JSR Publications

Available Online

J. Sci. Res. **1** (1), 121-127 (2009)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

A HPTLC Densitometer Determination of Sinapic Acid in Chandrasur (*Lepidium sativum)*

Preeti Sagar Nayak[1], Sunil. D. Upadhyaya, and Anubha Upadhyaya

Department of Crop and Herbal Physiology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur 482004, M.P, India

Received 30 September 2008, accepted in final revised form 21 November 2008

Abstract

A qualitative and quantitative analysis method was established to improve quality assessment standards for *Lepidium sativum* seeds. The sinapic acid was identified by comparing with standards and quantified simultaneously by High performance thin layer chromatographic (HPTLC). HPTLC of *Lepidium sativum* methanolic extract was performed on Silica gel $60F_{254}$ [20 cm × 10 cm] plates with butanol: acetic acid: water (4:1:5), as mobile phase. Quantitative evaluation of the plate was performed in the absorbance-reflectance mode at 326 nm. The sinapic acid was separated on a thin layer of silica gel and determined by HPTLC-photo densitometry. The proposed method is simple, sensitive and can be used for the routine assay.

Keywords: *Lepidium sativum*; Sinapic acid; HPTLC; Photo densitometry.

DOI: 10.3329/jsr.vlil.1196

1. Introduction

Phenolic compounds exist widely in plants. They are plant secondary metabolites and they have an important role as defense compounds. Although the exact contribution of these secondary metabolites is still unclear, phenolic compounds are known to be important in the survival of a plant in its environment [1]. Plants still remain a major source for drug discovery in spite of the great development of synthetic molecules. Consequently, the use of traditional plant extract in the treatment of various diseases has been flourished [2]. Extraction techniques need to take into account the location of phenolic acids in the plant. Most of the previous studies in literature reported the phenolic levels of grains, using various aqueous solutions of methanol, ethanol, and acetone to extract soluble phenolics [3, 4]. These studies assumed long extraction time. The use of finely powdered samples would ensure maximum extraction of phenolic compounds from grains. Actually, researchers used acidic or alkaline hydrolysis for extraction and for determination of

[1]*Corresponding author*: preetisagarnayak@rediffmail.com

polyphenolic compounds. But cinnamic acid derivatives, *p*-cumaric, caffeic and ferulic acids, were found to degrade under hot acidic conditions [5, 6]. Therefore it is proper to use different method for extraction of polyphenols, e.g. enzymatic extraction. The use of enzymatic extraction for determining phenolic acid in grains seems to be the best choice because of the specific working of enzymes. Enzymatic reactions have been reported to release phenolic acids (mainly ferulic and *p*-cumaric acids). These enzymes (e.g. pectinases, cellulases, amylases) are employed for the degradation of the carbohydrate linkages. Wojdylo and Oszmainski [7] obtained that individual phenolic acids, such as caffeic and ferulic acids, are positively associated with increased antioxidant activity. Caffeic and ferulic acids were the strongest antioxidants, whereas sinapic acid was the weakest. This may explain why extracts of oats with high contents of ferulic acid, had higher antioxidant activity. Phenolic acids behave as antioxidants, due to there activity of the phenol moiety (hydroxyl substituent on the aromatic ring). The antioxidant behavior depends on the three phenolics: etherified, glycosylated, and nonglycosylated which exist in cereals [6].

Lepidium sativum L. (LS), (Garden cress) locally known as "hab arrachad" is a native shrub belonging to Brassicaceae family. Rapeseed contains more phenolic compounds than any other oilseed plant [8]. *Lepidium sativum* seeds contain an alkaloid, glucotropaeloin, sinapin, sinapic acid, mucilaginous matter and uric acid [9]. The most significant of these are sinapic acid and its derivatives, most notable sinapine. The presence of sinapine and the related phenolic compounds such as sinapic acid has been a concern for oilseed breeders and processors [10, 11]. The seed are bitter, thermogenic, depurative, rubefacient, galactagogue, emmenagogue, tonic, aphrodisiac, ophthalmic and diuretic. They are useful as poultices for sprains and in leprosy, skin diseases, dysentery, diarrhoea, splenomegal, asthma [12]. Colorimetric methods [13, 14] when used to determine individuals' phenolics require specific reagents for colour development. Furthermore, it is difficult to avoid the interference from other compounds in theses systems. Some colorimetric methods and ultraviolet Spectrophotometric methods require purification procedures [15-16]. Gas chromatography [16, 17] requires the hydrolysis of phenolic esters and the derivatization of the resulting phenolics acids before determination.

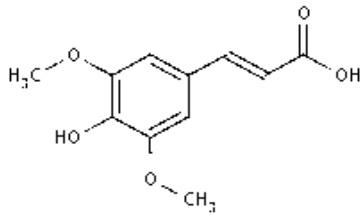

Fig. 1. Structure of sinapic acid.

As far as the pharmacological activities of *Lepidium sativum* are concerned, not much work have been demonstrated. The present study reveals the high-pressure thin layer

chromatography (HPTLC) based on analytical method for identification and quantification of sinapic acid (Fig. 1) in Chandrasur.

2. Experimental

Chemicals and Reagents

Methanol and other solvents used for HPTLC were of analytical grade. The standard sinapic acid was purchased from Natural Remedies Pvt. Ltd. (Bangalore, India).

Plant materials

The investigation was performed on dried seeds part of the plants and were collected from the botanical garden of JNKVV, Jabalpur. The experiment was conducted in the field in 2006 for the determination of secondary metabolites. The samples were collected in accordance to their date of sowing and in the different harvesting period from an experimental area of the University. According to the different date of sowing they were named as Chandrasur, i.e. (T1), (T2), (T3), (T4) and (T5). To investigate the variability of the material at different date of sowing, drug samples were collected at various times. The samples were dried at room temperature, subsequently milled into powder and stored in air-tight stopped glassware before subjected to physical and chemical evaluation of different properties. The experiment consisted of five treatments (different date of sowing) with four replication in a Randomized Block Design.

Treatment details: Sowing date

T1: Eighteen November
T2: Twenty eight November
T3: Sixth December
T4: Sixteen December
T5: Twenty Six December

Preparation of standard solutions

A solution of sinapic acid standard was prepared by dissolving 2 mg accurately weighed, in 5 mL methanol (Merck, Germany) in a volumetric flask. This is the stock solution. It was further diluted for preparing six points calibration curves.

Preparation of sample solution

One gram of the finely dried seed powdered of plant material was extracted two times with 25 mL of methanol on a water bath at 70^0C for 20 min. Each of the extracts from the same samples were combined, partially evaporated and concentrated to dryness under vacuum. After that the extracts were re-dissolved in methanol, combined in a 5 mL

volumetric flask and adjusted to the final volume with methanol. Prior to use, all the samples were filtered through 0.45µm filter.

Chromatography

A Camag HPTLC system equipped with an automatic TLC sampler (ATS_4), TLC scanner 3 and integrated software WINCATS version 1.4.1 was used for the analysis. Chromatrography was performed on 20 cm × 10 cm HPTLC plates coated with silica gel $60F_{254}$ (E.Merck Germany) of 200 µm layer thickness for the quantification of sinapic acid in *Lepidium sativum* . Standard and samples were applied to the plates as 8mm long bands, 8mm apart by use of a Camag Linomat (V) sample applicator equipped with a 100 µl microsyringe and an automatic TLC sampler (ATS_4) under a flow of N_2 gas.

Detection and Estimation of sinapic acid

The linear ascending development was carried out in a Camag glass twin through chamber (20 cm × 10 cm) previously saturated with 20 mL mobile phase with butanol : acetic acid : water (4:1:5) for 15 min at room temperature 25^0C. Plates were developed to a distance of 80 mm. Subsequent to the development; the TLC plate was dried in a current of air with an air dryer. The dried plate was dipped into freshly prepared p-anisaldehyde reagent followed by heating the plate at 110^0C for 10 min. Quantitative evaluation of the plate was performed in absorbance-reflectance mode at λ_{max} = 326 nm, using a slit width 6 × 0.4 mm, data resolution 100 mm step $^{-1}$, scanning speed 20 mm s^{-1} and baseline correction was used. Typical chromatograms of the standard and sample are shown in Figs. 2 and 3. Peak areas were recorded and a calibration plot obtained by plotting peak area against amount of sinapic acid applied.

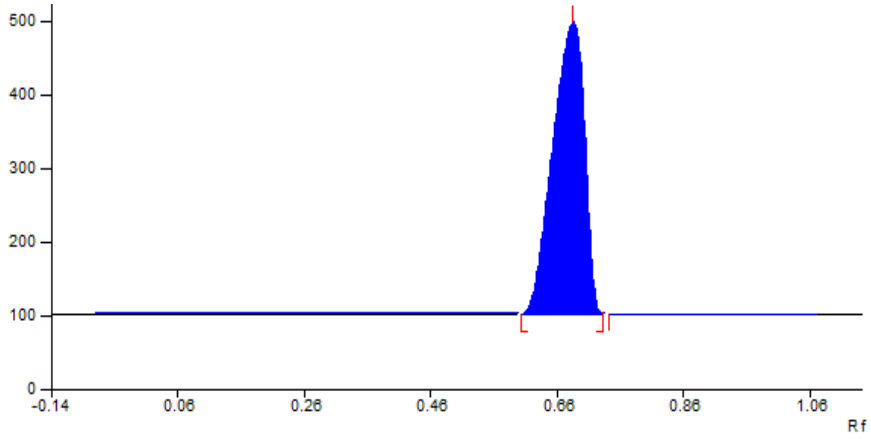

Fig. 2. Thin-layer chromatogram obtained from sinapic acid standard.

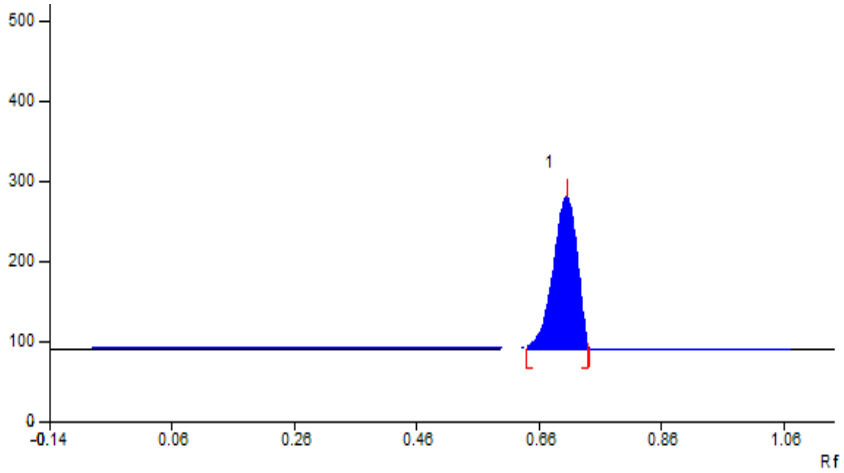

Fig. 3. Thin-layer chromatogram obtained from the sample.

Validation of the method

Linearity: Standard solutions (2 mg/5 mL) were prepared. Chromatograms were developed after application of 20, 40, 80, 160, 240 and 360 µg mL^{-1} of these working solutions. A six-point calibration was obtained for each analyte by fitting peak areas to the amount (µg) of the compounds by least squares regressions. The regression equations were $y = 20.434x + 4063.47$ (where y is the response and x the amount of sinapic acid). The correlation coefficient was found to be 0.9975 (Table 1).

Table 1. Linear regression data for calibration curves (n=6) of sinapic acid obtained from HPTLC

Parameter	Value
Linearity range	20-360 µg mL^{-1}
r^2	0.9975
Slope ± S.D	20.434 (± 1.11)
Intercept ± S.D	4063.47 (± 1.027)

Limit of Detection and Quantification*:* In order to determined limit of detection (LOD) and limit of quantification (LOQ), the blank methanol was spotted six times following the same method as explained. The limit of detection (LOD) and limit of quantification (LOQ) which could be detected were 10 µg mL^{-1} and 50 µg mL^{-1}, respectively.

Specificity: It was observed that the other compounds present in the constituents did not interfere with the peak of sinapic acid. Therefore the method was specific. The spectrum of standard sinapic acid spot in other sample was found to be similar or overlap.

Accuracy: It was measured by analysis of standard solutions spotted at different concentrations several times on the same day. The RSD was 1.7%, which is acceptable.

3. Results and Discussions

Various compositions of mobile phases were tested to get better resolution of sinapic acid. Experimental conditions were optimized for separation of sinapic acid from other components of the plant extract and Silica gel $60F_{254}$ HPTLC plates with butanol: acetic acid: water (4:1:5), as mobile phase resulted in the best separation. Under these conditions the R_f of sinapic acid was 0.68 and the compound was well resolved from other components of the extract. The methanolic extract of *Lepidium sativum*, when subjected to HPTLC, showed the presence of sinapic acid in all the samples. Comparison of the UV spectral characteristic of the peak for the standard of sinapic acid revealed the identity of sinapic acid present in the samples track. Validation of the method showed the linear range of 20-360 μg mL^{-1} for sinapic acid. Data presented in Table 2 illustrated that the second date of sowing was more suitable for more accumulation of sinapic acid in Lepidium seeds than other date of sowing. The probable reasons for the enrichment of these metabolites in the seeds may be attributed to environmental factors, genotypes, morphotypes or cultivation practices. Mann and Vyas [18] on *Plantago ovata* revealed that sown crop in 15th November showed significantly higher plant heights, number of leaves per plant and dry matter accumulation compared to the later sowing dates (25 November, 5 and 15 December). Singh *et al.* [19] reported that peppermint planted on 30th December was superior crop growth, comparing with other planting dates and that is attributed to favorable light and temperature conditions.

Table 2. Percentage of sinapic acid in different samples.

Sl. No.	Place	Different date of sowing	Sinapic acid %
1.	Jabalpur Chandrasur (T1)	18th November	0.4237
2.	Jabalpur Chandrasur (T2)	28th November	0.5590
3.	Jabalpur Chandrasur (T3)	6th December	0.4741
4.	Jabalpur Chandrasur (T4)	16th December	0.4391
5.	Jabalpur Chandrasur (T5)	26th December	0.4593
		Mean	0.4710
		S.E.m±	0.000284
		C.D @ 5%	0.000875

4. Conclusion

Although *Lepidium sativum* is widely used medicinal plant, no analytical method suitable for quality control was available in the literature. This method can be used for identification and quantitative determination of sinapic acid in *Lepidium sativum*.

Acknowledgement

The authors are thankful to ICAR for providing financial grant in the form of externally funded project 'Impact, Adaptation and Vulnerability of Agro Biodiversity to climate change' for carrying out the present investigation.

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