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DEVELOPMENT OF ULTRAVOILET SPECTROSCOPIC AND THIN LAYER CHROMATOGRAPHY METHOD OF BROCCOLI EXTRACT

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Abstract:

Broccoli contains sulforaphane, an isothiocyanate which is formed by the action of myrosinase enzyme on glucoraphanin. This sulforaphane has anticancer properties and can also help in treating many diseases like kidney disorders, skin diseases etc. The present paper highlights the development of UV and TLC method, which will be used in further research. The paper highlights the development of UV method in three media namely-Distilled water, phosphate buffer 7.4 and HCl buffer 2.3. This paper also highlights the Development of TLC method for the identification of sulforaphane in Broccoli extract.

Key words*: Broccoli, sulforaphane and glucoraphanin*

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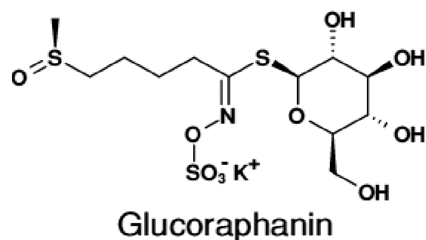
INTRODUCTION:

Vegetables are good sources of natural antioxidants and biologically active components. They help in supplying certain constituents that are deficient in other foods thereby playing an important role in human nutrition [1]. Brassicaceous vegetables such as broccoli, red cabbage contains high concentrations of minerals, vitamins and a special group of phytochemicals called glucosinolates[2]. Broccoli is a type of cruciferous vegetable which is classified under the italic cultivar group of species Brassica oleracea. It belongs to family Brassicaceae and was originated in Italy about 2000 years ago[3]. Glucosinolates, on hydrolysis by the endogenous enzyme myrosinase (thioglucoside glucohydrolase) yields a variety of bioactive products. Formation of these bioactive products like sulfate, D-glucose, oxazolidine-2-thiones, thiocyanates, nitriles, isothiocyanate depends on the chemical structure of glucosinolates and other factors like temperature and pH during enzmymic cleavage [4-6].

Sulforaphane (SFN) (4-methylsulfinylbutyl isothiocyanate) was first identified in broccoli extracts as a product of enzymatic- or acid hydrolysis of the corresponding co-(methylsulfinyl)-alkyl-glucosinolate (Glucoraphanin). Glucoraphanin is the inert glucosinolate precursor of sulforaphane which is hydrolysed by an enzyme called Myrosinase into biologically active isothiocyanate. This sulforapahne has been a great deal of interest and known to possess anticancer properties. Myrosinase enzyme is present in fresh broccoli as well in broccoli sprouts. It is also found in the microbial flora of the lower intestine of humans and animals, hence as the Glucoraphanin fully passes the GIT system, a significant amount of Glucoraphanin is expected to be hydrolyzed by myrosinase enzyme and become bioavailable as sulforaphane[7-9].



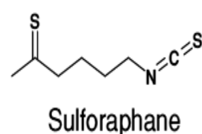


Fig1:Structure of sulforaphane and glucoraphanin[10]

Sulforaphane has huge cancer chemopreventive potential and it modulates cell sycle, cell death, invasion and metastasis, susceptibility to carcinogens and also possesses antioxidant properties [11]. It was found that sulforaphane can protect against carcinogens and other toxic electrophiles [7, 12, 13]. It was also found that sulforaphane exhibits anti-cancer, antimicrobial and anti diabetic properties in experimental models [14]. It can also reduce the incidence of number of forms of tumors, hence sulforaphane is a promising chemoprotective agent and hence has attracted many reseacher's interest [15-19].

Ultraviolet Spectroscopy

Technique insight

UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. The amount of ultraviolet or visible radiation absorbed by a substance in solution is measured by this instrument. Instrument which measures the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers.

Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds. The fundamental law that governs the quantitative spectrophotometric analysis is the Beer –Lambert law.

Beer's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. In other words, absorbance is proportional to the concentration.

Lambert's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially as it passes through a medium of homogeneous thickness. A combination of these two laws yields the Beer-Lambert law.

Beer-Lambert law: When beam of light is passed through a transparent cell containing a solution of an absorbing substance, reduction of the intensity of light may occur. Mathematically, Beer- Lambert law is expressed as

A=a b c

Where, A=absorbance or optical density

a=absorptivity or extinction coefficient

b=path length of radiation through sample (cm)

c=concentration of solute in solution.

Both b and a are constant so a is directly proportional to the concentration c

When c is in gm/100 ml, then the constant is called A (1%, 1 cm) [20]

$$A = A\frac{1\%}{1cm}bc$$

MATERIALS AND INSTRUMENT:

Fig 2: SCAIN-2301 double beam Ultraviolet spectrophotometer

The instrument used was SCAIN-2301 double beam Ultraviolet spectrophotometer. Broccoli extract was purchased from Connell Bros. Company (INDIA) private limited. Standard broccoli sprout tablet was purchased from Nutradeal from amazon. Acetonitrile (HPLC grade), Methanol (HPLC grade) , Dichloromethane and water of HPLC grade were purchased from SD fine Chemicals, India. All other chemicals used in the process were of analytical grade.

Method

Preparation of dilutions

The sample solution was prepared by dissolving 1g of broccoli sprout extract in 10mL of distilled water. 1mL of this sample solution was taken and 9mL was distilled water was added. The solution formed contained 10mg/mL drug. Further dilutions were made from this 10mg/mL sample stock solution. 0.5mL of this sample stock solution was taken in a measuring cylinder and distilled water was added to make up the volume up to 100mL. The solution formed is of 50ppm. Similarly solutions with 100ppm, 150ppm, 200ppm, 250ppm were made.

Dilutions of same concentration were made using phosphate buffer 7.4 and hydrochloride buffer 2.6 instead of distilled water.

Different aliquots were taken and λ max was found by UV (Perkin Elmer). It was found to be 240nm, 215nm, and 235nm for distilled water, phosphate buffer and HCl respectively. Absorbance was measured. The calibration curve was prepared by plotting absorbance versus concentration.

Thin Layer Chromatography

Technique Insight

Thin layer chromatography is a simple and a cost-effective method which has been used for several decades to routinely separate chemical and biochemical compounds [21].

It is used to separate non-volatile mixtures and is performed on a sheet of glass, aluminium foil or plastic which is coated with a thin layer of adsorbent material such as silica gel, cellulose or aluminium oxide (alumina). The layer of adsorbent is called stationary phase [22 ,23].

TLC is used for identifying compounds in a given mixture, progress of a reaction and to determine the purity of the compound. For example, it is used for identification of medicinal plant and their chemical constituent, detection of pesticides or insecticides in water and food sample, assaying the radiochemical purity of radiopharmaceuticals and many more.

The results are quantified by finding the retention factor or R_f. R_f is calculated by –

$$R_f = \frac{\text{Distance travelled by the substance being considered}}{\text{Distance travelled by the mobile phase or the solvent front}}$$

Those substances which resemble the mobile phase will have higher retention factor and those who have similar structure to stationary phase will have low retention factor [24].

Materials

TLC plate was purchased from MERCK Company and 20X20 cm in size. Methanol (HPLC grade), acetic acid, Dichloromethane were purchased from SD fine chemicals, India.

Method

Various solvent system were made for identification of broccoli extract. Different ratios of DCM and methanol were tried. Further to improve this combination, acid was added and the final solvent mixture was prepared by mixing Dichloromethane, methanol and acetic acid in following ratios- 9.5 : 0.5 : 0.5. 10mL of both methanol and acetic acid was mixed together with 190mL of Dichloromethane and the mixture was added to the TLC tank. The sides of TLC tank were lined with filter paper and the tank was covered using the lid. The filter paper was made wet and was allowed to cover the bottom of tank. This setup was allowed for saturation. TLC plate was marked with a help of sharp pencil and a line 1cm from the bottom was made [25].

The sample solution of broccoli extract was made by mixing 1g of sample in 5-10 mL of 0.1M HCl. The solution was filtered and was labelled as Sample. The standard solution was made by crushing 2 tablets of standard and mixing it with Dichloromethane. The solution was then filtered and labelled as Standard.

The sample solution of broccoli extract as well as the standard solution was spotted using glass pipette 1 cm from the bottom of TLC plate. The plate was placed in solvent tank and the tank was covered with lid. The plate was removed till the development was up to 1 cm top. The plate was air

dried and viewed under UV. The R_f values were calculated and compared with the standard.

RESULTS AND DISCUSSIONS:

The UV method was developed in different dilution solvents- distilled water, phosphate buffer 7.4, HCl buffer 2.3 and was validated according to the parameters. In TLC it was seen that the spot clearly occurred when DCM, methanol and acetic acid mixture were used. This study was done for the further research on Broccoli tablets which will be published in another paper. The results were under the specified limit.

UV spectrophotometry

Table 1: Absorbance of broccoli extract in Distilled water at λ = 240nm

Concentration (mg/ml)	Absorbance
0.2	0.16
0.4	0.42
0.6	0.68
0.8	0.85
1	1.03
2	2.17

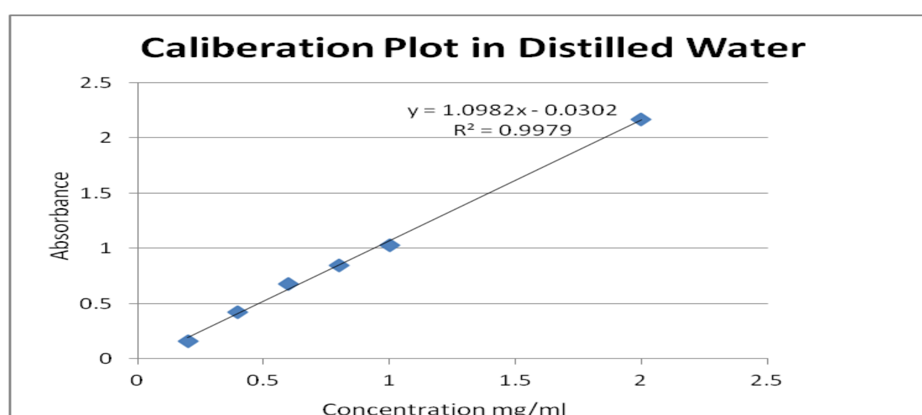


Fig 3: Standard graph of broccoli extract in Distilled water at λ = 240nm

Table 2: Absorbance of broccoli extract in Phosphate Buffer 7.4 at λ = 215nm

Concentration(ppm)	Absorbance
50	0.165
100	0.305
150	0.479
200	0.651
250	0.797

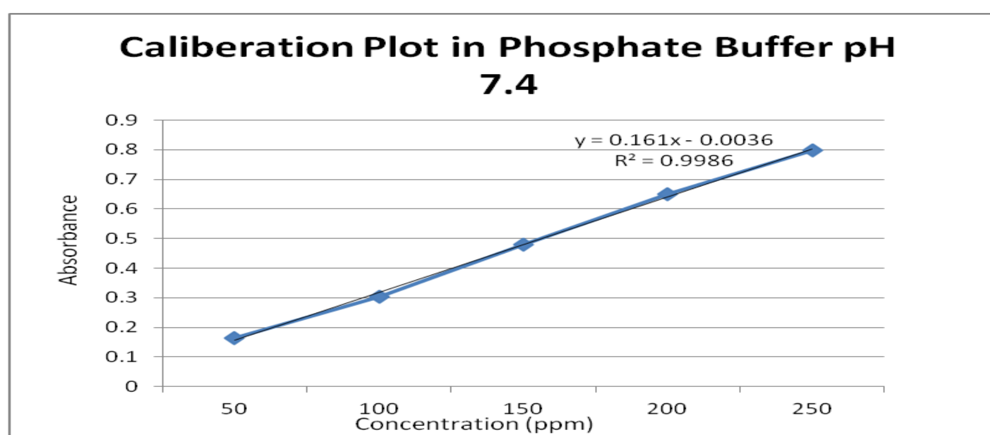


Fig 4: Standard graph of broccoli extract in Phosphate Buffer 7.4 at λ = 215nm

Table 3: Absorbance of broccoli extract in HCl buffer 2.6 at λ = 235nm

Concentration(ppm)	Absorbance
50	0.083
100	0.155
150	0.236
200	0.315
250	0.409

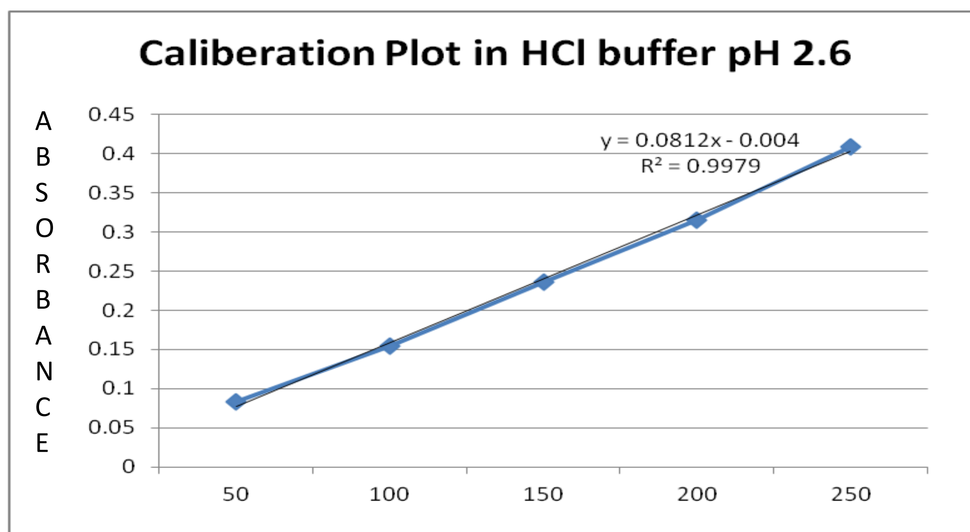


Fig 5: Standard graph of broccoli extract in HCl buffer 2.6 at λ = 235nm

Thin layer chromatography

Concentration (ppm)

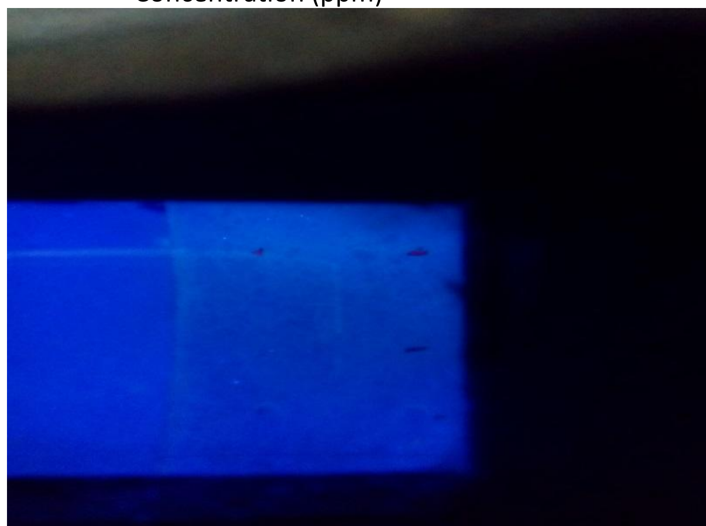


Fig 6: TLC of broccoli extract under UV

TLC remains to be an important method for qualitative analysis as man sample can be analysed at the same time and there are many dectection that can be used for identification of components. The Broccoli extract was first hydrolysed in HCl so that sulforaphane can become available. Glucoraphanin is present in extract and it only gets converted into sulforaphane in acidic medium. TLC was used to qualitatively identify the sulforaphane present in the extract fraction. It was seen that the spot was not clearly visible when the mobile phase of DCM and methanol combination was used. The spot was clearly visible in UV when DCM : methanol : acetic acid combination were used as mobile phase. The Rf value of standard was found to be 0.74 and the broccoli extract sample was found to be 0.73 by the formula mentioned above. Thus we can conclude that the given broccoli extract contains sulforaphane.

CONCLUSION:

Thus, it can be concluded that the most important analytical parameters of the broccoli extract have being developed and validated. Broccoli extract contains sulforaphane which offers huge potential and this broccoli extract can be used in formation of different formulations such as tablets, capsules etc. Bioavailability of Sulforaphane from broccoli extract can be increased by adding an enhancer such as piperine or this sulforaphane can be conjugated with other agents such as silver nanoparticles, gold nanoparticles and help in anticancer drug delivery. This study has being done for the reference for the project on "Formulation of broccoli tablet by direct compression". The developed ultraviolet spectroscopic method was validated and was done in different dilution media such as distilled water, phosphate buffer pH 7.4 and hydrochloride buffer pH 2.6 which will aid in further formulation development and in vitro and in vivo studies. The Rf value of Standard and the sample (broccoli extract) are very close, concluding that sulforphane must be present. Further studies will be done on broccoli extract and will be published in another article.

REFERENCES:

1.D. Zhang and Y. Hamauzu, Food Chem., 2004; 88: 503–509.
2.N. Bellostas, P. Kachlicki, J. C. Sørensen and H. Sørensen,Sci. Hortic., 2007; 114: 234– 242.
3."Broccoli" Merriam-Webster's Collegiate Dictionary 11th edition 2014; 156.
4.Bones A. M., Rossiter, J.: The myrosinate - glucosinolate system, its organisation and biochemistry. Physiol Plant, 1996; 97:194-208.
5.Cole. R.: Isothiocyanates, nitriles and thiocyanates and products ofautolysis of gluccosinolates in cruciferae. Phytochemistry, 1976; 15:759-762.
6.Fenwick, G. R., Ileaney R. K., Mawson R.: Glucosinolates: Intoxicants of plant origin, P. R. Checkee (Ed.), CRC Press, Inc., 1989; 17.
7.Zhang Y., Cho C., Pooner G. H., Talalay P.: A major inducer of anticarcinogenic protective enzymes from broccoli isolation and elucidation of structure. Proc. Natl. Acad. Sci., 1992; 89:2399-2403.
8.Kore A. M, Spencer G. F., Wallig M. A.: Purification of the w-(methyl-sulfinyl)-alkyl glucosinolate products. J. Agric. FoodChem., 1993; 41:89-95.
9.Yanaka A, Fahey J.W., Fukumoto A., Nakayama M., Inoue S., Zhang S., Tauchi M., Suzuki H., Hyodo I., Yamamoto M.: Dietary sulforaphane-rich broccoli sprouts reduce colonization and attenuate gastritis in Helicobacter pylori-infected mice and humans. Cancer Prev. Res. 2009; 2:353-360.
10.http://www.wildflowerfinder.org.uk/Flowers/C/Cabbage(Wild)/Cabbage(Wild).htm
11.Xu T., Ren D., Sun X., Yang G.: Dual roles of sulforaphane in cancer treatment, Anticancer Agents Med Chem. 2012; 12(9):1132-42.
12.Zhang Y., Kensler T. W., Cho C. G., Posner G. R, Talalay P.: Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. Proc. Natl. Acad. Sci. USA., 1994; 91:3147-3150.
13.Higdon JV., Delage B., Williams DE., Dashwood RH.: Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. Pharmacol. Res., 2007; 55:224-236.
14.Michaud Rene, Fabreguettes Georges, Bouchard Johanne, Howart David, Ameller Thibault, Foster Roy: Development and validation of a LC-MS/MS method for the quantification od D,L-sulforaphane in monkey blood.
15.Hahm E.R., Singh S.V.: Sulforaphane inhibits constitutive and interleukin-6-induced activation of signal transducer and activator of transcription 3 in prostate cancer cells. Cancer Prev. Res., 2010; 3:484-494.
16.Rausch V., Liu L., Kallifatidis G., Baumann B., Mattern J., Gladkich J., Wirth T., Schemmer P., Büchler M.W., Zöller M., Salnikov A.V., Herr I.: Synergistic activity of sorafenib and sulforaphane abolishes pancreatic cancer stem cell characteristics. Cancer Res., 2010; 70:5004-5013.
17.Dickinson S.E., Melton T.F., Olson E.R., Zhang, J., Saboda K., Bowden G.T.: Inhibition of activator protein-1 by sulforaphane involve interaction with cysteine in the cFos DNA-binding domain: Implications for chemoprevention of UVB-induced skin cancer. Cancer Res., 2009; 69:7103-7110.
18.Rudolf E., Andělová H., Červinka M.: Activation of several concurrent proapoptic pathways by sulforaphane in human colon cancer cells SW620. Food Chem. Toxicol, 2009; 47:2366-2373.
19.Sharma R., Sharma A., Chaudhary P., Pearce V., Vatsyayan R., Singh S.V., Awasthi S., Awasthi Y.C.: Role of lipid peroxidation in cellular responses to D,L-sulforaphane, a promising cancer chemopreventive agent. Biochemistry, 2010; 49:3191–3202.
20.Davidson AG (2002) Ultraviolet-visible absorption spectrophotometry. In Beckett AH, Stenlake JB, (4thedn), Practical Pharmaceutical chemistry. CBS Publishers and distributors, New Delhi, 275-278.
21.Cheng SC, Huang MZ, Shiea J.: Thin layer chromatography/mass spectroscopy, 2011; 1218(19):2700-11.
22.Harry W. Lewis & Christopher J. Moody (13 Jun 1989). Experimental Organic Chemistry: Principles and Practice (Illustrated ed.). 159–173.
23.Vogel A.I., Tatchell A.R., Furnis B.S., Hannaford A.J., Smith P.W.G.: Vogel's Textbook of Practical Organic Chemistry (5th ed.).
24.Reich, E.; Schibli A. (2007). High-performance thin-layer chromatography for the analysis of medicinal plants (Illustrated ed.). New York: Thieme.
25.http://shodhganga.inflibnet.ac.in/bitstream/10603/33055/13/13_chapter%203.pdf