EXTRACTION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS AND THEIR EFFECT ON BIOLOGICAL ACTIVITY

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Abstract

Objectives: No systematic way of extraction of bioactive compounds in the plant materials is universal as both traditional and modern techniques are effective depending on a number of decisive factors. They are the chemical composition of the plant matrix, the character of the bio active compounds and the extent of scientific knowledge and skill used in the extraction extracting the process. The main objective of the research work is extraction and characterization of bioactive compounds and their effect on biological activity. **Methods:** Extraction was done by solvent of 100ml of 30, 60 and 90 % Ethanol was prepared and keep still in the extractor. Purification of extract (onion, garlic and vinca) was done with the help of Column Chromatography. Identification of collected fractions was done with the help of Thin Layer Chromatography. The structure of isolated extract was elucidated by FTIR spectroscopy. **Result:** Results show that plant extracts show the better results as compared to standard. Show better results. Conclusion: From the study it was concluded that the Quercetin, Allicin and vinblastine show the better result and better effect on biological activity.

Keywords: Extraction, Bioactive ingredients, Quercetin, Allicin and vinblastine

1. Introduction

Bioactive compounds are natural products in plants, animal organisms and microorganisms which have favourable biological activities in living things. They are not even essential nutrients but play a very crucial role in health promotion and disease prevention. The common ones are polyphenols, flavonoids, alkaloids, saponins, terpenoids, and carotenoids. These products have various properties; which include antioxidant, anti-inflammatory, antimicrobial anticancer, and anticancer properties.[1,2] They find a broad application in pharmaceuticals, functional foods, cosmetics and nutraceuticals. The sources are fruits,

vegetables, herbs, grains and marine life. Critical extractive methods are of great importance in extracting them.[3,4]

Quercetin is a flavonoid that is determined naturally and is found abundantly in onions especially onion skins and red or yellow types. It is said to have great antioxidant activity, anti-inflammatory effect, and antihypertensive effect. The compound is important in neutralization of free radicals and shielding cells against oxidative harms. Onions are a good food source of quercetin, where mostly found in the form of quercetin glycosides. Depending on the type and processing and preparation method, the level of quercetin varies in onion. Quercetin can be sourced in onions to be utilized in future nutraceuticals and therapeutic products to help in managing disorders such as cardiovascular diseases, diabetes, and cancer. In general, onions can be viewed as a very basic natural quercetin source and can be useful in dietary as well as pharmaco applications.[5,6]

Allicin is a sulphur-based organic compound produced when garlic (*Allium sativum*) is chopped or crushed, which turns the amino acid alliin to allicin in the presence of alliinase. It is attributed to the unique smell of garlic and most of its health benefits. Allicin has broad spectrum antibacterial activity in that it acts on bacteria, viruses and fungi. It is also antioxidant, anti-inflammatory agent, anticancer agent, and cardioprotective agent. Allicin however is very unstable and easily breaks down into other sulfur compounds, which may also have a therapeutic effect on garlic. Its inconstancy causes restriction in direct therapeutical exact utilization yet preparations in capsules and controlled distribution systems are under research as approaches to settle on its dependability and engross availability. The raw garlic or one lightly cooked is recommended in everyday use because of the presence of allicin.[7,8]

Vinblastine is a strong alkaloid that can be derived out of a Madagascar periwinkle plant. It is a popular chemo agent in the treatment of many cancers. The mechanism of action of vinblastine is that it connects to tubulin and suppresses the formation of the microtubules and consequently stops cell division and causes apoptosis of high proliferating cancer cells. Its preparation is a complicated process of biosynthesis in plant and its extraction and purification are critical operations. Vinblastine might also induce side effects including bone marrow suppression even though it has therapeutic effects and hence it should be administered cautiously.[9,10]

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2. Materials and Methods

2.1 Preparation of extract

In this experiment, 30, 60 and 90 % ethanol solutions were prepared and added to Soxhlet extractor. The standard amount of 15 grams of the powdered plant matter (onion, garlic and vinca) was weighed inside a filter paper thimble which was inserted into the extractor. Using each ethanol concentration, extraction was done about eight hours. The extracts were set

aside and cooled, then filtered and evaporated on a water bath at a temperature of 50^0C to give dried residues. These dried extracts were kept at 4^0C further to be analysed and used.

2.2 Quantification of extracts

The weight of 10 mg of the standard and sample was put into a 100 ml volumetric flask and approximately 50ml of methanol was added as the diluent. To prepare the mobile phase we mixed filtered and degassed methanol and water in the ratio of 70:30. Sonication of the solution was done on the five-minute time interval, which was cooled at room temperature and adjusted to the appropriate volume using a diluent. The column was loaded with blank diluent, standard solution, first test sample solution one by one at time. Chromatographic conditions and chromatographed it at 228 nm.

2.3 Purification of extract

The extraction was cleaned up by column Chromatography. The solid phase consisted of silica gel and ethyl acetate and the blank column was washed with ethyl acetate 10 minutes. A cotton pad would be placed at the bottom of the column and tightly pack the silica gel (stationary phase) which was moistened using ethyl acetate (mobile phase). About the terminus of the column a collecting tube or flask had been placed, to receive the elute. The stationary phase was packed with five grams of extracts added on top. We began to elute using the solvent-Hexane: Ethyl Acetate (80:20) and switched the polarity of the solvent, the polarity being 70:30 to 10:90 at the flow rate of 10 ml/min.

2.4 Identification of extract

To determine what the collected fractions were, we resorted to the Thin Layer Chromatography. We pipetted the obtained fractions by using capillaries on TLC plates (10x6 cm). The toluene: ethyl acetate: methanol (4:0.5:0.5) solvent system was used in TLC. The plates were then placed in a room full of iodine vapours so that the color of the spot may be viewed (yellow). We sprayed the formed plate with a 5% ethanolic solution of ferric chloride so that we could observe what color the dots were in the sample as well as in the standard Quercetin. We determined the retardation factor (Rf value) of the extracted sample and compared it against the standard present as well. We calculated the values of Rf of the spot.

2.5 Characterization of Isolated extract

The FTIR spectroscopy also indicated with clarity what the structure of separated extracted material was. A sample on a holder containing 2µg was placed in the beam of the IR source. The detector interprets an analog signal and converts it into a spectrum. Signals were examined on a computer to determine the peaks and a graph recording was created. The standard and isolated extract were recorded using FTIR spectra at 400 to 4000 cm^{-1}.

3. Results

3.1 Quercetin from Onion Extract

Quercetin was detected in Onion at the retention time in between 3.9 to 4.2 min by HPLC and Quercetin yield was showed. HPLC chromatograms of standard and samples are presented. Soxhlet extraction showed lower Quercetin yield with 30% EtOH. Quercetin yields significantly increased with Ethanol concentration and power level.

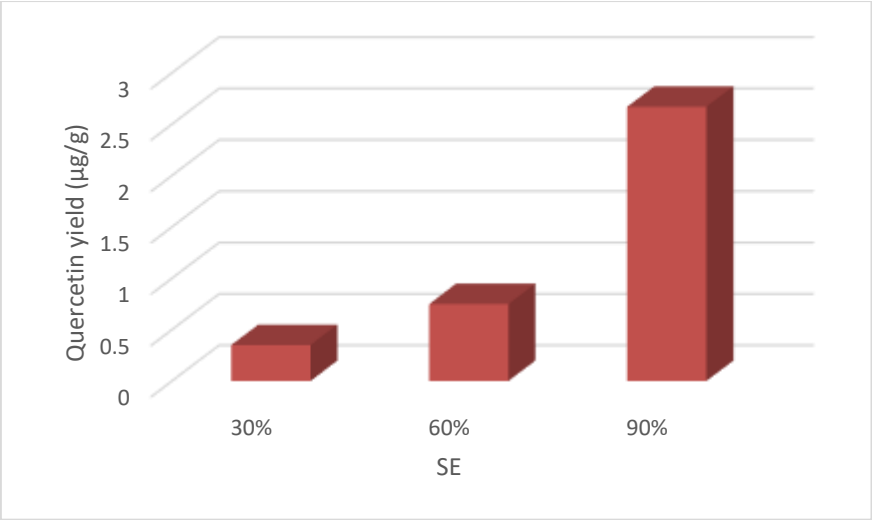

Figure 1. Yield of Quercetin in Onion skins by Soxhlet extraction method

3.1.1 Chromatogram of Onion extract prepared by Soxhlet extraction and standard Quercetin

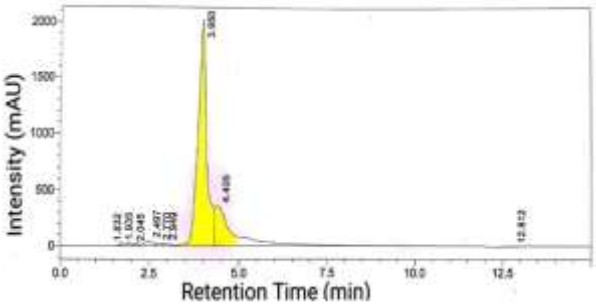

Figure 2. Standard Quercetin

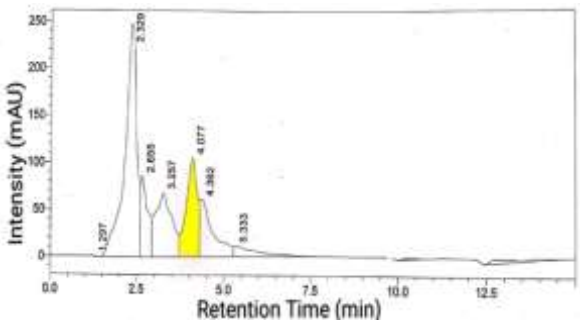

Figure 3. 30 % EtOH by Soxhlet

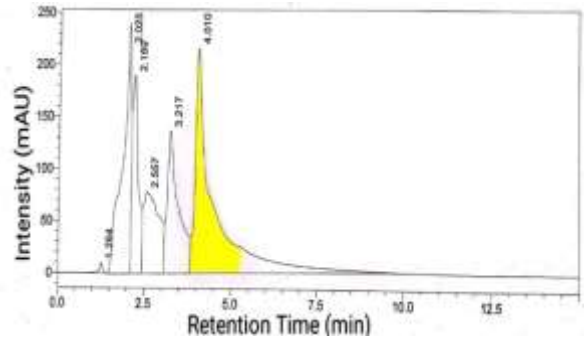

Figure 4. 60 % EtOH by Soxhlet

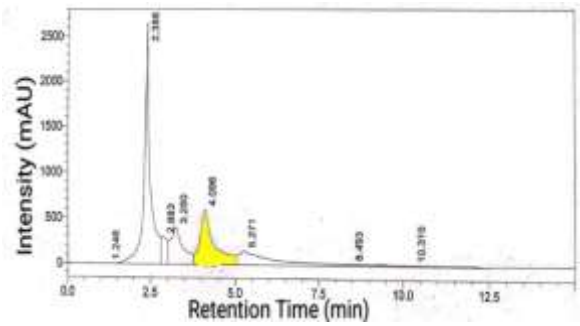

Figure 5. 90 % EtOH by Soxhlet

3.1.2 Purification and Identification of Quercetin

On drying fractions yielded yellow colored powder, gave positive test for Quercetin. Onion extract gave the highest quantity of Quercetin.

Figure 6. identification of quercetin

3.1.3 FTIR of Quercetin

The isolated Quercetin was structurally characterized using FTIR spectroscopy methods.

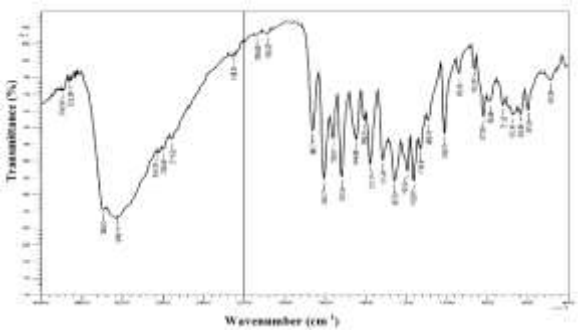

Figure 7. Standard Quercetin

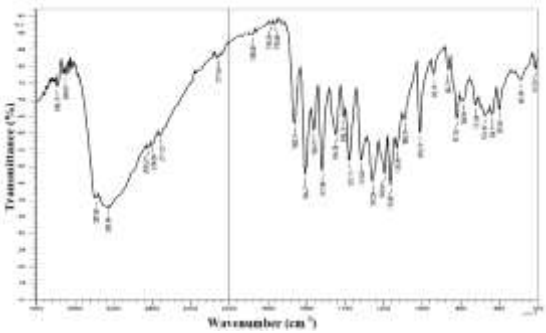

Figure 8. Isolated Quercetin from Onion

3.2 Allicin from Garlic Extract

This study claim, fresh garlic was extracted by Soxhlet extraction method with three solvents distilled water, 1:1 ethanol-water mixture, and 100 percent ethanol. The two-hour procedure of the extraction process. Depending on which solvent you use, the yields of that garlic extract would normally be between 0.1 to 2 grams. The best extract yield was obtained using the mixture of ethanol and water as a solvent because it has moderate polarity that makes it effective in dissolving various compounds. Conversely, lower quantities were obtained in distilled water and 100 percent ethanol, marking the effect of polarity on the solvent on extraction efficiency.

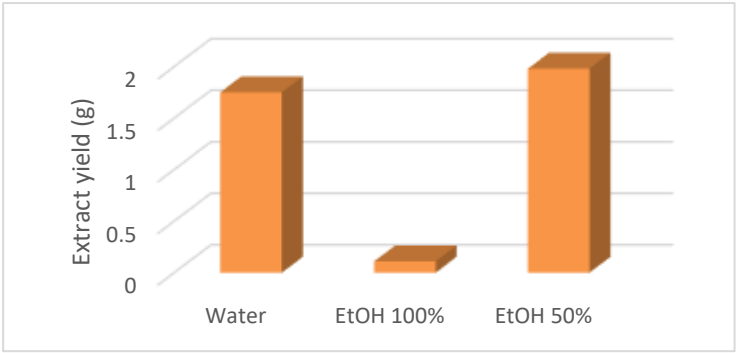

Figure 9.Yield of Allicin from garlic by Soxhlet extraction method

3.2.1 Chromatogram of Allicin

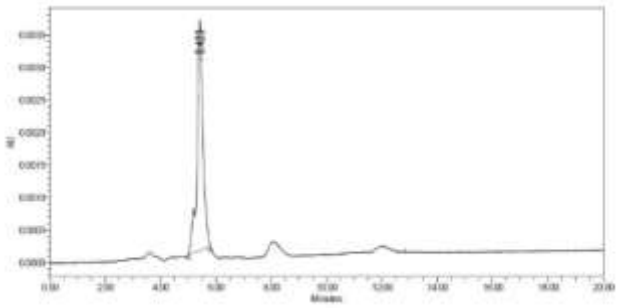

Figure 10. Allicin standard at concentration

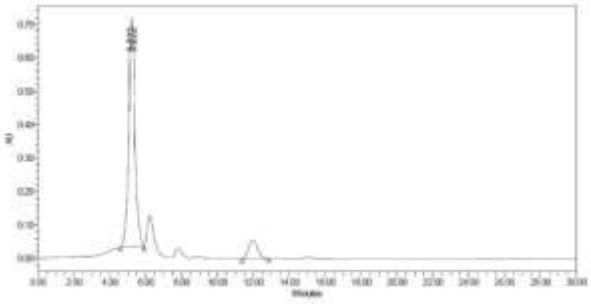

Figure 11. garlic extract consisting of allicin

3.2.2 Purification and Identification of allicin

The chromatogram conditions used in the HPTLC analysis further elucidate the analytical method employed to characterize the allicin content in the final product. The mobile phase providing optimal conditions for the separation and detection of allicin on the chromatographic plate.

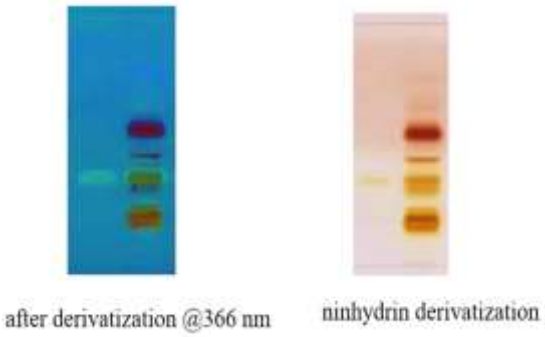

Figure 12. TLC of Allicin

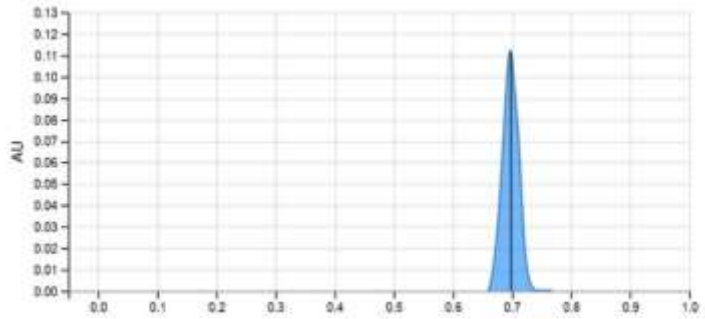

Figure 13. Allicin peaks by HPTLC

3.2.3 FTIR of Allicin

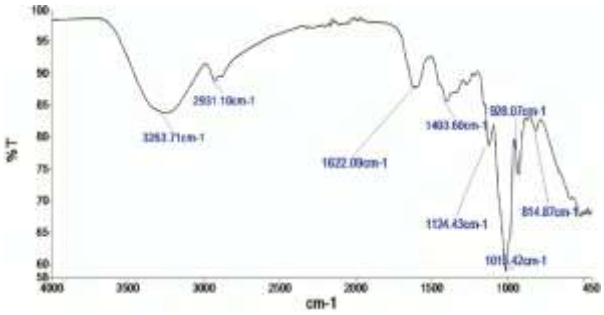

Figure 14. FTIR Spectra of Standard Allicin

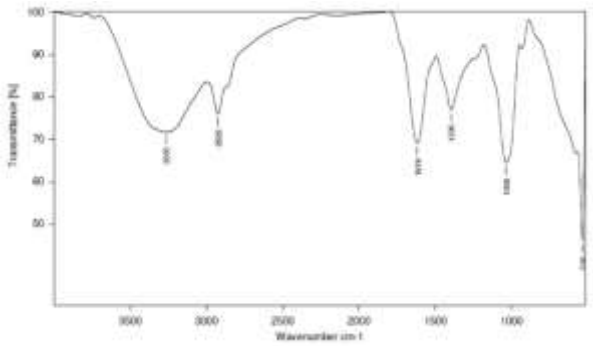

Figure 15. Isolated Allicin from garlic extract

3.3 Vinblastine from Vinca

As it can be seen in the table, in 15 hours the most concentrate obtained in the Soxhlet extraction was 19.67 mg of 30 grams of plant material. This is an indication of how the use of longer extraction time contributes to better recovery of the plant constituents through Soxhlet method.

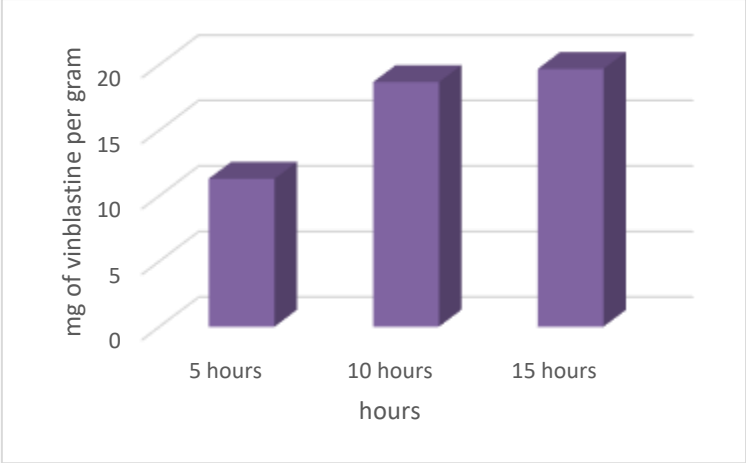

Figure 16. Soxhlet extraction for the extraction of Vinblastine

3.3.1 Chromatogram of Vinblastine

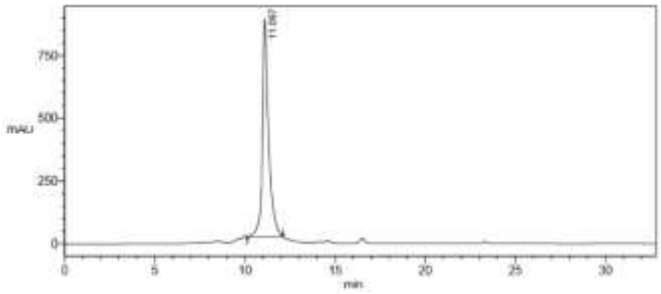

Figure 17. Chromatogram for Vinblastine standard

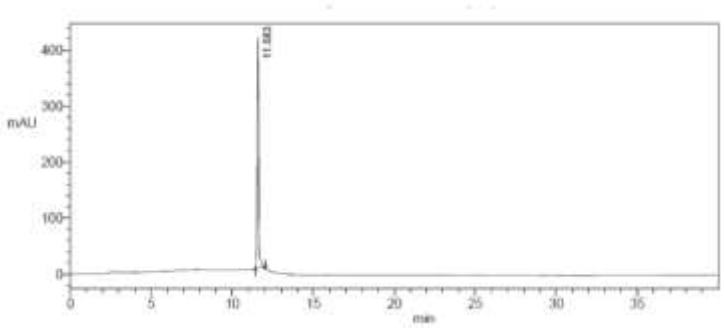

Figure 18. Isolated Vinblastine

3.3.2 Purification and Identification of Vinblastine

Figure 19. TLC chromatogram at 225 nm

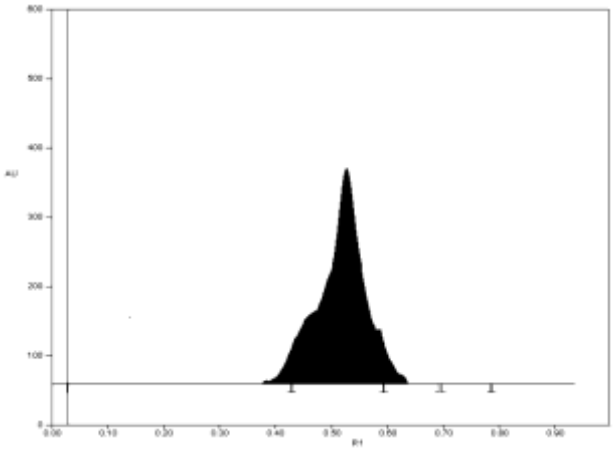

Figure 20. HPTLC chromatogram of Vinca rosea extract containing vinblastine

3.3.3 FTIR of vinblastine

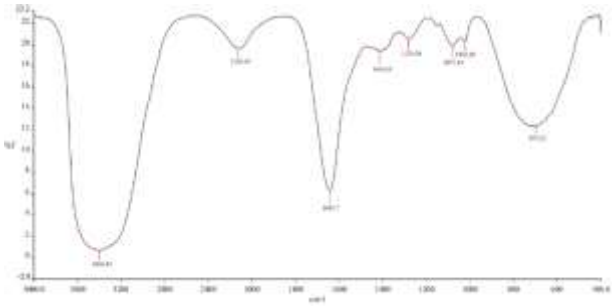

Figure 21. FTIR Spectra of Standard vinblastine

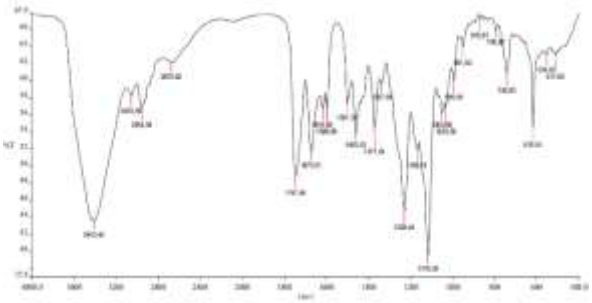

Figure 22. Isolated vinblastine from vinca extract

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4. Discussion

Quercetin was detected in Onion at the retention time in between 3.9 to 4.2 min by HPLC and Quercetin yield was showed. HPLC chromatograms of standard and samples are presented. Soxhlet extraction showed lower Quercetin yield with 30% EtOH. Quercetin yields significantly increased with Ethanol concentration and power level. From the TLC analysis, Quercetin was identified. It showed spots which coincided with that of the reference Quercetin.it illustrates that the garlic extract was obtained using the Soxhlet extraction with various solvents, i.e., distilled water, ethanol– water (1:1), and 100% ethanol. Generally, the used solvents yielded between 0.1 and 2g of extract. The derivatization reagent utilized in the analysis comprised 0.6 g of ninhydrin dissolved in a mixture of isopropanol and glacial acetic acid (190:10), facilitating the visualization of allicin spots on the plate. From the FTIR of methanolic extract showed the presence of functional groups. Plainly the greatest measure of concentrate acquired was 19.67mg per 30 gram of plant material following 15 hours of extraction. The homogeneity of the purified compounds was confirmed by HPLC analysis, which showed a single, symmetrical peak with RT 11.097 min and 11.53 min on C18 symmetry column of vinblastine. For the analysis samples were spotted in TLC plate, vinblastine has Rf of 0.49.

5. Conclusions

Quercetin was detected in Onion at the retention time in between 3.9 to 4.2 min by HPLC and Quercetin yield was showed. HPLC chromatograms of standard and samples are presented. Soxhlet extraction showed lower Quercetin yield with 30% EtOH. Quercetin yields significantly increased with Ethanol concentration and power level. Quercetin was identified. Rf value for standard Quercetin was approximately similar with Quercetin isolated from samples which confirms the purity of isolated compound. Under Soxhlet extraction, distilled water, ethanol-water mixture (1:1) and 100 percent ethanol were the solvents used in an amount of 200 mL. The extract yield was 0.1 to 2 grams. The best yield was realized in the ethanol-water mixture because the ratio was well-balanced in terms of polarity, which increases solubility. On the other hand, less was obtained using deionized water and 100 ethanol confirming the role played by the polarity of the solvent during extraction. The chromatogram conditions used in the HPTLC analysis further elucidate the analytical method employed to characterize the allicin content in the final product. chromatogram conditions

ensure the reliability and accuracy of the HPTLC analysis in determining the allicin concentration in the extracted and purified product. The study employed FTIR, HPLC, TLC as methods of undertaking chemical composition and purity analyses of vinblastine. FTIR spectrum detected the presence of different functional groups with significant peaks obtained at 3263.71 cm -1 (COOH group), 2931.10 cm -1 (NH2 group), 1622.09 cm -1 (C=C group) and 1015.42 cm -1 (related to oxygen functional groups) and 928.07 cm -1 (C-S bond). Purity of the compound was determined by HPLC analysis which gave a single sharp and symmetrical peak with retention time at 11.097 and 11.53 minutes on the used C18 symmetry column to confirm its purity and uniformity and indicating zero impurities. Also, TLC analysis revealed that Rf value of vinblastine was 0.49. The transparency and space between the spots as well as the high peaks resolution showed that the sample did not have any direct impurities. On the whole, the overall FTIR, HPLC and TLC results proved the identity and the functional structure of the vinblastine compound and ensured the purity of the compound as well.

Abbreviations

The following abbreviations are used in this manuscript:

FTIR	Fourier transform infrared spectroscopy
HPLC	High Performance liquid chromatography
TLC	Thin layer chromatography

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