TLC Analysis of Ethanol Extract of Fresh Leaves of Celery (*Apium Graveolens* L.) Grown In Jos, Nigeria

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Abstract: ***Apium Graveolens*** **L., is a member of the Apiaceaefamily. Apiaceae is a large family of mostly aromatic flowering plants whose leaf is commonly used as vegetables worldwide. The fresh leaves of Celery (*Apium Graveolens* L.) were crushed extracted with ethanol by Maceration and Fractionated with n-hexane and ethyl acetate. Thin Layer Chromatographic (TLC) analysis of the fractions of n-hexane and ethyl acetate were performed, important phytochemicals such as flavonoid, terpenoid and naturally occurring phhthalides were identified in the various fractions. The presence of flavonoid was revealed in n-hexane fraction with two spots whose $R_{.f}$ values are (0.82 and 0.58). Terpenoid presence was revealed in n-hexane fraction with two spots whose R_f values are (0.79 and 0.61) and also based on their observed colour change under ultraviolet light probably due to reaction with (vanillin-H_2SO_4). Phthalide was observed in ethyl acetate fraction with four spots under UV/10% H_2SO_4 with R_f values of (0.97, 0.67, 0.51 and 0.41). The findings provided the evidence that *Apium Graveolens*L. is a potent source of some medicinally important phytochemicals and natural products which justifies its use as medicinal plant and food flavourings. This can be further investigated for the isolation and structural analyses of the biological active phytochemical components for medicinal application.**

Keywords: ***Apium Graveolens*****, Celery plants, Phytochemical screening, Phthalide, Vanillin-H_2SO_4.**

I. INTRODUCTION

Celery is a member of the Apiaceaefamily. Apiaceae is large family of mostly aromatic flowering plants named after the type of genus *Apium.* Celery, *Apium graveolens* L. (Apiaceae*)*,is native to Mediterranean regions like Asia, Africa and Europe [1], it's grown mainly in coastal regions. Celery is widely cultivated in the temperate zones as an important garden crop and the leaves, stalks are relished as a popular vegetable [2].

The plant genus has had a long history of its medicinal uses and *Apium graveolens* contains variety of bioactive components such as terpenoids phenolic acids, alkaloid, tannins and flavonoids which have numerous biological and pharmacological properties such as hyperglycemic, analgesic, anti-inflammatory, anti-hypertensive, anticancer, antineurogenesis, anti-platelet, weight lost, natural diuretic, reduce menstrual pain, aid in digestion [3]. The plant can also be liquidized and be taken as a juice for joint and urinary tract inflammations, rheumatoid arthritis, cystitis, or urethritis, for weak conditions and nervous exhaustion [4].

Use of medicinal plants to treat common ailments has been prevalent since ancient times and different parts of the plants were used for public health. This is logically reasonable because the use of natural treatments is cost-effective [5].*Apium graveolens* is an important plant with great Ayurvedic medicinal properties. The medicinal properties of celery also include diuretic and sedative activities Ayurvedic physician (Vaidyas) used celery seed to treat people with cold, flu, water retention, poor digestion, various type of arthritis and certain disease of liver and spleen [6]. The methanolic extract of celery seed was found to be effective paracetamol-induced [7]and carbon tetra chloride-induced [8].

Celery (*Apium graveolens* L., Apiaceae) is a medicinal herb used as food, and also in traditional medicine. It contains aromatic substance in the roots, stem and leaves. The healing properties of celery are due to the essential oils and flavonoid mostly apeginin and apiin[9]. Worthy of note is that celery contains a flavonoid called apigenin, which has been shown to induce death in cancer cells [10]. In fact, celery leaves and stalks are usually eaten alone or incorporated into recipes as many menus across almost every culture in Nigeria. Celery leaves offer nutritional value that can boost our health [5]. Interestingly, the findings in a prior literature suggest that the celery plant grown in Jos, Nigeria, contains phthalides which have been reported to be responsible for the bioactivity of celery plants [11].

Since ancient times, phthalidecontaining plants have been used worldwide as herbal remedies in traditional medicines, dietary supplements, and food flavorings, for example the leaf, stalk and fruits of *Apium graveolens* have a long history of use as healthy food and food flavorings in both Western and Eastern countries [12].

Celery is cultivated in the northern and northcentral part of Nigeria especially in Jos plateau state possibly due to the favourable soil and climate [11]. The celery plant extracts grown in Jos, Nigeria showed strong antioxidant capacity. The extracts can be considered as a good source of natural antioxidants and antimicrobials. Based on the result of free

radical scavenging activity observed, the crude ethanolic extract exhibit stronger antioxidant activity as compared to standard ascorbic acid. The presence of flavonoids, terpenoids, anthocyanin and carotenoid among other secondary metabolites may be responsible for its antioxidant property and its medicinal application. [13]. Due to both economic reasons and high activity of n-hexane extract it has been successfully employed for a large-scale extraction of the volatile oils from celery plants [14].

Over the year's considerable efforts have been dedicated into the investigations of celery plants grown in other parts of the world especially in Asia using various analytical methods such as High-performance LiguidChromatography, HPLC and GC/MS [15, 16], infrared (IR) and ultraviolet (UV) spectrophotometry [14, 17] TLC [18].

However, surprisingly, despite the high promising therapeutic potentials of the pharmacological and pharmaceutical properties of the celery plants grown in Nigeria, to the best of our knowledge such level of works have not been reported on celery plants cultivated in Nigeria. In this study we describe the thin layer chromatography (TLC) analytical determinations of the ethanol extracts of the fresh leaves of *Apium graveolens* L. cultivated in Nigeria. Although, previous literatures have demonstrated the gc/ms investigation of the constituents of phthalides from the essential oils of the leaf and stalk of the celery plants cultivated in Nigeria [11] and also described the antioxidant activity of Nigerian celery plants [13], only a limited number of works have been reported on the celery plant grown in Nigeria. Many more researches are required on this very valuable plant to exhaustively explore the potentials of the pharmaceutical compounds of the Nigerian grown celery plants which could be useful in medicine, food and pharmaceutical industries.

II. MATERIAL AND METHODS

Whatman No.1 filter, pre-coated aluminum TLC sheets, glass rod, 2.5litre wide mouth bottle, separatory funnel, beakers, conical flask, scissors, pencil, developing tank, retort stands, capillary tube, vacuum pump, measuring cylinder, steam bath, volumetric flask, analytical balance, mortar and piston, UV lamp (365nm), oven, Buchner funnel, ice bath, spatula, muslin cloth.

2.1 Solvents and Chemicals

Ethanol, methanol, n-hexane, ethyl acetate, anhydrous sodium sulphate, Aluminum chloride, vanillin, concentrated ammonia, sulphuric acid, distilled water, acetic anhydride and iodine crystal. Which are all analytical grade chemicals and solvents.

2.2 Collection and Identification of Celery Plant Grown In Jos.

The fresh leaves of *Apium graveolens* L were collected from Yelwa ward (Jos North local government area of Plateau State, Nigeria) in the month of June, 2018. The Celery plant was identified and authenticated at the department of Horticulture, Federal College of Forestry Jos, Plateau State, Nigeria, and voucher specimen number (FHJ/18/014) was deposited in the Herbarium of the same department.

2.3 Sample Preparation and Extraction

The freshly collected leaf samples were washed, pounded into smaller sizes using agate mortar and pestle. The properly crushed samples were immediately transferred into a stoppered container that had been previously washed with n-hexane and ethyl acetate and oven dried. This was extracted using HPLC graded ethanol by maceration at room temperature for two days (48 hours) with frequent agitation. The mixture was strained into a beaker using muslin cloth and filtered with Whatman no.1 filter paper. The filtrate then concentrated using Rotary Evaporator (R-205) at 60^{o}c to obtain a crude extract of the leaf, which was properly transferred and kept in an air-tight round bottom flask for further use.

III. FRACTIONATION OF THE CRUDE EXTRACT.

3.1 n- *Hexane Fraction*

The crude ethanolic extract of the leaf (10g) was dissolved in 50ml of n-hexane. It was then placed on a steam bath at 50^{o}C with occasional stirring, then allowed to cool for 20 minutes and filtered, the process was repeated four (4) times and a residue was obtained, the 200ml of n-hexane fraction was concentrated with rotary evaporator and properly stored for further analyses.

3.2 Thylacetate Fraction

In a crude ethanolic leaf extract (10g) was added 50 ml of ethyl acetate, it was then placed on a steam bath at 50^{o}C with occasional stirring, it was then left to cool for 20 minutes and filtered the procedure was repeated four (4) times and a residue was obtained, 200ml of ethyl acetate fraction was placed in round bottom flasks, concentrated using the rotatory evaporator and the fraction properly stored for further analyses.

3.3 Preparation of Reagents

(i) 10% ethanolic H_2SO_4.

A 10ml of H_2SO_4 was added very slowly with constant stirring using a glass rod to a 90ml of ethanol.

(ii) Vanillin-H_2SO_4

A 0.5g of vanillin was dissolved in 8ml of ethanol and 2ml of concentrated H_2SO_4 under constant stirring

with cooling in an ice bath (0 °C).

IV. CHROMATOGRAPHIC ANALYSIS

4.1 Thin Layer Chromatography (TLC)

(i) TLC plate.

Commercially obtained silica gel pre-coated thin layer chromatographic plate was cut into (2x10cm) strips, the

solvent front and origin marked with a pencil and used for the TLC analyses.

(ii) Developing Tank.

Two different sets of solvent system with varying ratios were prepared with a total volume of 20ml depending on the ratio of solvent mixture to ensure that the solvent does not go above 0.5cm of the TLC plate. The solvent tank was lined with filter paper, covered with an air tight lid, and left to stand for 20 minutes to ensure that the tank was saturated with the solvent system.

(iii) Plate Development.

Clean micro-capillary tubes were used to spot each sample, n-hexane fraction and ethyl acetate fractions on the prepared TLC plate. The spots were allowed to air dry and then placed in the already prepared solvent tank, ensuring that the spots are properly placed at the baseline on the TLC plate. The plate was then left undisturbed to allow the solvent front move by capillary action up to about ¾ the height of the plate. The plate was then removed and allowed to dry for subsequent treatments with locating agents.

4.2 Visualiztion of Spots

(i)The use of iodine vapour.

The air dried plate was placed in a tank saturated with iodine vapour for the location of the components' spots.

The spots were traced with pencil and their respective R_f values calculated.

(ii) The use of Vanillin- H_2SO_4

The air dried plate was sprayed with vanillin- H_2SO_4 using a spray gun, left to dry for 5 minutes, then kept in an oven at 105^{o}C for 15 minutes until full development of colours. The plate was then removed, and the coloured spots were traced with pencil and their respective R_f values were determined.

(iii)The use of UV light (365nm) and 10%H_2SO_4

The air dried plate was viewed under UV light (365nm) and the components were located by varying colours and the spots traced. The same plate was stained with **10%** H_2SO_4 using a spray gun, the plate was left to dry for 5 minutes then viewed under UV light and colour changes were observed. The R_f value of each component was calculated.

V. RESULT AND DISCUSSION

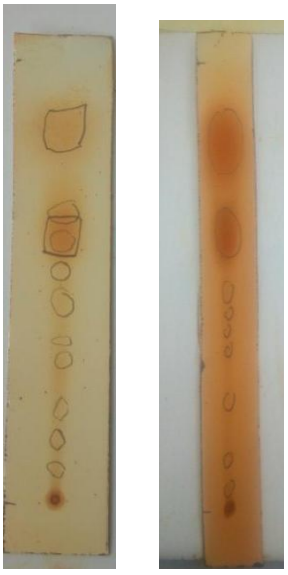
5.1 Result of TLC Analysis

Secondary metabolites in plants can be obtained by extraction methods, one of which is maceration. The mechanism of maceration is by pulling or removing metabolites by immersing them using the appropriate organic solvents [19].

The fresh celery leaf oil extracts were subjected to TLC analysis. First, EtOAc:n-hexane (1:3 and 1:2) were employed as model solvents ratios to probe the optimal solvent system with n-hexane extract , both solvent systems provided excellent separation of the obtained components, with two prominent spots observed in all cases with iodine vapour as the locating reagent (Fig 1). Their R_f values were then determined (Table 1, entry 1 & 2). Table 2, presents the outcome of the TLC analysis of ethyl acetate fraction using EtOAc:n-hexane (1:2) as the solvent system. Four components were observed, probably reactions with the locating agent (10% sulphuric acid) could possibly be responsible for the formation of the fourfluorescent blue colour (Fig 2) components' spots observed with corresponding R_f values of 0.97, 0.67, 0.51, and 0.14 which could likely be attributed to the naturally occurring phthalide derivatives. Table (3) demonstrates the result of the TLC analysis of n-hexane fraction treated with vanillin-H_2SO_4. Five components were observed with the solvent system ofEtOAc:n-hexane (1:3). The purple colour component with R_f value 0.91 suggests the presence of terpenoid, the yellow components with R_f values 0.80, 0.55 and 0.44, and the dark green colour components with R_f value 0.75 suggest the possible presence of flavones. While Figures 1, 2 and 3 illustrates the chromatoplates of the TLC analysis of the n-hexane fraction and ethyl acetate fractions showing colour reactions using different locating reagents.

Table 1: Results of TLC Analysis of n-hexane Fraction using Iodine Vapour as Locating Agent

Solvent system	No of Components	R_f values
EtOAc:n-hex (1:2)	2	0.88
		0.65
		0.88
EtOAc:n-hex (1:2)	2	0.65

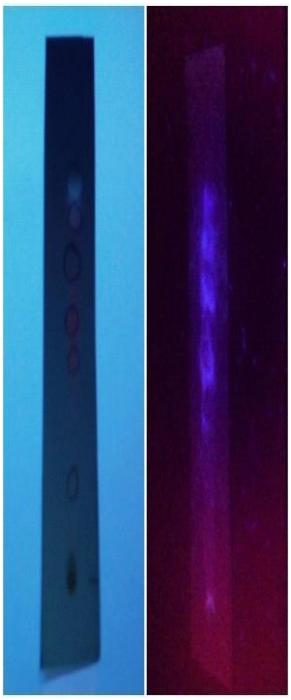


(a) [EtOAc:Hex(1:3)] (b) [EtOAc:Hex(1:2)]

Figure 1.The Chromatoplates of n-Hexane Fraction in Iodine Vapour using two different Solvent Mixtures.

Table 2: Results of TLC Analysis of Ethyl Acetate Fraction using 10% H_2SO_4as Locating Reagent

Solvent system	No of Components	UV light	10%H_2SO_4/ UV	R_f Values
EtOAc:n-hex (1:2)	4	Blue	Fluorescent blue	0.97
		Pale blue	Fluorescent blue	0.67
		Grey		
		Red	Fluorescent blue	0.51
			Fluorescent blue	0.41



UV/10%H_2SO_4

Figure 2: The Chromatoplates of Ethyl acetate Fraction with UV/10% H_2SO_4. Solvent System of EtOAc:Hex (1:2).

Table 3: Results of TLC Analysis of n-Hexane Fraction using Vanillin-H_2SO_4 as Locating Agent

Solvent system	No of Components	Vanillin-H_2SO_4	R_f Values
EtOAc:n-hex (1:3)	5	Purple	0.91
		Yellow	0.81
		Dark green	0.75
		Yellow	0.55
		Yellow	0.44

Figure 3: The Chromatoplate of n-Hexane Fraction with Vanillin-H_2SO_4. Solvent System: 16 = EtOAc:Hex (1:3).

Figure 4: Uprooted Celery Leaves and Stalk (*Apium graveolens* L.) Grown in Jos.

VI. CONCLUSION

This study describes the TLC analysis of ethanolic extracts of *Apium graveolens* L cultivated in Nigeria to investigate the presence of very important class of bioactive chemical components which exhibit promising therapeutic and medicinal values. Phthalide, terpenoids and flavones were identified from the celery (*Apium graveolens*) plant grown in Nigeria. The aforementioned identified phytochemicals have recently sparked growing interest in natural product and pharmaceuticals due to their promising therapeutic properties

and may continue to be of vital use in modern medicinal applications.

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