

Extraction and Separation of Glucosinolates from *Brassica Oleraceae var Rubra*

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Abstract: Glucosinolates occur in a variety of Crucifers and among various classes of crucifers, genus *Brassica* contains a rich content of glucosinolates. Glucosinolates are found to have detrimental activity against various types of cancer such as breast, lung and colon. The intact glucosinolates were separated by cold maceration and the fractions were separated using Column, Thin layer (TLC) and Paper chromatographies. The fractions were further analyzed using chromogenic reagent and the Rf values were calculated. The purified fraction were further subjected to HPLC for purity assessment and confirmed with the standard graphs. It was concluded that the yield depends on the different stages of plant development. Any pure extraction study has to be performed by taking the plants life cycle stage and age of the plant as a parameter to optimize the yield.

Key words: *Brassica oleraceae* • Glucosinolates • Separation • Myrosinase • Chromatography

INTRODUCTION

In recent years, plant research has grown to a greater scale with scope in the treatment of various diseases. The nutrition campaign “five per day” (for better health) which is supported by governmental, economical authorities and health organizations in several countries encourages the population to eat five or more servings of fruit and vegetables every day [1]. Epidemiological studies demonstrated that vegetable consumption decreases risk of various diseases, especially cancers of various types [2]. Among the plant groups *Brassica* research continues to attain greater pace for the past 15 years [3]. Vegetables of the *Brassica* genus (broccoli, cabbage, cauliflower, radish, mustard, etc.) have received much attention, because they are reported to have anticancer activity both *in vitro* and *in vivo* [4].

Broccoli sprouts are widely consumed in many parts of the world. There have been no reported concerns with respect to their tolerance and safety in humans [5]. Agudo [6] reported that the consumption of cruciferous vegetables and hence of glucosinolates, is relatively low within Europe, which in turn is lower than in North America and several Asian populations.

A considerable number of epidemiological studies revealed an inverse relationship between consumption of *Brassica* vegetables (broccoli, red cabbage, Brussels sprout, kale, cauliflower, cabbage) and risk of cancer in

various human organs [1]. Crucifers are important sources of glucosinolates (GLs), [7] whose de-generated products like isothiocyanates were attributed to chemo-preventive activity [8]. Glucosinolates are anionic, hydrophilic plant secondary metabolites and play an important role in the prevention of cancer and other chronic and degenerative diseases [9]. However Abdull Razis and Bagatta [8] showed that the intact glucosinolates are capable of carcinogen-metabolizing enzyme systems [10]. Glucosinolates may breakdown to form isothiocyanates and/or nitriles in plant material during processing by the action of the endogenous enzyme myrosinase (thioglucoside glucohydrolase) or within the gastrointestinal tract by the action of commensal microflora [11]. Aires *et al.* [12] demonstrated the potential of these glucosinolates and their respective enzymatic hydrolysis products on bacteria isolated from the human intestinal tract [13]. Various collaborative research states that Indole compounds will be produced as a consequence of breakdown of glucosinolates by enzyme myrosinase which is present in intact tissue. Many researchers support that the chemopreventive effect of *brassica* vegetables and their constituents in various animal and clinical experiments [14]. Red cabbage (*Brassica oleraceae var rubra*) contains similar amounts of glucoraphanin and glucobrassicin but, in addition, appreciable amounts of glucoiberin, progoitrin, sinigrin, gluconapin and glucoerucin, while neo-glucobrassicin occurred at trace levels [2].

Our study focus on the extraction of *Brassica oleracea* var *rubra* using methanol by cold maceration and separation of the constituents by various chromatography techniques.

MATERIALS AND METHODS

Plant Materials: *Brassica oleracea* var *rubra* was obtained from the local grocer and the leaves were separated, washed and air dried. Plant material was lyophilized and subsequently macerated with 80% methanol (4ml/g original fresh weight) and extracted for 18 hr before filtration. The plant material was re- extracted twice with cold 80% methanol for 4hr each time with frequent swirling. The filtrates were combined, concentrated under reduced pressure at 30°C and subjected to column chromatography [14, 15].

Chromatographic Investigation

Column Chromatography: The obtained residue (total glucosinolate) of each plant was further purified on silica column using the mobile phase ethyl acetate.

Thin Layer Chromatography (TLC): The fractions were separated using the solvent mixture as hexane: ethyl acetate (80:20%) for separation of *Brassica oleracea* var *rubra*.

Paper Chromatography of the Glucosinolates:

The purified glucosinolates of each plant (broccoli and red cabbage) was subjected to paper chromatography on (Whatmann 3mm) using the solvent system butanol: ethanol: water (4:1:4, two runs) and descending technique. The developed chromatograms were air dried and then the outer margins of each chromatogram were sprayed with ammoniacal silver nitrate reagent. The separated bands were cut off and diluted separately with methanol (80%) to obtain the pure compounds. The compounds were identified using chemical tests.

Isolation of Myrosinase from Mustard Cake: Myrosinase was isolated from *Aspergillus* spp using mustard extract medium. To 100 ml of potato dextrose broth, fresh culture of *Aspergillus* spp was added and shaken for 15 min with intermittent heating initially and continued further for 3 hrs. The culture was filtered and centrifuged; the supernatant was collected and stored. Protein test was done using ninhydrin test [16].

Chromogenic Reagents: Compounds were detected by means of ferric nitrate in solution of nitric acid and myrosinase solution (pH 7) as previously described [14, 15]. The compounds were developed on TLC plates and sprayed with myrosinase (pH 7) with ascorbic acid and allowed to dry for 2 hrs and spraying with ferric nitrate reagent.

HPLC Analysis: HPLC was performed with Zorpax Eclipse XDB C₁₈ 5 µm 4.6 mm × 150mm column. The mobile phase was acetonitrile: water (20: 80). The sample (20 µl) was injected in to the column by means of an auto-injector and peaks were monitored at 229 nm. The chromatograms were recorded for 30 minutes. Mobile phases were pumped at 1.5 ml/ min [17-19].

RESULTS

Determination of Total Glucosinolates: The residue of glucosinolates after extraction was estimated to be 1.8 and 1.7 g, respectively. The fractions from the column were collected and were subjected to TLC for further analysis.

Thin Layer and Paper Chromatography: The chromatograms from the column chromatography were air dried and designated red cabbage as RC1, RC2, RC3 and RC4. The R_f values of different samples are given in Table 1.

Based on the R_f values of the fractions and upon spraying with ferric nitrate reagent the red brown colour may indicate the presence of glucosinolates.

Table 1: Chromatographic properties of Glucosinolates

Solvent system	R _f values for compounds			
	RC1	RC2	RC3	RC4
Butanol:ethanol:water	0.46	0.66	0.76	0.62
Chromogenic reagent				
Ferric nitrate in nitric acid	Brownish	Bluish	Bluish	Bluish green
Myrosinase (pH 7)+ Ferric nitrate in nitric acid	Brown	Reddish brown	Reddish brown	Reddish brown

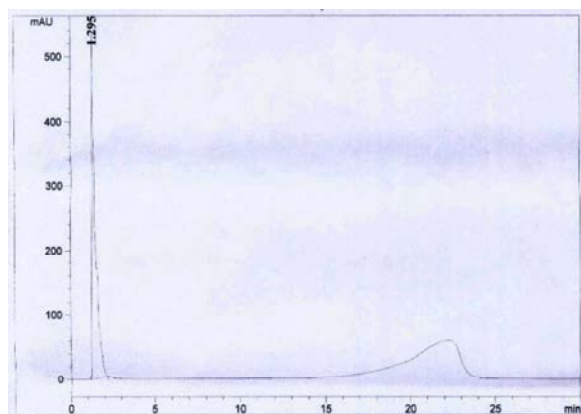


Fig. 1: HPLC for sample RC1

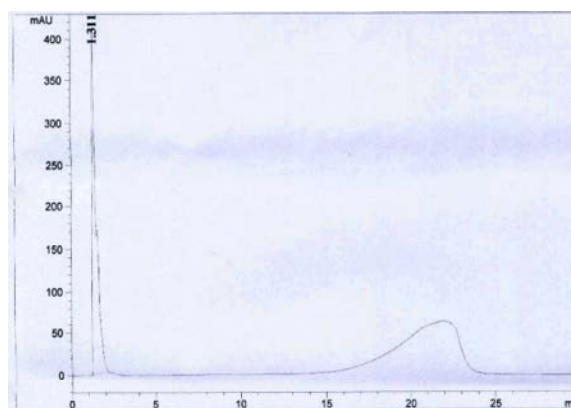


Fig. 3: HPLC for sample RC3

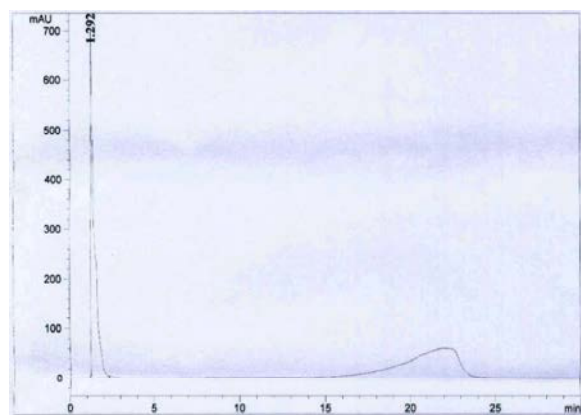


Fig. 2: HPLC for sample RC2

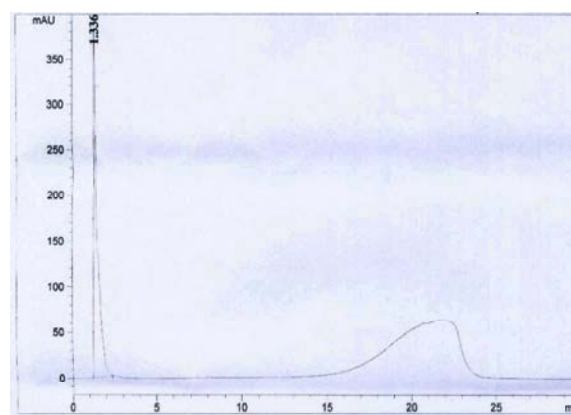


Fig. 4: HPLC for sample RC4

HPLC Analysis: The fractions from chromatography were individually subjected to analysis and the graphs are depicted in the picture. Since internal standards are not available the glucosinolates are confirmed by comparing the retention times of peaks with the standard graphs from the literature [20]. The HPLC graphs are shown below.

DISCUSSION

The objectives of the current extraction and purification were to analyze the extracted compounds further to study the anti-cancer activity. Marcelo Fonseca Xavier studied the optimization of column process and optimization conditions for extraction of anthocyanins from red cabbage [18]. Michael Meyer made a comparative study of glucosinolate levels in commercial broccoli and red cabbage. [1] McNaughton discussed about the creation of a food composition database for the estimation of dietary intakes of glucosinolates [21].

Kim and Durret states that the Levels of leaf glucosinolates are regulated during plant development.

The level of glucosinolates increases in response to mechanical damage or insect feeding. [22] Zhou *et al.* used a three step process to purify main glucosinolate from *Brassica oleracea*. The steps involved, extraction with methanol, separation and purification by chromatographic column on alumina support; and followed by a reversed-phase separation by octadecyl (C18) silica [23]. Barillari *et al.* measured the two important glucosinolate viz., Glucoraphasatin (GRH) and glucoraphenin (GRE) from *Raphanus sativus* sprouts and seeds during different stages of development. They found that in comparison to the seeds, the GRE content in sprouts decreased with development. Whereas, GRH content increased steadily up to a 25-fold increases. [24].

Different glucoraphenins have role in different stages of plant development and the extraction optimization has to be done based on the plants age and stage of development for commercial extraction studies, which is a scope for future study. Further scope also includes studying the various processes like anti-cancer and antimicrobial activity and also extraction of active compounds of importance.

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