



Comparative Phytochemical Profiling of Garlics (*Allium sativum* L.) and Onion (*Allium cepa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author SNAMZ designed and performed the study and wrote the manuscript. Author NIO designed and supervised the study, involved in the lab works and edited the manuscript. Author IAW co-supervised and provided guidance in lab works and author HFM assisted and involved in the conduction of study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This research aims to perform the extraction of garlic samples and onion as well as to carry out qualitative phytochemical screening by using Thin Layer Chromatography (TLC) and phytochemical analyses for the detection of carbohydrates, flavonoids, and alkaloids.

Study Design: Thin Layer Chromatography (TLC) and some preliminary qualitative phytochemical tests to detect the presence of carbohydrates and reducing sugars, flavonoids, and alkaloids were carried out to compare and identify the chemical compositions in black and white garlic as well as onion.

Place and Duration of Study: Pharmaceutical Chemistry Department laboratory, Faculty of Pharmacy Universiti Teknologi MARA Puncak Alam. The whole study was conducted and completed in 12 months.

Methodology: Black garlic (BG) is derived from raw white garlic (WG) that is modified by treating it with highly controlled temperature and humidity. In addition to the black and white garlic powder samples, this comparative study was also performed on smoked garlic and onion. The extraction of

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black garlic (BG), white garlic (WG), crushed smoked garlic (CSG), and onion was performed by using ethanol and ethyl acetate. The comparative phytochemical profiling was conducted by using TLC and qualitative phytochemical analyses were done using standard methods.

Results: The presence of amino acid in the ethanolic extracts of black garlic (BG), crushed smoked garlic (CSG), and onion were detected. Ethyl acetate extracts might contain triterpene. Phytochemical screening tests showed the presence of reducing sugars and alkaloids in ethanolic extracts of black garlic (BG), while white garlic (WG) contains flavonoids, alkaloids, and a trace amount of carbohydrates. Both extracts of onion showed the presence of carbohydrate, reducing sugars and flavonoids. Meanwhile alkaloids are only detected in ethanolic extract of onion.

Conclusion: The extracts of garlicks and onion samples in this study had shown appreciable presence of some important phytochemical compounds in *Allium* species such as amino acids, flavonoids, alkaloids and carbohydrates.

Keywords: *Phytochemistry; pharmacognosy; Allium sativum; Allium cepa; black garlic; onion.*

1. INTRODUCTION

Allium vegetables are recognized among the most widely used and important crops around the world. Among the most commonly used species from the Allium genus are garlic (*Allium sativum* L.) and onion (*Allium cepa* L.). Allium vegetables are also acknowledged to be therapeutically important plant species. It has been verified in a number of studies that both garlic and onion possess a broad range of pharmacological activities such as anti-inflammatory, anti-cancer, anti-microbial, anti-oxidant, anti-asthmatic as well as neuroprotective [1-5]. These various pharmacological activities are attributed to the phytochemical constituents [6] which contains a high amount of organosulfur bioactive compounds (principally alliin [(+)-S-allyl-L-cysteine sulfoxide] and gamma-L-glutamyl peptides), saponins, and antioxidants (flavonoids, polyphenols) [1, 7].

Naturally, white garlic is having pungent scent, and unpleasant flavor. Thus, owing to this unpleasant property, it was modified in different forms to be more palatable [8]. One of the forms of modified and processed garlic is black garlic in which thermal processing is used. In this process, the temperature within the range of 60-90°C is exerted on the garlic to produce black appearance which requires certain period of time to complete this thermal exposure process [9,10]. This kind of modification process is originated from Asian countries before just recently introduced to other regions of the world few decades ago [10,11].

The process to transform white garlic to black garlic involved Maillard reaction [8] and results in different organoleptic properties of black garlic as compared to raw white garlic in terms of texture, flavor and odour apart from the difference in

colour. Black garlic has been identified to be more tender and chewy in texture, sweet and less odorous [12]. During the thermal exposure process, the breaking down of polysaccharides is taking place thus producing monosaccharides reducing sugar, fructose and the reaction with amino acid will result in the browning hue of the black garlic [13]. Apart from the difference in physicochemical features, black garlic also differs from raw white garlic in terms of its antioxidative compounds [10], sugar, pyruvate, and ash contents [12,14].

The present study is primarily aimed to compare the phytochemical profiles of these two forms of garlicks (fermented black garlic and raw white garlic). In addition to different garlic samples, a sample of onion extract (*A. cepa*) was also assessed to compare the bioactive compounds. This study was performed to carry out extraction of the samples by using ethanol and ethyl acetate as the solvents. Thin Layer Chromatography (TLC) and different reagents were used in the present study to detect phytochemical compounds such as sulphur-containing amino acid S-allyl cysteine (SAC), carbohydrates, flavonoids, and alkaloids.

2. MATERIALS AND METHODS

2.1 Preparation of the Sample

Four samples were used in this study: black garlic (BG), white garlic (WG), crushed smoked garlic (CSG), and onion powder. BG was obtained from the fermented black garlic homemade supplier. The peeled fermented BG cloves were heated in the oven at 75°C, three times and were ground to turn BG into powder form. The powdered fermented black garlic was sieved. As for WG powder, smoked garlic, and

onion powder were purchased from local market. The smoked garlic were crushed by using garlic crusher to obtain the crushed smoked garlic (CSG). All of the samples were weighed approximately 5 grams using analytical weighing balance.

2.2 Preparation of Allium Extracts

Two extraction solvents were used which are 100 ml of ethanol and ethyl acetate. The samples were soaked for 1 hour in the extraction solvents within water bath to intensify the extraction. Then, they were filtered by using Whatman No.1 filter paper. The filtrates were evaporated using rotary evaporator. The crude extracts were collected into evaporating dish to be used in the next assays.

2.3 Thin Layer Chromatography (TLC) of Allium Extracts

The method for TLC assay is adapted from Itakura et al. [15] and Belemkar et al. [16]. The polar mobile phase was prepared by using glacial acetic acid: propanol: water: ethanol (20:20:20:20 v/v/v/v), while nonpolar mobile phase was prepared using a mixture of hexane and acetone (70:30), marked up to 100 ml. The TLC silica gel 60 plates were used as stationary phase. TLC plates then were observed under UV light (254 nm) and R_F values were calculated. For the detection of amino acid derivative compounds, ninhydrin and anisaldehyde were used as spraying agent.

2.4 Qualitative Phytochemical Screening of Allium Extracts

Both extracts of the samples were used to qualitatively determine several bioactive compounds such as carbohydrates, flavonoids, and alkaloids. The presence of these compounds were determined by using method described in [17] with some adjustments and modifications.

2.4.1 Test for carbohydrates and reducing sugars

1 ml of Benedict's reagent were added into 1 ml extracts solution to test the presence of carbohydrates (monosaccharides and disaccharides). The mixture was boiled for 3 to 5 minutes and the colour changes were observed. Barfoed's reagent was used to detect any presence of monosaccharides or reducing sugars.

2.4.2 Test for flavonoids

0.2 g powder of black and white garlic, CSG, and onion powder were dissolved in 1% sodium hydroxide (NaOH). Then, 10% of hydrochloric acid (HCl) will be added and any colour changes will be observed.

2.4.3 Test for alkaloids

2 ml of the sample solutions were mixed with 1 ml of 1% HCl. The mixtures were heated in water bath for 2 minutes. The mixtures were filtered by using Whatman No. 1 filter paper. Six drops of Dragendorff reagent were added into 1 ml of the filtered solutions. The changes in colour were observed.

3. RESULTS AND DISCUSSION

3.1 Thin Layer Chromatography of Allium Species Extracts

As presented in Fig.1, the TLC of the ethanol extract (left plate) using polar mobile phase showed the development of the spots under the UV light (254 nm) in BG powder, CSG, and onion powder, while WG powder did not show any spot. The spot of the BG powder appeared as tailing. The R_f value for the BG powder was 0.78 while for CSG and onion powder shared the same R_f value which was 0.85. In ethyl acetate extract using non-polar mobile phase (right), only BG powder and CSG showed the spots under UV light while the other two samples did not show any appearance of the spot. The R_f value of BG powder was 0.22 while for CSG, there were 4 spots detected; from the lowest spot, the R_f values were 0.46, 0.49, 0.60, and 0.87, respectively.

Fig. 2 shows development of the ethanolic-extract samples on the TLC plate after spraying with different spraying agents, which are ninhydrin and anisaldehyde. With ninhydrin spraying agent, all samples showed purple to brown development on the plate after air drying, except for WG powder extracts which did not show any spot. While anisaldehyde spray showed appearance of the purple to green development in all samples.

For the ethyl acetate extract, development of the samples on the TLC plate by using anisaldehyde was observed as clear and separated spots except for CSG, while development using ninhydrin reagent was undetected (Fig. 3).



Fig. 1. Spots under the UV light (254 nm) of the ethanolic extract samples (left) and ethyl acetate-extracted samples (right); for each plate, from left to right, S1 (WG powder), S2 (BG powder), S3 (CSG), S4 (onion powder)

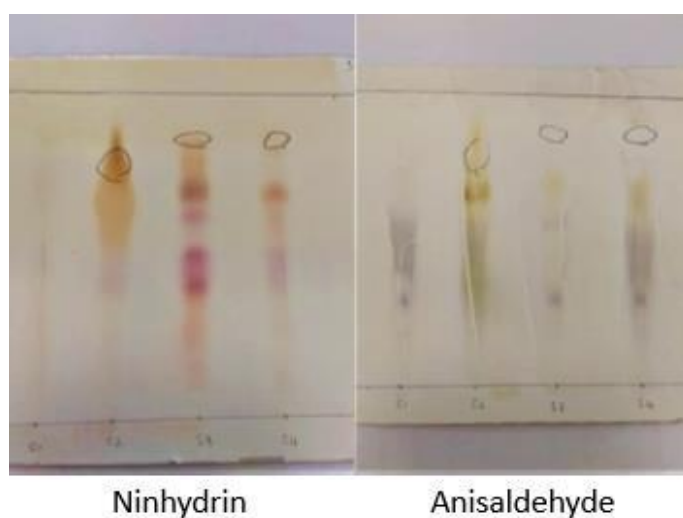


Fig. 2. TLC of different *Allium* ethanolic extract samples; for each plate, from left to right, S1 (WG powder), S2 (BG powder), S3 (CSG), S4 (onion powder), using ninhydrin reagent (left) and anisaldehyde (right) reagents

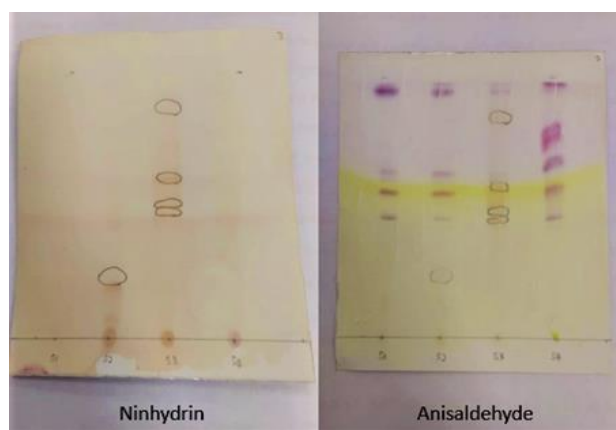


Fig. 3. TLC development of ethyl acetate- extracted *Allium* species using different reagents. For each plate, from left to right, S1 (WG powder), S2 (BG powder), S3 (CSG), S4 (onion powder)

Both solvents (ethanol and ethyl acetate) are commonly used in the research to extract the medicinal plants. In this study, powdered BG, WG, onion, and CSG were extracted by using two distinctive solvents which are ethanol and ethyl acetate. Visualisation using ultraviolet (UV) light is the quick and non-destructive method for early detection of compounds in TLC. UV light can detect aromatic and highly conjugated compounds as these compounds can strongly absorb UV radiation [18]. For the TLC in ethanol extract samples, CSG and onion have similar Rf value that might indicate that these two have same compound or different compounds with the same Rf value. The compounds also have high polarity since the RF values were high; BG (0.78), CSG and onion (0.85). In ethyl acetate extract, only BG and CSG showed spots under UV light. BG has a spot with lower Rf value than in ethanol extract indicate that this compound has high polarity. TLC showed that CSG might have a higher number of conjugated or aromatic compounds compared to BG and onion. Flavonoid and phenolic acid are examples of compound that can absorb UV light as they are commonly exists as conjugated form in the natural plants [19,20]. Thus, the compounds observed under the UV light might be flavonoid and phenolic acids. However, this is not in agreement with the study conducted by Kim et al. [21] that revealed the BG consists of high amounts of total flavonoids and phenolic acid as compared to the fresh garlic.

Ninhydrin is a non-specific, widely used spraying agent to detect the presence of primary and secondary amino acid. It reacts with free amino group to form 'Ruhemann's purple'; which gives the purple coloured spots in the presence of amino acid. This reagent alone cannot distinguish the types of amino acid, except for proline and hydroxy-proline which gives bright yellow colour [22–25]. While for amino acid Asparagine, it gives a brown colour to the sample as it has free amide group [26].

Polar mobile phase was used in conducting TLC for the ethanol extracts which was the mixture of glacial acetic acid, propanol, water, and ethanol with the same proportion. TLC results showed that there was compound revealed in ethanolic extracts upon spraying with the ninhydrin reagents, except in WG powder. The purple or violet spots formed indicates the presence of amino acids in the sample. CSG showed more and intense

purple spots compared to the other samples which might suggest that smoked garlic has a higher amino acid content than in other samples. WG powder did not showed any appearance of pink or purple coloured spot upon spraying with anisaldehyde reagent, and this might indicate that WG powder did not contain amino acid. This is in contrast with the previous studies that showed a higher amount of amino acid content in white garlic compared to the black garlic [14,27–29]. It has been reported by Czompa et al. [14] that a high amount of other amino acids such as cysteine, which is the precursor of the sulphur-containing compound, tyrosine, threonine, glycine and serine are all reduced in the black garlic production. The reason for this is probably because of Maillard reaction that occurs between amino acid and carbohydrates, in which this amino acid is consumed and produces an intermediate browning product [14,29]. This is manifested by complex formation of sugar-cysteine and sugar-tyrosine which can exhibit higher antioxidant capacity in black garlic [14].

The third purple spot identified in CSG sample might be allicin; a sulphur-containing amino acid, which indicated in the study conducted by Muoio et al. [30]. While BG did not show any purple spot upon spraying with anisaldehyde reagent, thus indicated that this mobile phase might not be suitable for the detection of S-allyl cysteine (SAC) in the BG powder. Black garlic possesses an abundant amount of water-soluble S-Allyl cysteine (SAC) which is the sulphur-containing amino acid, the primary active ingredient that contributes to the high antioxidant capacity of the black garlic. This organosulfur compound is much more stable and odourless. The amount of SAC in black garlic is five to six times higher than in white garlic [31].

SAC production from γ -Glutamyl cysteine is catalyzed by the enzyme γ -glutamyl transpeptidase. This enzyme does not act productively in dried white garlic bulb and this might be due to the location factor; the enzyme is located in the cell membrane of garlic while its substrate, γ -Glutamyl cysteine, is located in the vacuole [32], thus this explains the reason why white garlic provides a little amount of SAC content. In non-treated white fresh garlic, it contains a high amount of γ -Glutamyl cysteine that will be naturally converted into alliin by hydrolyzation and oxidization reaction. Alliin then will be

further converted into allicin which exhibit unpleasant odour and cytotoxic effect. Meanwhile, in black garlic, SAC is produced instead of alliin from γ - Glutamyl cysteine. The amount of allicin in black garlic is reduced to a lower extent due to the inactivation of enzyme allinase by the heat during thermal processing of black garlic, thus eliminate the distinct odour of black garlic [33].

The purple colour on the plate of the BG, CSG, and onion samples were all in the same distance, and this might indicate the presence of same amino acid detected in those samples. In BG sample, the spot was large as the sample applied was too concentrated. The development of the colour in the plate was similar to the study conducted by Umar et al. [34], which presented with purple colour at short distance while brown colour at higher retardation factor (Rf) value. This observation indicates that the amino acids represented by the brown colour are more polar than the purple colour since the mobile phase we used was polar.

For the ethanolic extracts sprayed with anisaldehyde reagent, purple and green spots were observed in all of the samples. The first purple spots were observed with the same Rf values in all the sample extracts except for BG as the sample was too concentrated that the tailing formed. These spots were having the same Rf value which means that they might be similar compounds and has low polarity since the Rf value was small. Anisaldehyde reagent can be used to detect the presence of saponin [15,34], which are the glycosides of

the steroids and triterpenes, and also carbohydrates [35]. According to Grande et al.[35], different colour of spots showed different compounds after heating; blue for monoterpenes, purple for triterpenes, and grey for steroids. This reagent tends to react with most functional groups and nucleophiles except for alkenes and alkynes.

Mobile phase used in ethyl acetate extraction of the samples was the mixture of hexane and acetone with the ratio of 70:30, respectively. In this extraction, the ninhydrin-sprayed plate yielded no coloured spot. Thus, this might indicate that ethyl acetate extracts were inefficient to extract the amino acids from the garlic and onion. Meanwhile, for anisaldehyde-sprayed samples showed a good separation of spots for all samples except for CSG. BG and WG samples have purple-colored spots with the similar Rf value, while onion has different additional spots compared to the other samples. The purple spots might indicate the presence of triterpenes [35].

3.2 Qualitative Phytochemical Screening of Allium Species Extracts

Preliminary phytochemical screening tests are qualitative tests which only detect the presence of the compounds without knowing the exact amount of the compound in the sample. These tests commonly utilize the reagents for the detection. This study performed a few screening tests which are Benedict's test, Barfoed's test, alkaline reagent tests, and Dragendorff test.

Table 1. Shows the result of qualitative phytochemical analysis tests for the ethanolic and ethyl acetate extracts of BG powder, WG powder, CSG, and onion powder

Phytochemical Test	Sample							
	Black Garlic Powder		White Garlic Powder		Crushed Smoked Garlic		Onion Powder	
	E	EA	E	EA	E	EA	E	EA
Benedict's Test	+	+	+	-	+	+	+	+
Barfoed's Test	+	+	+	-	-	-	+	+
Flavonoids	ND		+	+	+	+	+	+
Alkaloids	+	-	+	-	+	-	+	-

E: Ethanolic extract; EA: Ethyl acetate extract

(+): Positive/present; (-): Negative/absent; (ND): No detection

3.2.1 Tests for carbohydrates and reducing sugars

Benedict's test for ethanolic extract of BG, WG, CSG, and onion revealed the presence of reducing sugar in the samples. Ethanol extract of BG showed brick red precipitate formation and brownish green precipitate solution appeared in CSG and onion extracts. For WG, the green solution was observed. In ethyl acetate extract, BG extract showed greenish yellow precipitate, CSG, and onion extracts showed green precipitates in the solution, and WG had no colour changes. Meanwhile, Barfoed's test showed appearance of brick red precipitate in all ethanolic extract samples, except for CSG. In ethyl acetate extract, brick red precipitate was only observed in BG and onion extracts while for CSG and WG, there were no colour changes observed.

Benedict's test is the semi qualitative tests since it can detect the rough amount of reducing sugars (monosaccharides and some disaccharides) according to the colour revealed. Benedict's tests showed the presence of reducing sugar in all samples, except for WG in ethyl acetate extract. The colour changed to brick red precipitate in the BG ethanolic extract and this indicates there was a high amount of reducing sugars in the sample with more than 2.0 g% sugar concentration (36). As for ethyl acetate extract, the colour was greenish yellow precipitate which indicates a small amount of reducing sugars detected in the BG sample due to the low polarity of the solvent. While in WG ethanolic extract, only a trace amount of reducing sugar was detected and in ethyl acetate extract, no free sugar present. In CSG there were also traceable amount of reducing sugars observed.

Black garlic cloves possess a high amount of reducing sugar such as fructose (most abundant), glucose, xylose (12), arabinose, galactose, while the amount of non-reducing sugar, sucrose, is lower compared to the white garlic (7,12,28,37). The reason for this may be due to the hydrolysis of fructan into glucose and fructose during thermal processing, hence the content of fructan is lower in black garlic (7). Moreover, the degradation of other polysaccharides in black garlic may occur due to the high temperature and acidic conditions (28). Increase in acidity of black garlic throughout thermal processing may cause

hydrolysis of sucrose into fructose and glucose, which reveals the abundant amount of fructose and glucose with the lower amount of sucrose (28). This also reveals the reason of the sweet flavour of black garlic. The content of sugar also decreases if the thermal processing period was further increased (28). Onion sample also showed green precipitate in both extracts that indicates a low amount of reducing sugars; less than 0.5g% (36), in the sample. In the study conducted by Badessa et al. (38), quantitative phytochemical screening of the ethanolic extract of onion bulbs (*Allium cepa*) showed a significant amount of carbohydrates in the onion samples.

Barfoed's test can be used to detect the monosaccharides (reducing sugar) in the presence of di- and polysaccharides under acidic conditions (39). This is because of only monosaccharides can react faster in the lower pH environment to give a positive result. Further heating of the sample may cause cleavage of glycosidic linkage, resulting in the breaking of disaccharides to monosaccharides. In this study, Barfoed's test also revealed the presence of reducing monosaccharides in BG, WG, and onion for ethanolic extract, meanwhile in ethyl acetate extract, only BG and onion contains monosaccharides. In this study, CSG revealed the absence of monosaccharides in both ethanolic and ethyl acetate extracts for this test, while it showed a traceable amount of reducing sugars in Benedict's test. CSG might contain a small amount of reducing disaccharides but does not contain monosaccharides.

3.2.2 Test for flavonoids

WG powder, CSG, and onion powder showed positive results with the appearance of yellow coloration upon addition of NaOH, and turn to colourless back upon addition of HCl. While for BG powder, the presence of flavonoid could not be detected.

Due to the dark-coloured sample of BG, the presence of flavonoids could not be determined in this study. Previous studies conducted by Hee et al. [29] and Angeles et al. [40] revealed a significantly higher amount of flavonoids in black garlic compared to white garlic which are responsible for high antioxidant activity of black garlic. It was found that the amount of phenolic content in black garlic; including flavonoid content, was significantly increasing [40] throughout the

heating process until day 21, and eventually increased slightly until the aging process lasts [37]. This showed similar result in a finding that reported a high antioxidant content, which are polyphenol and flavonoid contents, in 21 days as compared to 35 days of manufacturing process [41]. Total polyphenol and flavonoid compounds may be derived from the compounds during browning reaction, such as 5-hydroxymethylfurfural (5-HMF), uridine, and adenosine, which have been observed in different fraction (chloroform: methanol) of ethyl acetate extracts of black garlic [42]. The examples of flavonoids that had been detected in black garlic are catechin, epicatechin, quercitrin, and myricetin [21]. The flavonoids also present in the onion sample. A study conducted by Abdulkadir et al. [3] revealed the presence of flavonoids in the ethanolic extract of onion sample. The extraction of essential oil from onion also showed the presence of flavonoids in the sample [43].

3.2.3 Test for alkaloids

For ethanolic extract, all of the samples showed the appearance of reddish orange precipitate upon the addition of Dragendorff reagent. While for ethyl acetate reagent, all the samples showed negative result.

Dragendorff test was used to identify the appearance of alkaloid in the samples. All the ethanolic extracts showed positive result but not in ethyl acetate extracts. This might be due to the polarity of the alkaloids present in the samples which favour the extraction in polar solvent. A number of alkaloids has been reported to present in black garlic such as 2-acetyl pyrrole which is the products of Maillard reaction [9,15] that is responsible for the pleasant aroma of the black garlic [42]. Another alkaloid found was tetrahydro- β -carboline derivatives, which is the antioxidant compound in the black garlic [28, 41]. Tetrahydro- β -carboline might be derived from the reaction between amino acid L-tryptophan and also α -oxo acid, for instance pyruvic acid, or aldehyde that were produced from Maillard reaction [28]. The finding of this study is also supported by Abdulkadir et al. whereby the qualitative phytochemical screening of ethanolic extract of onion showed positive detection of alkaloids [3].

4. CONCLUSION

Extraction and detection of potential phytochemical constituents of BG, WG, CSG,

and onion were successfully performed in this study. The extraction of the BG in this study did not show the presence of sulphur-containing amino acid. CSG extraction probably contains sulphur-containing amino acid, allicin. The preliminary phytochemical tests revealed the presence of simple carbohydrates (except for CSG) and flavonoids (except for BG) in most of the samples tested, while alkaloids were only identified in the ethanolic extracts of the samples. The results from this research are useful to serve as a preliminary study and to provide insight into the potential application of black and white garlcs as well as onion in pharmaceutical preparations and functional food industries supported by further extensive studies in these areas.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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