IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 18, Issue 2 Ser. II (Mar. – Apr. 2023), PP 16-22
www.Iosrjournals.Org

Quantification of Secondary Metabolites and Chromatographic Analysis of *Allium Cepa*, Liliaceae Ethanolic Extract

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Abstract
Introduction*: Allium cepa is among the most popular and widely grown vegetable crops globally. Because of its peculiar flavor, it is the third most significant spice with a high economic value. It has medicinal qualities in addition to culinary use. Several previous research has been published in support of its medicinal claims. About 80% of the world's population relies mostly on traditional medicine, with the majority of these therapies comprising plant extracts and their active components.* ***Aim:*** *the study's goals were to determine the phytochemical constituents of Allium cepa and perform preliminary thin layer chromatography (TLC) analysis on the ethanolic extract.* ***Method:*** *Allium cepa bulbs were collected, cleaned, and macerated in methanol for extraction. Phytochemical screening and TLC analyses were conducted.* ***Results****: phytochemicals such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins, and reducing sugar were present in the plant through phytochemical analysis. TLC examination revealed 15 spots that indicated the presence of several phytochemicals and chemical constituents in the plant.* ***Conclusion****: The phytochemical analysis revealed that Allium cepa bulbs are a significant source of secondary metabolites. The TLC profile produced an amazing result, indicating the presence of many chemical constituents, responsible for its medicinal properties.*

Keywords*: Allium cepa, phytochemicals, herbs, medicinal plant, TLC.*

Date of Submission: 02-04-2023 Date of Acceptance: 13-04-2023

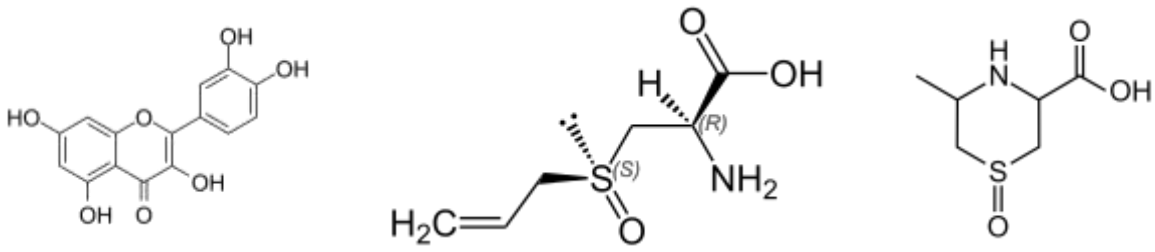
I. Introduction

Allium cepa is a well-known medicinal herb, with several reported pharmacological properties from previous research. Medicinal herbs or plants have medicinal characteristics or chemicals or synthesize metabolites to create valuable medicinal agents [1]. Every substance created or collected from a living organism is referred to as a natural product, this is not limited to just plants or animals, but also those obtained from bacteria, viruses, fungi, etc. When not in adequate quantities, they can be synthesized chemically, after designing a possible synthetic pathway(s), and this have played important role in the development of medicinal chemistry and drug design [2]. Plants are significant in traditional medicine, and a greater part of the world's populace depend on ethnomedicine for the prevention and treatment of several ailments [3].

In most developed countries, the rural populations lack access to basic health care, hence rely heavily on medicinal plant remedies to treat their illnesses [4]. Plant, animal or micro-organisms secondary metabolites modify their action in the living system using the same mechanisms as that are observed in the conventional medications [5]. Therefore, the herbal medicinal treatments have the same molecular mechanism compared to orthodox medicines [6]. *Allium cepa*, commonly known as onion, is a popular therapeutic and culinary spice all over the world, that contains significant bioactive chemicals such as thiosulfates, phenolic acids, flavonoids, and many others [7]. According to previous investigations, the plant has a variety of functions, including anticancer, anti-diabetic, antihypertensive, antibacterial, cardiovascular, and antioxidant activities [8], [9].

Figure 1: Bulbs of *Allium Cepa*

Allium Cepa grows across Asia, the United States, Africa (including Nigeria), and other parts of the world [10], [11], [12]. Despite the fact that it is a biennial plant, it is grown annually. Height differences range from about 15 to 45 cm. The leaves are yellowish to azure-green and grow alternately in a flat, fan-shaped swath. Onion has one flattened side and is soft, hollow, and cylindrical. Each leaf is flattened, with a white sheath that grows from the onion bulb's basal plate. From the plate's underside, a string of fibrous roots extends a short distance into the earth [13], [14]. The food storage in the leaf bases expands and the bulb swells as the onion matures [15]. *Allium cepa* pH is generally about 5.5, and can be propagated through seeds or sets of half-developed bulbs [16], [17]. Most *Allium cepa* comprise 89% water, 9% carbohydrate, 4% sugar, 1% protein, 2% fiber, and 0.1% lipids, with vitamins like thiamine (4%), riboflavin (2%), niacin (1%), pantothenic acid (2%), and pyridoxine (2%). Other reported compositions include fluoride (1.1%), magnesium (3%), manganese (6%), phosphorus (4%), potassium (3%), and zinc (2%), [18]. *Allium cepa* has different medicinally important chemical constituents like quercetin (Figure 2A), alliin (Figure 2B), cycloalliin (Figure 2C), as well as glycoquine, glucose, xylose, galactose, mannose, allylsulfides, thiosulfinates, etc., that are behind its medicinal use, previously reported [19], [20], [21].



A B C

Figure 2. Some chemical constituents of *Allium cepa*

Allium cepa has been used medicinally to treat bronchial asthma (thiosulfinate), some types of cancer (Quercetin), diabetes (glycoquine), lower blood triglycerides, and enhance the number of high-density lipoproteins (HDL), also known as good cholesterol, in the blood, thereby preventing arteriosclerosis [22]. Blood circulation is greatly improved by preventing arteriosclerosis and thrombosis. *Allium cepa* consumption has also been demonstrated to reduce the risk of cardiovascular (including heart attack), lung, urinary, renal, and liver diseases [23]. People with pneumonia, asthma, cough, sinusitis and other respiratory conditions are strongly advised to consume lots of *Allium cepa* [24]. *Allium cepa* has also been found to boost libido [25], enhance digestion, immune system, and bone density in older women, and it is beneficial in cases of bleeding piles, as well as anemia [26]. *Allium cepa* juice can be used to prevent bug bites, repel moths, applied to on the skin scalp to promote hair growth, as well as to reduce freckling on the face [27]. It's also been used to polish

glass and copperware and to keep iron from rusting [28]. In North America, sliced onions are battered and deep-fried and served as onion rings [29], [30]. TLC is an analytical technique, used to separate non-volatile liquids. A suitable adsorbent (stationary phase), a solvent or solvent mixture (mobile phase or eluent), and the test sample are used in a multistage distribution method. Following the application of the sample to the plate, capillary action pulls the solvent or solvent system up the plate, causing the test substance to ascend at different rates on the TLC plate, resulting in separation [31]. It is used to determine the purity of substances, evaluate fatty acids, identify pesticides or chemicals in food and water, assess fiber dye composition in forensics, and assay radiopharmaceutical purity [32], [33]. TLC is one of the fastest, least expensive, simplest, and most straightforward chromatographic techniques [31], [34], [35]. The study's goal was to assess the phytochemical components and TLC fingerprinting of *Allium cepa*.

II. METHOD

Sample Collection and Extraction

The *Allium cepa* bulbs were obtained at random from Elele Main Market, Elele, Rivers State, Nigeria. Extraneous items were discarded, including filth, dry and decaying skin. The onion bulbs were cleaned in fresh distilled water, the outer layers were manually peeled off, and the inner sections were rewashed in distilled water before drying, pulverized into a powder, and weighed. 25 grams of powder were macerated in 120 mL of methanol for about 96 hours. It was then filtered. After being evaporated to dryness in a water bath, the filtrate was weighed. The percentage yield was estimated.

Phytochemical Screening

Phytochemical analysis: The tests were carried out following the techniques specified by Harborne [36]. The methanol extract of the powdered plant sample was screened for tannins, flavonoids, anthraquinones, saponins, terpenoids, steroids, cardiac glycosides, carbohydrates, proteins, reducing sugar, and alkaloids.

Carbohydrates

Molisch's test: The powdered extract was boiled and filtered with distilled water (10ml). Molisch's reagent was added to the filtrate, with slow addition of concentrated sulphuric acid down the test tube's edge to create a lower layer. Carbohydrate's presence was shown with a purple interfacial ring [37].

Saponins

Approximately 20ml distilled water was added to 2.0g powdered plant extract, and boiled for 2 minutes in a hot water bath, sample was filtered, and allowed to cool, for the following tests:

i. Frothing test: 5 ml of the filtrate was diluted with 15 ml distilled water and shaken vigorously. The production of steady foam suggests the presence of saponins [38].

ii. Emulsion test: about 2 drops of olive oil were swiftly shaken into 10ml of foaming solution. The formation of an emulsion shows the presence of saponins [39].

Tannins

About 2.5g of powdered plant extract was boiled in 20ml of water, and filtered for the following tests:

i. Ferric chloride test: Few drops of ferric chloride was added to filtrate (3ml), and formation of a greenish-black precipitate indicates the presence of tannins [38].

ii. Lead acetate test: The filtrate was mixed with 3ml of lead acetate solution. The presence of tannins is indicated by the formation of precipitates [38].

Flavonoids

About 2g of powdered extract was boiled in a water bath for 3 minutes with 10 ml ethyl acetate, allowed to cool, and filtered:

i. Ammonium test: small amount of filtrate was shaken with 2ml of weak ammonia solution. Layer separation was permitted. Flavonoids' presence was indicated with a golden tint in the ammonical layer [40].

Alkaloids

About 2g of the powdered plant extract was mixed with 20 ml of ethanol (50%), boiled for 10 minutes in a water bath, cooled, and filtered.

i. Dragendorff's test: Few drops of Dragenndorff's reagent (Bismuth potassium iodide solution), a brick red coloration indicates the presence of alkaloids

ii. Mayer's test: Few drops of Mayer's reagent were added to each filtrate. A milky precipitate with indicates the presence of alkaloids.

iii. Picric acid test: Few drops of 1% picric acid solution were added to each filtrate, a yellowish coloration indicates the presence of alkaloids

iv. Wagner's test: Few drops of Wagner's reagent (iodo-potassium iodide solution), were added to each filtrate, and a reddish-brown coloration indicates the presence of alkaloids [41].

Steroids

The extract (1g) was dissolved in approximately 9ml ethanol, then refluxed for 3 - 5 minutes before being filtered. In a hot water bath, the filtrate was reduced to 2.5 mL, and 5 mL of hot distilled water was added, filtered after 1 hour to remove the waxy debris. 2.5 ml of chloroform was used in a separating funnel to remove the filtrate. To form a layer, 1 ml of concentrated sulphuric acid was mixed with 0.5 ml of chloroform extract in a test tube. The presence of steroids was indicated by a reddish-brown interface [41].

Terpenoids

About 0.5ml of chloroform extract was evaporated to dryness and heated for 10 minutes in a water bath with 3ml of sulphuric acid. A grey coloration indicated the presence of terpenoids [41].

Reducing sugar

Fehling's solution test: 5ml equal volume mixture. Fehling's solutions 1 and 2 were mixed into 5ml of the extract before being heated for 5 minutes. A color change indicates the presence of lowering sugar [41].

Cardiac glycoside

Raymond's Test: 1g of powdered plant extract was mixed with 2 ml ethanol and 0.1 ml of 3,5-dinitrobenzene (1%) in ethanol. 2 – 3 drops of a 20% sodium hydroxide solution were added to the solution. The violet tint suggested the presence of cardiac glycoside [41].

Thin Layer Chromatographic Analysis

Following the extraction method, the extract was dissolved in dichloromethane in a beaker. A small spot of the solution containing the test sample was spotted on the TLC plate using a capillary tube about 1.5 cm from the bottom border. To avoid interfering with the sample's interaction with the mobile phase and DCM was allowed to completely evaporate. The different mobile phase solvents include ethanol (10ml), petroleum ether: ethyl acetate (9:1), hexane: ethyl acetate (9:1), and benzene: ethanol (9:1), respectively. This procedure was repeated to ensure that there was enough analyte at the starting point of the plate to produce a visible result. The solvent front and sample were clearly marked. After viewing the plate in an iodine tank, the retention factor (Rf) was calculated. This technique was replicated with several eluents (mobile phase), including ethanol (10ml), petroleum ether: ethyl acetate (9:1), and benzene: ethanol (9:1).

III. RESULTS

Table 1. The percentage yield of *Allium cepa*:

Powder	Weight of Powder	Volume of Methanol	Weight of Extract	Percentage Yield
Allium cepa	25 g	120 ml	11 g	44%

Table 2. Results of Phytochemical Screening: *Allium cepa.*

S/N	Phytochemical	Reagents	Observation	Inference
1.	Alkaloids	Dragendorff's Mayer's Wagner's Picric acid	Red precipitate Cream precipitate Reddish brown precipitate Yellow precipitate	++ ++ ++ ++
2.	Flavonoids	Ammonium Aluminum chloride	Yellow color in the ammonia layer Yellow color in the aluminum layer	++ ++
3.	Cardiac glycosides	Raymond's	Violet color	++
4.	Tannins	Lead acetate Ferric chloride	Blue precipitate Greenish-black precipitate	++
5.	Saponins	Frothing Emulsion	Stable foam Formation of emulsion	++
6.	Steroids	Concentrated HCl	Purple color	++
	Terpenoids	Concentrated HCl	Purple color	--
7.	Carbohydrates	Molisch	Purple ring	++
8.	Reducing sugar	Fehling's	Brick red	++

Key: ++ = present, -- = absent

Table 3. Results from Thin Layer Chromatographic Analysis of *Allium cepa* R_f values

Ethanol (10ml) mobile phase		**Petroleum ether: ethyl acetate (9:1)**		**Benzene: ethanol (9:1)**		**hexane: ethyl acetate (7:3)**	
Visually (Daylight)	Ultraviolet Light (254nm)	Visually (Daylight)	Ultraviolet Light (254nm)	Visually (Daylight)	Ultraviolet Light (254nm)	Visually (Daylight)	Ultraviolet Light (254nm)
0.16 (b)	0.16 (b)	Not visible	0.11 (p)	Not visible	0.15 (db)	0.21 (db)	0.21 (db)
0.44 (y)	0.33 (p)		0.23 (p)		0.30 (b)	0.38 (b)	0.38 (b)
	0.44 (y)		0.96 (p)		0.74 (b)		0.49 (b)
	0.79 (p)						0.61 (p)
	0.98 (p)						

Key: lb – light brown, y – yellow and p – purple, db – dark brown, b – brown, db – dark brown, b – brown, by – brownish yellow, o – orange.

IV. DISCUSSION

From the initial 25g powdered plant materials and 11g yield, the percentage yield of Allium cepa after the thorough extraction process was 44%. (Table 1). The phytochemical screening of *Allium cepa* methanol extract yielded positive results for alkaloids, flavonoids, cardiac glycosides, tannins, steroids, carbohydrates, and reducing sugars. Table 2 displays the results of the phytochemical screening of *Allium cepa* methanol extract. The phytochemical screening revealed the presence of several chemical compounds in methanol fraction (extract) via direct maceration with methanol, demonstrating methanol as a solvent that can dissolve potential chemicals of the plant. The retention factor values and chromatogram findings of the methanol extract employing 10ml of ethanol, petroleum ether: ethyl acetate (9:1), hexane: ethyl acetate (9:1), and benzene: ethanol (9:1), as mobile phases, and the aluminum plates of TLC (silica gel 60 F254) as stationary phase, then ultraviolet (UV) light of 254 nm was used as visualization medium. The total number of spots obtained across all solvent systems was around 15 spots (Table 3).

The ethanol chromatogram result (10ml) as the mobile phase yielded 5 spots utilizing ultraviolet light as the medium of visualization, there were three purple spots with distinct Rf values including 0.33, 0.79, and 0.98, while the other two spots were brown (0.16) and yellow (0.44), respectively. A light brown (0.16) and a yellow color (0.44) were also seen with bare eyes. The visual examination and UV yielded identical results in two scenarios, showing the presence of five potential compounds in the ethanol fractions; consequently, Rf of 0.16 and 0.44 may be related compounds with similar physicochemical characteristics. Chromatogram results using petroleum ether: ethyl acetate (9:1) as mobile phase yielded 3 spots all purple but different Rf values ranging from 0.11, 0.23, and 0.96 under ultraviolet light as visualization medium indicating the presence of either a single or more compounds. Nothing was seen with bare eyes (Table 3).

Chromatogram result using hexane: ethyl acetate (7:3) using ultraviolet light as visualization showed 4 spots including a dark-brown (0.21), brown (0.38), light-brown (0.49), and a purple (0.61) spot, respectively. Two spots were also observed when viewed with the bare eyes; a dark brown (0.21), and a brown (0.38) spot, respectively. This also indicates the presence of four compounds, as Rf 0.21 and 0.38 was seen both with bare eyes and under UV lamb, thus may have similar physicochemical properties. Chromatogram result using benzene: ethanol (9:1) under ultraviolet light as visualization showed 3 spots, a dark-brown (0.15), brown (0.30), and light-brown (0.74) spots, respectively, but none under bare eyes. Various phytochemicals give different R_f values in different solvent systems (Table 3). All of the foregoing was rated as the finest solvent systems for plant the quantification, with ethanol and the combination of n-hexane and ethyl acetate taking nearly five and four spots, respectively.

These solvent systems would produce more pure isolates than the others after subsequent isolation. The difference in the Rf values of phytochemicals gives an important clue in establishing their polarity and aids in the selection of suitable solvent system for the extraction of pure components from the plant. This simply validates previous reports on the plant for its various chemical constituents, which include quercetin, alliin, cycloalliin, glycoquine, oligo-fructose, quercetin-3-glucoside, isorhamnetin-4-glucoside, selenium, xylose, glucose, galactose, allylsulfides, thiosulfate, flavonoids, flavanols, [19], [20], [21]; that is responsible for its purported therapeutic benefits such as bronchial asthma, cancer [26], [42]; diabetes, blood hypertriglyceridemic reduction, and rise in high-density lipoproteins (HDL) levels [22].

V. CONCLUSION

The phytochemical results revealed that *Allium cepa* bulbs has potential medicinal and pharmacological properties. Flavonoids, for example, have been discovered to be effective inhibitors of numerous enzymes, such as xanthine oxidase and cyclooxygenase, implying that the plant has further medicinal properties. Alkaloids, cardiac glycosides, saponins, steroids, carbohydrates, and other compounds have therapeutic properties when present in plants. The TLC profiling produced a remarkable result, indicating the presence of a variety of beneficial chemical constituents (phytonutrients) of the plant.

Conflict of Interests/Competing Interests
The authors hereby declare that, there is no conflict of interest in the manuscript as presented.

Funding
No external funding was received in conducting this research.

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