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Original Research Article

STUDY OF GROWTH, SECONDARY METABOLITIES AND GLUCOSINOLATE CONTENT IN CARDAMINE HIRSUTA V/S BRASSICA JUNCEA (INDIAN MUSTARD)

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Abstract: Comparative studies of secondary metabolites and glucosinolate contents in *Cardamine hirsuta* L. v/s *Brassica juncea* (Indian mustard) were carried out. Physiological characteristics and analysis of secondary metabolites and glucosinolate content in *Cardamine hirsuta* L.and Brassica juncea was done simultaneously. Physiological characteristics of *Cardamine hirsuta* L. with Brassica juncea.Methods were compared with the help of U.V spectrophotometer, HPTLC and qualitative chemical analysis. Since both plants belong to Brassicaceae family, mustard plant was selected to compare physical, chemical and biological properties of *Cardamine hirsuta* L. Their different phytoconstituents were identified and compared qualitatively as well as quantitatively. Out of many important phytoconstituent glucosinolate was further quantified simultaneously. Secondary metabolites was identified and compared qualitatively while glucosinolate content was quantified and compared. The study revealed that raw Cardamine seeds and leaves showed maximum level of glucosinolates compared to the raw mustard seeds and leaves samples.

Keywords: *Cardamine hirsuta* L., qualitative analysis, glucosinolates.

Introduction: The family Brassicaceae formerly Cruciferae, is the mustard family of flowering plants. This family includes various plants of economic importance which have been cultivated and domesticated extensively by human civilization.

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In genus Brassica, cabbage, broccoli, Brussels sprouts, Napa cabbage, turnip, radish, and white mustard have been altered for agriculture otherwise most members of Brassicaceae grow wild as weed with short lifecycles [1].

Mustards sprout and grow very fast. It flowers and settle with seeds early in the season to provide proper moisture to the growing

embryos. Certain sulfur-containing chemicals with pungent bitter taste are produced within the plants themselves, which are known as glucosinolates. The family specific chemicals are the part of mustard-oil glycosides (glucosinolates) which defend the plants against herbivores and microorganisms [2].

Glucosinolates and their metabolites also act as anti-tumor agents. The glucosinolate-myrosinase system is considered as one of the best studied plant chemical defenses. Beside this, the glucosinolates are non-toxic. It may be a wise approach to incorporate these edible and medicinal associates into our diets. Studies have shown that increased intakes of cruciferous vegetables are linked with a reduced threat of prostate cancer, colon cancer, lung cancer and breast cancer [3] [4].

Research studies routinely show that wild varieties generally include higher levels of nutritional and medicinal compounds than their cultivated counter parts. The genus *Cardamine* is from the family Brassicaceae. Plant kingdom contains diverse organic chemicals which are of pharmaceutical and industrial level interest. They are mainly categorized into secondary metabolites.

Primary plant metabolites can be considered metabolites which are very much essential for plants and actively participate in plant metabolism. Plants have been used in traditional medicines since ancient times and even today 40% of medicines prescribed in USA contain compounds derived from them. Secondary metabolites accumulate by plants in microgram to picogram quantities than the primary metabolites. Secondary metabolites from plants directly or indirectly play an important role to combat human diseases. As a result the secondary metabolites are generally high value products than the primary metabolites, as they are used in drug manufacture by the pharmaceutical industries [3].

This project was designed to systematically identify and quantify these compounds (secondary metabolites) by standardized HPTLC and by basic qualitative phytochemical analysis. In this study, the HPTLC method was used to analyze the glucosinolate profiles of *Cardamine hirsuta* L. and *Brassica juncea*. Beside this, the growth Cardamine hirsuta L. was also studied.

Materials & Methods:

Part I - To understand the growth cycle of the wild weed *Cardamine hirsuta* L.

Ripe yellow pods were collected, burst open and sun dried for a week from, Homi Bhabha Centre for Science Education, TIFR, Deonar, Mumbai campus. (Wt. of collected seeds approx. 500mg) Approximately 300 ripe seeds were kept in plastic tray for germination. Four leaves stage of the seedlings were achieved and then the seedlings were shifted to small cups with same soil and water supply.

Part II – To study the physiological characteristics of *Cardamine hirsuta* and *Brassica juncea.*

Cardamine hirsuta:

- *Cardamine hirsuta* whole plants was collected from HBSCE campus. (wet wt. 180g)
- Whole plant was collected without pods and flowers washed with tap water and dried on filter paper overnight.
- Dried plant was then weighed and kept in hot air oven at 70˚C for 20mins.
- Dried plant is finely crushed in motar pestle to obtain fine powder.
- For organic plant extract, this dry *Cardamine hirsuta plant powder* is then soaked in 80% methanol overnight in ratio 1:10. 15g of *Cardamine hirsuta* powder was soaked in 150ml of methanol.

- Aqueous extracts were prepared in the same ratio using 1g powder plant sample in 10ml of D/W.

Brassica juncea:

- *Brassica juncea* plant (only stem and leaves) was collected from local market. (wet wt. 124g)
- Plant was cleaned, washed and dried on filter paper overnight (wet wt. 124g).
- The plant was then kept in hot air oven at 90˚C for 45mins.
- Dried plant was weighed and then finely crushed in a food mixer.
- Crushed powder of plant sample was then soaked in 80% methanol in the ratio 1:10. 15g of dry *Brassica juncea* powder was soaked in 150ml of methanol.
- Aqueous extracts were prepared in the same ratio using 1g powder plant sample in 10ml of D/W.
- The plant organic and aqueous samples of *Cardamine hirsuta* and mustard were used for phytochemical analysis as per the tests.

Phytochemical Analysis:

➢ Test for Phenols, Tannins, Terpenoids, Anthraquinones, Carbohydrates, Steroids, Reducing Sugars (Fehling's test), Flavonoids, Phlobatannins, Alkaloids, Cardiac Glycosides (Keller-Killiani test) and Saponins were performed following Talreja and Moon, 2014.

Part III - Extraction and estimation of Glucosinolates from *Cardamine hirsuta* and *Brassica juncea* seeds using U.V. Spectrophotometer.

- *Cardamine hirsuta*

- Ripe dried seeds were crushed in a motar pestel in ratio 1:20. 1g of *Cardamine hirsuta* seed crushed in 20ml of distilled water and soaked overnight.
- Sample was filtered with filter paper next day to obtain clear solution.

- *Brassica juncea*

- Dry mustard seeds are grind in a food mixer till powdered. 1g dry powder was then soaked in 20ml of distilled water overnight.
- Sample was filtered with filter paper next day to obtain clear solution.

- UV Spectrophotometer was used.
- Baseline was prepared using D/W.
- Aqueous extracts of *Cardamine hirsuta* and *Brassica juncea* were analyzed separately by the instrument from the wavelengths 200-750nm. The wavelength of obtaining total glucosinolate content was from 230nm.

Part IV – Extraction and estimation of Glucosinolates from *Cardamine hirsuta* and *Brassica juncea* plant using HPTLC [6,7,8,9,10,11,12].

1. Sample preparation:

- Whole *Cardamine hirsuta* plants was cleaned (without pods and flower), washed and dried in hot air oven for 70° C for 15mins.
- Dried plant was then crushed in a clean and dry motar and pestle to obtain fine powder.
- 1g of dry *Cardamine hirsuta* extract was then mixed in 20ml of 80% methanol and soaked for 2 weeks.
- Solution was heated in evaporator for about 10mins.
- Solution was then filtered with filter paper.
- The solution was then placed in 2.5 microfuge tubes and then placed in cold centrifuge at 21°C at 10,000rpm for 15mins.
- The upper organic layer was then separated and used.

2. Standard preparation:

- Whole dried *Brassica juncea* plant without seeds and flowers was cleaned, washed and dried in hot air oven at 90°C for 45mins.
- Dried plant was then crushed to obtain fine powder.

- 1g of dry *Brassica juncea* extract was then mixed in 20ml of 80% methanol and soaked for 2 weeks.
- Solution was heated in evaporator for about 10mins.
- Solution was then filtered with filter paper.
- The solution was then placed in 2.5 microfuge tubes and then placed in cold centrifuge at 21°C at 10,000rpm for 15mins.
- The upper organic layer was then separated and used.

3. Developing solvent system: A number of solvent systems were tried, for extracts, but the satisfactory resolution was obtained in the solvent for Hexane: ethyl acetate (4:1) organic & aqueous extracts respectively.
4. Application: Application of bands of each extract was carried out using spray technique. Sample were applied on pre-coated silica gel 60F254 Aluminum sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.
5. Development of Chromatogram: After the application of sample, the chromatogram was developed in Twin trough glass chamber 10 x 10 cm saturated with solvent Hexane: ethyl acetate (4:1) for organic& aqueous extract for 20mins.
6. Detection of Spots: The air-dried plates were viewed in ultraviolet radiation. The chromatogram was scanned at 230nm for organic extracts of *Cardamine hirsuta* and *Brassica juncea* using CAMAG scanner IV. [8, 10, 11and 12].

Results:

Part I - To understand the growth cycle of the wild weed *Cardamine hirsuta L*

Cardamine hirsuta life cycle was studied by it various stages that included 6 main important stages of the plant. Seven principal stages are germination and emergence (stage 0), leaf production (stage 1), stem extension (stage 2), flower bud development (stage 3), flowering (stage 4), pod development (stage 5), seed development (stage 6).

- Day 1 – seeds
- Day 5 –emergence of radicle and plumule
- Day 7 - first two leaves (cotyledons 0.5cm in ht)
- Day 23 - 3leaf
- Day 29 - 4leaf
- Day 33 - 5 leaf
- Day 64 - Whole plant with fruits (flower ,pods ,seeds)

Part II - Physiological characteristics has been studied of *Cardamine hirsuta* and *Brassica juncea.*

Table No.1 Phytochemical analysis of *Cardamine hirsuta* and *Brassica juncea*

*Key; +: Presence; - : Absence

PHYTOCHEMICAL CONSTITUENTS / PLANTS	***CARDAMINE HIRSUTA***		***BRASSICA JUNCEA***	
	Observation	+/-	Observation	+/-
Phenols	-	-	-	-
Tannins	Brownish green color	+	Brownish green color	+
Terpenenoids	Reddish brown color	+	Reddish brown	+
Anthraquinones		-		-
Carbohydrates	Violet ring	+	Violet ring	+
Steriods	Red color layer	+	Red color layer	+
Flavonoids	Yellow color	+	Yellow color	+
Phlobotannins	Red precipitate	+	Red precipitate	+
Alkaloids	White cream	+	White cream	+
Cardiac glycoside	Brown ring	+	Brown ring	+
Saponins	emulsion	+	emulsion	+

PART III - Extraction and estimation of Glucosinolates from *Cardamine hirsuta* and *Brassica juncea* seeds using U.V. Spectrophotometer.

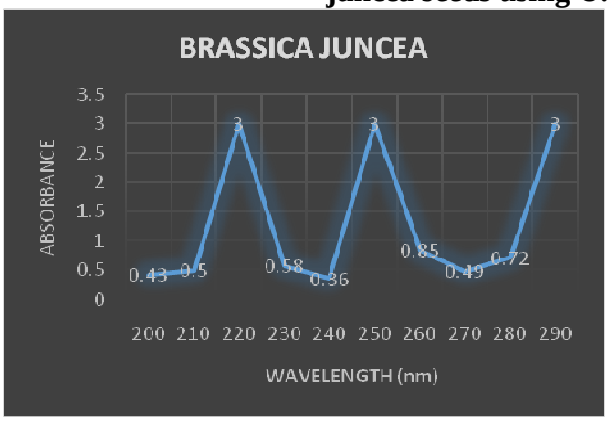

Fig.1. Graphical representation of aqueous extraction of *Brassica juncea* seeds in U.V spectrophotometer from 200-300nm. *Blue color line- *Brassica juncea.*

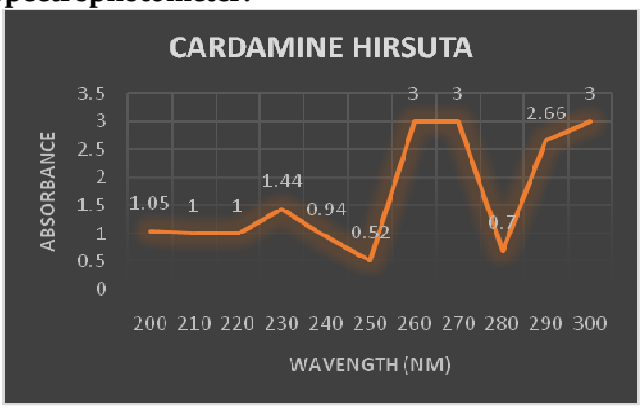

Fig.2; Graphical representation of aqueous extraction of *Cardamine hirsuta* seeds in U.V spectrophotometer from 200-300nm. * Orange color line – *Cardamine hirsuta*

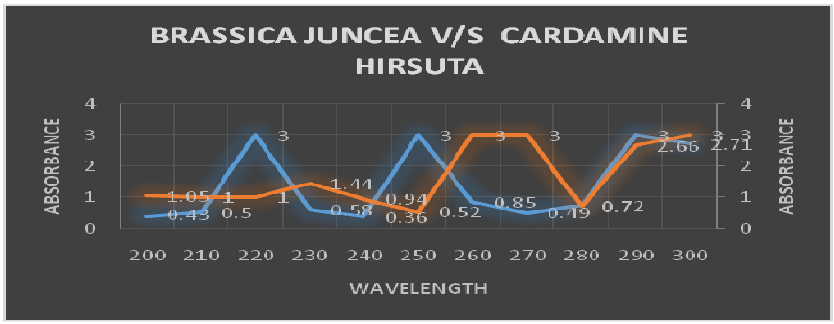

Fig.3; Comparative graphical representation of U.V spectrophotometer of *Brassica juncea* v/s *Cardamine hirsuta* scanned for glucosinolates from 200-300nm.
*Blue line- *Brassica juncea*; Orange line – *Cardamine hirsute*

Part IV – Extraction and estimation of Glucosinolates from *Cardamine hirsuta* and *Brassica juncea* plant using HPTLC.

1. TLC plate observed under UV trans illuminator showing bands for Glucosinolate in both *Brassica juncea* (track 1)and *Cardamine hirsuta* plant extracts (track 2) at Rf 0.24 respectively.

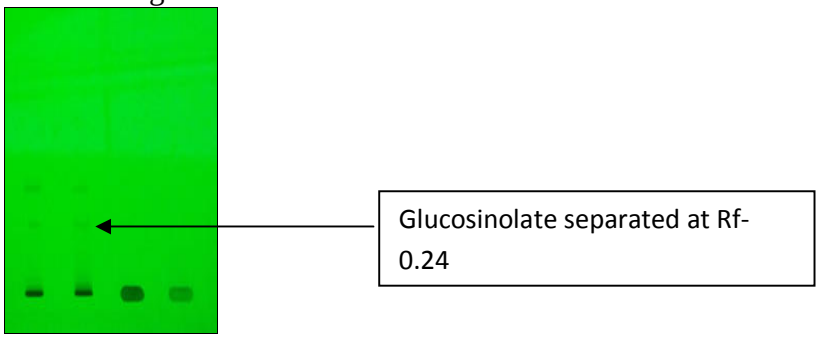

Fig.4; TLC plate under U.V light showing two separated bands in the Track no. 7 (*Brassica juncea)* and track no. 8 (*Cardamine hirsuta)*. The first two bands in the both the tracks indicates the separated glucosinolate and 230nm.

2. Three dimensional overlay of HPTLC densitogram showing comparison between Glucosinolate content of *Cardamine hirsuta* and *Brassica juncea.*

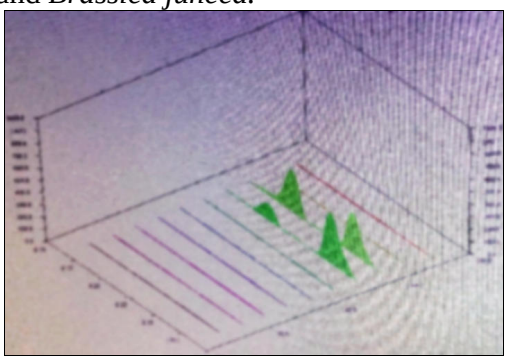

Fig.5; Three dimensional overlay of HPTLC densitogram showing comparison between Glucosinolate content of *Cardamine hirsuta* and *Brassica junce*

Table no. 2. Quantification of glucosinolates in *Brassica juncea* and *Cardamine hirsuta* plants by HPTLC method.

Track no	Rf	Area under curve	Assigned substance
7	0.24	767.7 (18.88%)	Glucosinolate (*Brassica juncea* plant organic extract)
8	0.24	2179.2 (50.69%)	Glucosinolate (*Cardaminehirsuta* plant organic extract)

Conclusion: The growth of *Cardamine hirsuta* plant was observed and studied morphologically and the plant was observed and photographed each stage of the plants life cycle while it was estimated to grow in approximate 64 days [5]. In the present study, phytochemical profiling of *Brassica juncea and Cardamine hirsuta* extract was performed. Distribution of secondary metabolities contents in leaves of *B. juncea* and *Cardamine hirsuta* is shown in Table 1. Results clearly indicated the presence of various phytochemicals like tannin, flavonoid, alkaloid, anthocyanidin and phenols. This project focused on glucosinolates derived from *Brassica juncea* L and *Cardamine hirsuta* L. leaves and seeds. The study revealed that raw *Cardamine hirsuta* seeds showed the highest levels of glucosinolates compared to the *Brassica juncea* seeds. The results of this study showed that *Cardamine hirsuta* is a rich source of glucosinolates. The compound was detected at 230nm using the UV Spectrophotometer [13, 14].

Though further work to characterize the other chemical constituents and perform quantitative estimation with marker compounds is also necessary these data can also be considered along with the other values for fixing standards to this plant. Organic extracts of plants of Brassica *juncea* and *Cardamine hirsuta*leaves was separated for comparing its Glucosinolate content. Thus it can be concluded that *Cardamine hirsuta* contains 50.69% Glucosinolate comparatively more than *Brassica juncea* extract which is 18.88%. Comparing Table no. 4 and 5 it can be concluded that the Rf. Value of glucosinolate in *Cardamine hirsuta* and *Brassica juncea* seeds organic extract is approximate to the range. The study revealed that raw Cardamine seeds and leaves showed the highest level of glucosinolates compared to the raw mustard seeds and leaves samples when analyzed by U.V. spectrophotometer and by HPTLC methods [10, 11,12].

Discussion: This study deals with the morphological study of *Cardamine hirsuta* plant which is the basis to know the life cycle to grow the plant with appropriate necessities and fruitful yield and for systematic study. *Cardamine hirsuta* was used in comparison of the commercial *Brassica juncea* plant to compare the secondary metabolite contents. Phytochemical screening of both the plants was carried out qualitatively. It is reported that Mustard has antibacterial, antifungal and anticancerous activity [13]. These activities may be attributed to various phytochemicals present in the extract. Tannin is reported to have antimicrobial activity and antibacterial activity. Alkaloids are reported to act as antioxidant [14].

Further a specific secondary metabolite; Glucosinolate of great importance was then chosen and quantified. Comparative study of glucosinolate levels in commercial *Brassica juncea* and wild weed *Cardamine hirsuta* was carried out. The dietary intakes of glucosinolates showed various benefits to health. The Levels of leaf glucosinolates are known to regulate during plant development. Mechanical damage or insect feeding. A three step process to obtain main glucosinolate from *Brassica junecea and Cardamine hirsuta* was carried out. The steps involved extraction with methanol, separation and purification by chromatographic column. HPTLC was performed to separate key component of Cardamine *hirsuta* and *Brassica juncea* extract i.e. glucosinolates, which are reported to have anticancerous activity. This can be indicated from the known Rf values of the compounds. We have found the presence of glucosinolate at 0.24 rf values which correspond to standard Rf values of 3-methylsulfinylpropyl GS (0.27). Also it has been noted that Hexane: ethyl acetate gave good results. Methanolic organic extract was used to detect the presence of glucosinolates in different extracts of Card *amine hirsuta* and *Brassica juncea*. This study further validates *Cardamine hirsuta* as an anticancer therapeutic plant. This weed can be included in diet for its therapeutic benefit. Future work with respect to the toxicity studies and *invivo, invitro* anticancer activity of *Cardamine hirsuta* for cancer is envisaged.

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