

Qualitative Analysis of Cruciferous Vegetable Extracts Useful for Estrogen Metabolism for Diindolylmethane (DIM)

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Abstract

3,3'-Diindolylmethane (DIM) is a compound derived from the digestion of indole-3-carbinol, found in Cruciferous vegetables (Broccoli, Cabbage, Cauliflower,) which promotes Estrogen metabolism in females. It has been known to help in the reduction of heavy blood flow during menstruation especially in females with uterine fibroids. Dim-plus® a herbal supplement contains Vitamin E, DIM, Phosphatidly choline, Spinach, Cabbage, and Broccoli powder. The purpose of this research is to extract and identify DIM present in Broccoli, Spinach and Cabbage obtained in local markets in Lagos, Nigeria and compare it with the reference standard DIM-plus®. Identification of compounds qualitatively by TLC showed different R_f values which were compared with the reference to identify compounds with similar R_f values. Extracts were subjected to HPLC analysis to confirm the presence of DIM in the Cruciferous vegetables. Based on the TLC and HPLC analysis it was confirmed that the vegetables crude extracts contains detectable amounts of DIM.

Keywords: Cruciferous Vegetables, Diindolylmethane, Estrogen, Herbal Medicine, HPLC, Uterine Fibroids

1. Introduction

Herbal medicine which is also called botanical medicine or phyto-medicine, refers to the use of plant parts or mixtures of plants extract(s) for medicinal purposes [1]. examples of such products includes the leaves and roots of *Annona senegalensis*, stem of *Cissus populnea*, the seeds of *Garcinia kola*, the whole plant of *Momordica charantia*, the leaves and the roots of both *Newbouldea* leaves and *Rauwolfia vomitoria* [1]. Medicinal plants are defined as plants with one or more of its parts containing compounds that can be used for therapeutic purposes or used as precursors for the synthesis of various drugs. Medicinal plants contain nutrients as well as numerous biologically active compounds such as carbohydrates, proteins, enzymes, fats and oils, minerals, vitamins, alkaloids, quinones, terpenoids, flavonoids, carotenoids, sterols, simple phenolic glycosides, tannins, saponins, polyphenols etc. [2]. Sometimes the living system may require more nutrients than can be received from daily meals and hence the need for dietary supplements.

A dietary supplement is a product that contains a “dietary ingredient” intended to add further nutritional value to (supplement) the diet. It may be one, or a combination of the following substances; a vitamin, a mineral, herb or other botanical, an amino acid, a di-

etary substance used by people to supplement the diet by increasing the total dietary intake, a concentrate, metabolite, constituent, or extract [3]. Dietary supplements may be found in many forms such as tablets, capsules, soft gels, gel caps, liquids, or powders. Some dietary supplements can help ensure that you get an adequate dietary intake of essential nutrients; others may help reduce risk of disease and some others complement the functions of the body systems e.g. hormones. A hormone is a naturally occurring substance secreted by specialized cells that affects the metabolism of behavior of other cells possessing functional receptors for the hormone. A group of hormones, called estrogens, are responsible for the development of female secondary sex characteristics; the development of the breast tissue and the proliferation of the uterine lining. Estrogen helps prepare the body for ovulation. Excess oestrogen is common amongst women in North America.

Stress, caffeine intake, synthetic estrogens in birth control pills and hormone replacement therapy and xeno-estrogens from cleaning products, plastics and cosmetics are among some of the causes of excess levels of estrogen in the body. Due to these environmental factors, many women suffer from “Estrogen Dominance”. [4]. This manifests as stubborn weight gain, anxiety, premenstrual symptoms of breast tenderness, acne, irritability, fatigue and brain

fog. Estrogen dominance can contribute to worsening of health conditions such as infertility, fibrocystic breast, repeated miscarriages, uterine fibroids and endometriosis as well as increase the risk of developing certain cancers. Estrogen detoxification can be achieved effectively through a healthy diet that aims at improving estrogen clearance in the liver and regulation of the action of estrogen at the cellular level. Diets which can be taken includes Cruciferous vegetables; cabbage, cauliflower, broccoli, brussel sprouts, kale, spinach, collard greens and other leafy greens are rich in a nutrient called indole-3-carbinol, or 13C. Indole -3-carbinol gets converted to Di Indolyl Methane (DIM) in the body, which is responsible for clearance of excess estrogens in the liver .

Diindolylmethane is found in many Brassica vegetables through the parent compound glucobrassicin. Ingested glucobrassicin is catalyzed via the enzyme myrosinase (stored in vegetables) and turns into indole-3-carbinol which is rapidly digested into both DIM and various other metabolites in the human stomach via acid mediated condensation reactions [5]. We aim to carry out quality assurance chemical analysis of a known medicinal dietary products/supplement; DIM-plus purported to contain *Brassica oleracea* var. *italica*, *Brassica oleracea*, and *Basella alba* using phytochemical analysis including High Performance Liquid Chromatography (HPLC).

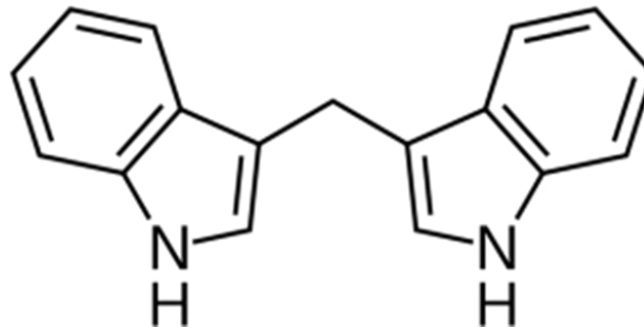


Figure 1: Chemical structure of Diindolylmethane (DIM)

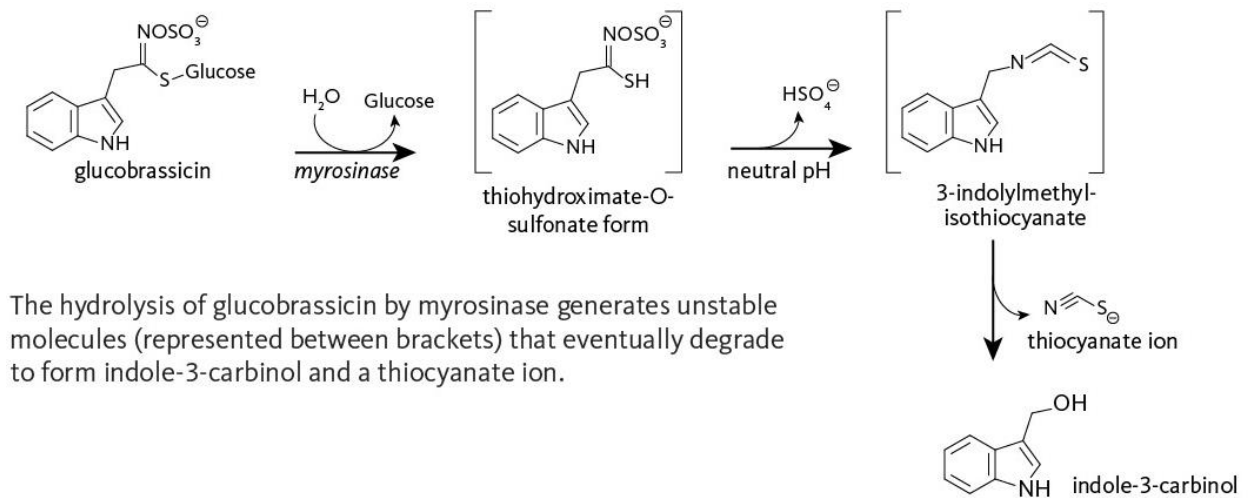


Figure 2 Bio synthesis pathway of Indole 3-carbinol [6]

DIM has been implicated in modifying pre-existing estrogen steroids into other metabolites [7]. The process of 2-hydroxylation, likely secondary to aryl hydrocarbon receptor (AhR) activation, may increase the ratio of 2-hydroxyestrone to 16 α -hydroxyestrone which is thought to be a less estrogenic profile of estrogen steroids. The processes of 4-hydroxylation and 16-hydroxylation do not appear significantly affected.

2. Methodology

2.1 Plant Materials: *Brassica oleracea* var. *italica* (Broccoli) BOI, *Basella alba* (Indian spinach) BA, *Brassica oleracea* (wild cabbage) BO were purchased fresh from the vegetable garden city, Idi-araba. The species were authenticated at the Department of Botany, University of Lagos, Akoka, Lagos state, Nigeria. With authenticated number: *Brassica oleracea* var. *italica* BOI (LUH 7587), *Brassica oleracea* BO (LUH 7589), *Basella alba* BA (LUH 7588). The plants were oven dried at 40°C, then reduced to powders using an industrial grinder. BOI, BO and BA powders were

macerated in 80% Methanol and extracted with intermittent swirling. The dried extracts were collected, weighed and preserved in a dry sample bottle until further use.

2.2 Preparative Thin Layer Chromatography (PTLC)

Preparative TLC was carried out on silica gel coated plates (Merck aluminum sheet silica gel 60F.254), the fractions were separated using the solvent mixture as Hexane: Ethyl acetate (80:20 %) After drying the plates, visualization was achieved at UV 254nm and 366nm and spray reagent containing distilled water 8%, Chilled acetone 2%, Perchloric acid 1%, Anis aldehyde 0.5% (8: 2: 1: 0.5)

was used. After spraying, heat was applied to dry at about 110 C for 3-5 minutes and the colored spots obtained were, recorded and the Rf was calculated. The TLC plate was observed under UV-light at 366nm and 254nm wavelengths.

2.3 HPLC Analysis

The column used is Zorbax XDB of 5 μ . The mobile phase was acetonitrile: water (40 : 60)% using isocratic runs at flow rate of 0.5/minutes. The detector used is UV at wavelength of 254 nm and the column dimension is 150 \times 4.6 m.

3. Results

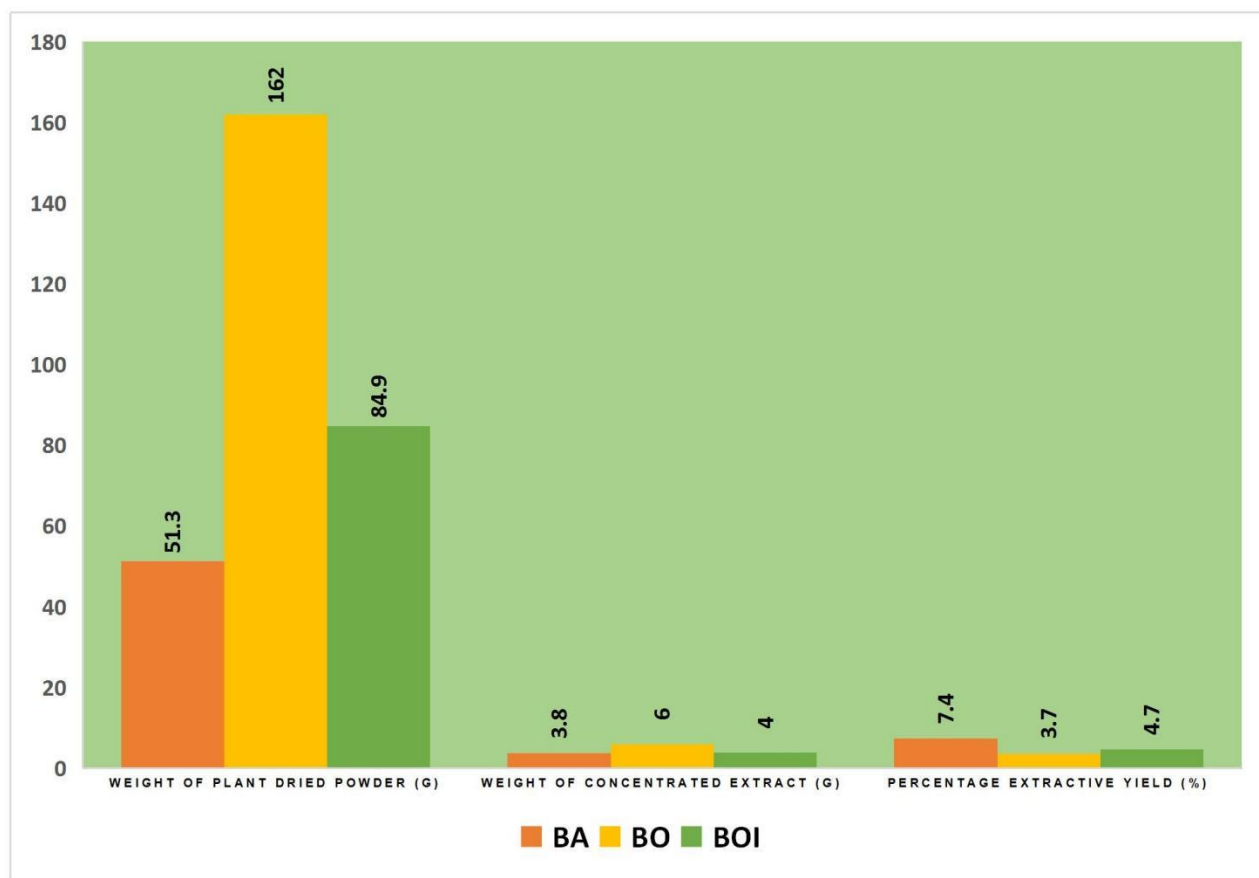


Table 1: Extractive Values of Crude Extracts of Selected Vegetables

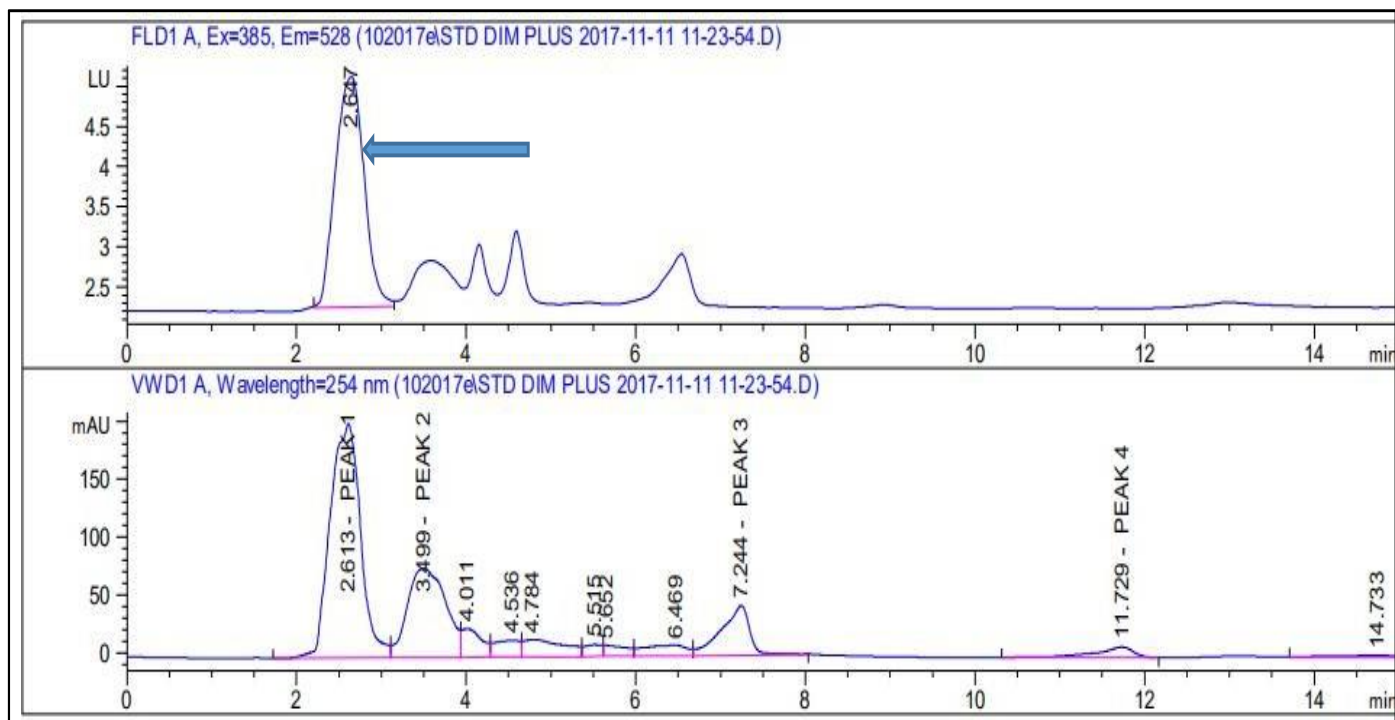


Figure 3: Dimplus Calibration Curve Obtained from HPLC. Arrow: DIM peak

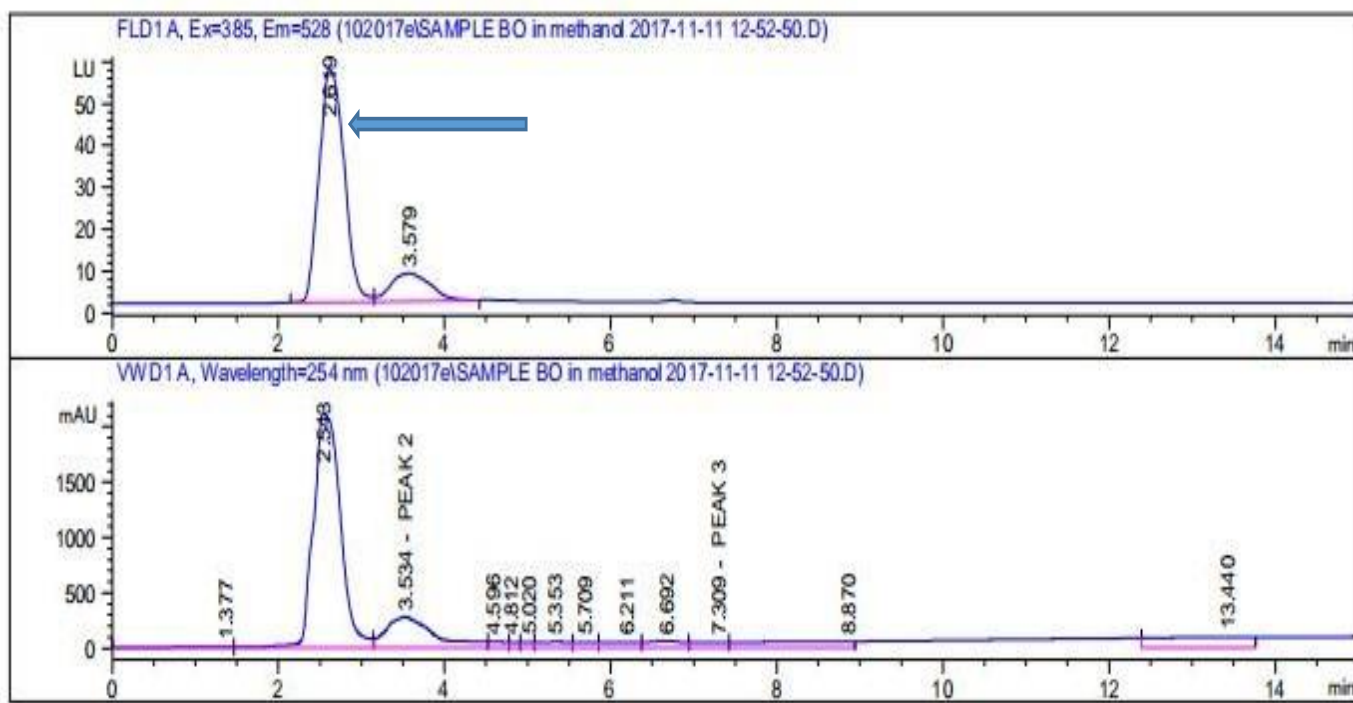


Figure 4: Brassica Oleracea BO Calibration Curve Obtained from HPLC. Arrow: DIM peak

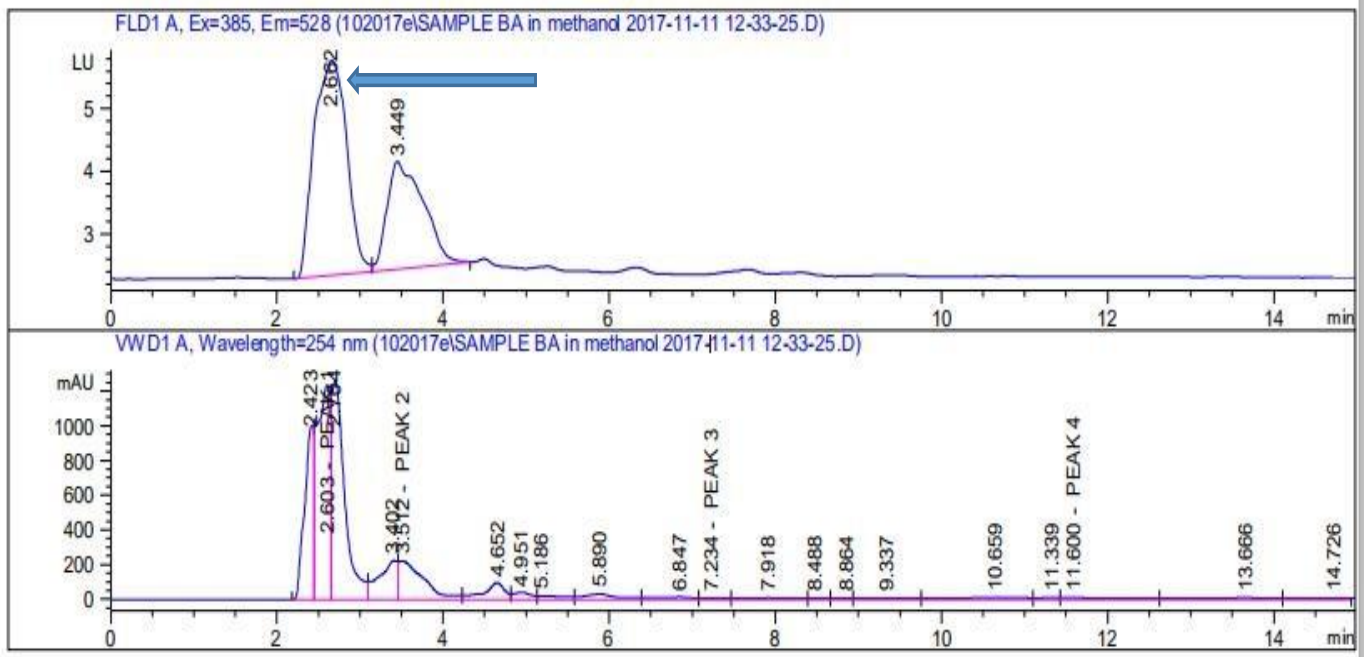


Figure 5: *Basella Alba* BA calibration Curve obtained from HPLC. Arrow: DIM peak

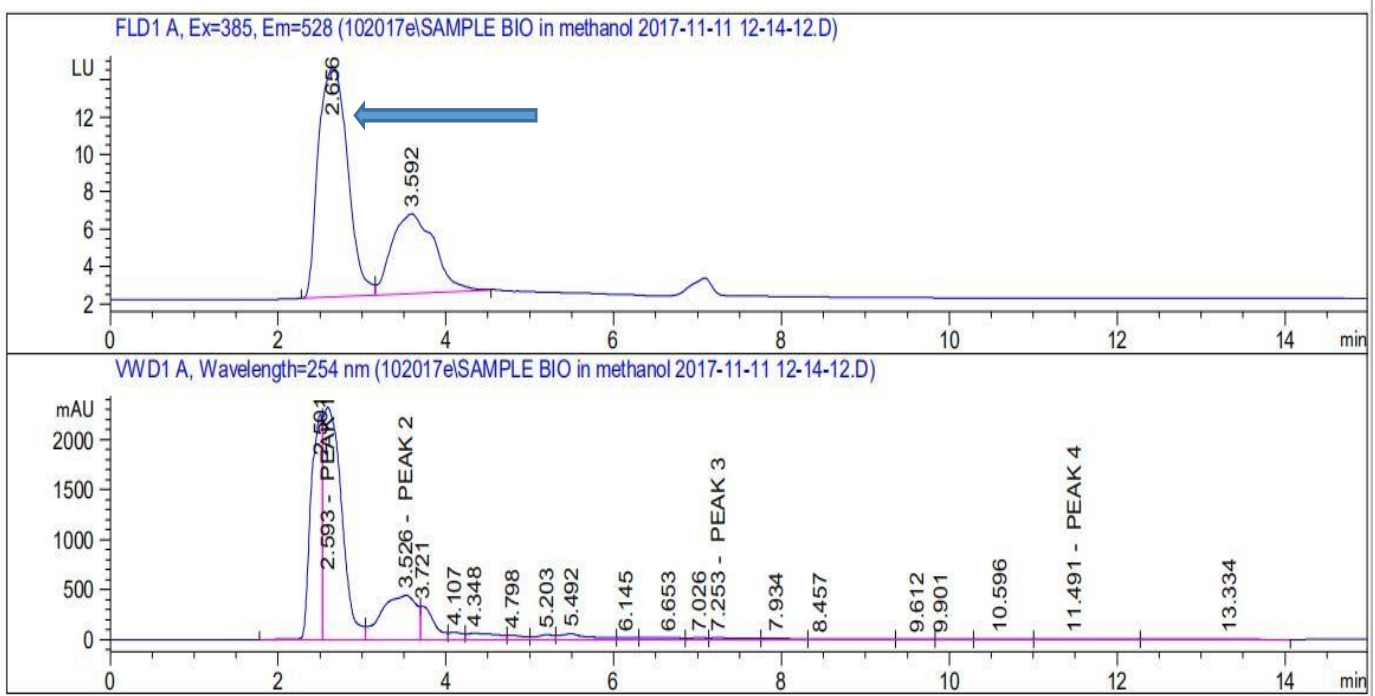


Figure 6: *Brassica Oleracea* var. BIO Calibration Curve obtained from HPLC. Arrow: DIM peak

4. Discussion and Conclusion

Vegetable consumption has always been known to be healthy and nutritious. In recent studies, the added value of phytoconstituents of vegetables has made the consumption doubly useful. DIM, an alkaloid is known for its many useful bio-activities in humans [8]. The discovery of its presence in locally sourced cruciferous vegetables in Tropical Africa is therefore worthy of note. The HPLC

analysis of the extracts of BO, BOI, BA, VE, DP and DI (Figures 3-6) showed that each of the plant extracts contained DIM. From these results, Table 1 shows that the extractive yields are small hence the necessity for frequent consumption of the vegetables in order to have significant health impact. This was already noted as the nanoparticles of DIM are already being formulated in order to increase the concentration consumed it can be concluded

that the qualitative analysis of the methanolic extracts of *Basella alba*, *Brassica oleracea*, *Brassica oleracea* var *italica* successfully showed presence of DIM and their consumption is whole heartedly encouraged for great nutrition and health [9-69].

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