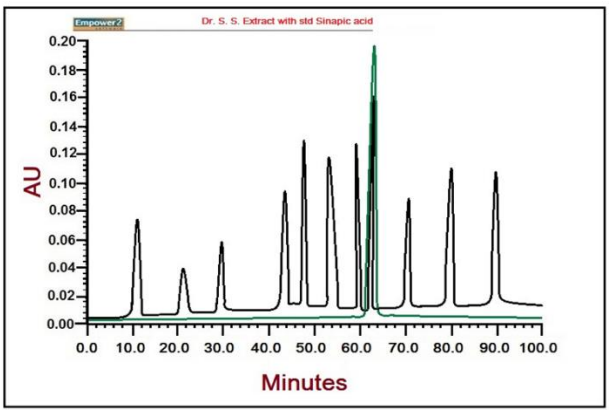


**Figure 2: HPLC analysis of *Brassica juncea* seeds extract with sinapic acid Table 1 Chemical identification of sinapic acid from FT-IR spectrum**

| Sl. No | Wave number ($cm^{-1}$) | Main attributes | Functional group |
|---|---|---|---|
| 1 | 3386 | –O–H stretching vibration | Phenol OH |
| 2 | 3219 | Benzene ring =C–H stretching vibration | Benzene ring |
| 3 | 2813 | Methoxy group | 0 |
| 4 | 2641 | –O–H stretching vibration | –COOH |
| 5 | 2282 | –O–H stretching vibration | –COOH |

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| | | | |
|---|---|---|---|
| 6 | 1686 | –C=O stretching vibration | –O–C=O |
| 7 | 1592 | –C=C stretching vibration | –O–C=O |
| 8 | 1548 | Benzene ring C=C stretching vibration | Benzene ring |
| 9 | 1388 | –O–H bending vibration | Phenol OH |
| 10 | 1227 | –C–OH stretching vibration | Phenol OH |
| 11 | 972 | =C–H out-plane bending vibration | Ar–C=C |
| 12 | 728 | Benzene ring =C–H out-plane bending vibration | Benzene ring |

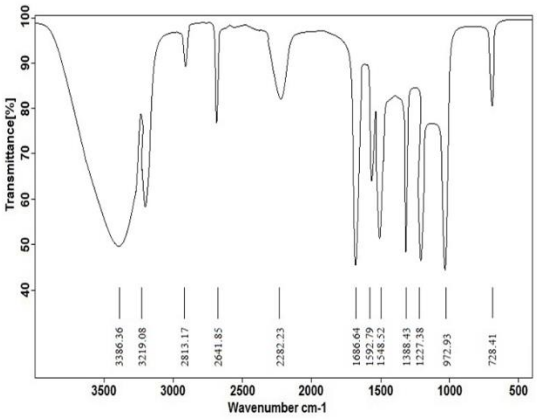


**Figure 3: IR spectra of sinapic acid**

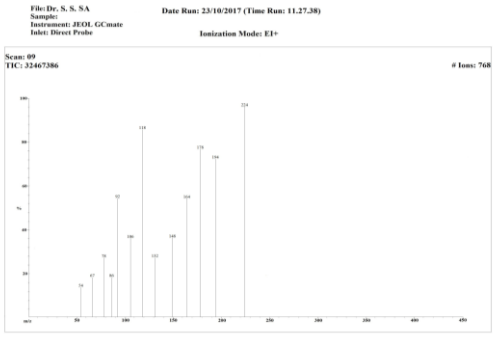

**Figure 4: Mass spectrum of sinapic acid**

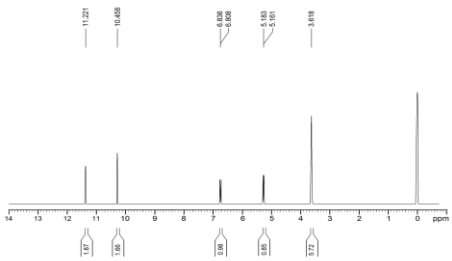

**Figure 5: $^{1}$H NMR spectra of sinapic acid**

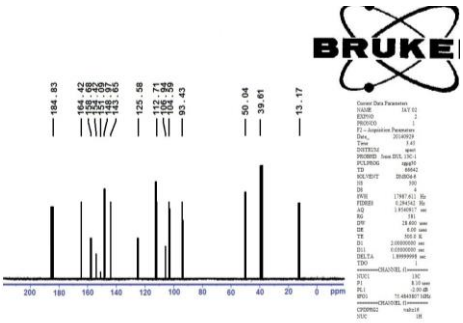

**Figure 6: $^{13}$C NMR spectra of sinapic acid**

## DISCUSSION

The foremost steps to utilize the biologically active components from plant resources include extraction, pharmacological screening, isolation and characterization of lead molecules, toxicological evaluation and assessment of pharmaceutical efficacy. Successful isolation of biologically active compounds

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from plant materials largely dependent on the type of extraction procedure employed. For optimal extraction, different solvent schemes were often employed ranging from non-polar to polar. The specific polarity of the solvent results in successful extraction of active molecules of the similar polarity.

The extraction of phenolic acids from brown mustard seeds have been carried out with different solvent systems and the ethanolic extraction systems were reported to have best results. Accordingly, the ethanolic extract was used in the present study to separate the hydroxycinnamic acids present in the *Brassica juncea* brown mustard seeds. The peak purity of each hydroxycinnamic acid present in the seeds ethanolic extract was assessed by diode assay detector (DAD). Comparison of HPLC chromatograms of standard hydroxycinnamic acids was compared with peaks in the HPLC chromatogram of mustard seeds extract. The determination of individual hydroxycinnamic acids was confirmed by various spectral studies such as FT-IR, mass, $^{1}$H NMR and $^{13}$C NMR. The present study involving the extraction of the hydroxycinnamic acids from the brown mustard seeds is an attempt to rationalize the existing knowledge within the framework of modern scientific principles practices and techniques.

**SINAPIC ACID**

**FT-IR SPECTRA**

The FT-IR spectrum of the isolated compound (Figure 3, Table 1) showed a strong peak at 3386 $cm^{-1}$ which is owing to the stretching absorption of hydrogen bonds between –OH groups; the weak peak around 3220 $cm^{-1}$ was attributed to the stretching vibration of benzene ring =C–H group; the strong peak at 1548 $cm^{-1}$ is due to the presence of stretching vibration of benzene ring C=C group; the strong peak at 1388 $cm^{-1}$ is owing to the presence of O–H bending vibration, and the center peak at 1227 $cm^{-1}$ was attributed to C–OH stretching vibration of phenol. In addition, a strong peak at 1686 $cm^{-1}$ is the proof for the presence of carbonyl group [75].

**MASS SPECTRUM OF THE ISOLATED COMPOUND**

The mass spectrum of the isolated compound is showed in figure 4. The isolated compound exhibited a molecular ion peak at 224 atomic mass unit (amu) which corresponds to the exact calculated molar mass of isolated compound having molecular formula ($C_{11}H_{12}O_5$) and the other fragment ions are at m/z 194, 178, 164, 148, 132, 118, 106, 92, 86 and 78 corresponding to $C_{10}H_{10}O_4$, $C_{10}H_{10}O_3$, $C_9H_8O_3$, $C_9H_8O_2$, $C_9H_8O$, $C_9H_{10}$, $C_8H_{10}$, $C_7H_8$, $C_4H_6O_2$ and $C_6H_6$.

**RESULTS OF NMR SPECTROSCOPY**

The $^{1}$H NMR spectrum showed fine singlet at δ 3.61 (6H) attributed to methoxy protons (Figure 5). Doublets around δ 5.1 and 6.8 ppm correspond to CH protons. Moreover, the singlets δ 10.4 and 11.2 ppm are owing to the presence of hydroxyl protons in the compound.

$^{13}$C-NMR spectrum exhibited eleven signals for each of eleven carbons present in the isolated molecule (Figure 6). The appearance of carbonyl group at δ 184 in the $^{13}$C NMR spectrum suggested the presence of the acid group in its structure. The methoxy group carbon signals were observed in the region of 50.04 ppm. The other carbon signals are due to the presence of aromatic and aliphatic carbons.

**Conclusion**

The HPLC analysis revealed the presence of hydroxycinnamic acids such as *p*-Coumaric acid, caffeic acid, ferulic acid, chlorogenic acid and sinapic acid in the *Brassica juncea* seeds extract. The authenticity of the individual phenolic acids was confirmed by spectral studies such as FT-IR, Mass spectra, $^{1}$H NMR and $^{13}$C NMR. The presence of sinapic acid in brassica juncea seeds further strengthen the biological property of the extract.