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Original Research Article

Phytochemical Screening, Thin-layer Chromatographic Studies and UV Analysis of Extracts of *Citrullus lanatus*

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ABSTRACT

The present study was carried out to determine the phytochemical constituents, thinlayer chromatographic profile and UV analysis of *Citrullus lanatus* leaf extracts. The leaves of *C. lanatus* were collected, dried, pulverized and extracted with methanol using maceration method. The extract was concentrated in *vacuo* with the aid of rotary evaporator to afford a greenish crude methanol extract (ME). The resulting extract was successively partitioned into hexane, chloroform, ethylacetate and n-butanol fractions respectively. The fractions were subjected to general phytochemical screening and thin-layer chromatography using standard procedures. The fractions were scanned in the wavelength ranging from 200-750nm using Optima Tokyo Japan (SP/3000PLUS) spectrophotometer and characteristics peaks were detected. Phytochemical screening revealed the presence of saponins, alkaloids, flavonoids, phenols, steroids and triterpenes which varies in other fractions. Thin-layer chromatographic studies using different solvent systems revealed homogenous spots with different R_f values. The UV profile showed different peaks ranging from 220-750nm with different absorptions respectively. The results of the study indicated that the leaf of *Citrullus lanatus* contains secondary metabolites and suitable mobile phase for each fraction have been developed.

Keyword: *Citrullus lanatus*; phytochemical; TLC; UV analysis

INTRODUCTION

Traditional medicine involved the use of plants, animals and mineral-based medicines to treat and prevent illnesses or maintain well-being [1]. According to the World Health Organization WHO, about 80% of the world's population relies on traditional medicine [2]. Most

traditional practioners rely on herbs (plants) which contain some bioactive secondary metabolites including tannins, flavonoids, saponins, alkaloids [3]. These constituents produce definite physiological action on the human body [4].

Citrullus lanatus (Cucurbitaceae) commonly known as watermelon is a prostrate annual plant with several herbaceous firm and stout stems up to 3m long. It produces a fruit that is about 93% water, hence the name "water melon". It is widely distributed in the temperate regions of Africa, central Asia and Mediterranean [5]. It has been cultivated in Africa over 4,000 years [6]. The leaves are a good antimalarial, analgesic, anti-inflammatory, mosquitocidal, and antimicrobial agents and can also be used in the treatment of gonorrhea (Personal Communication).The present study investigates the qualitative phytochemical screening and, analyses TLC plates of suitable mobile phase and UV spectroscopy for solvent extracts from the leaves of *C. lanatus* plant.

Materials and Method

Collection and Identification of Plant Material

The plant material was collected from Illela Local Government Area, Sokoto State, Nigeria in December 2014. It was authenticated by Namadi Sanusi at the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria by comparing with the voucher specimen (No: 1266).

Preparation of the Extract

The leaves were shade dried, pulverized, labeled and stored at room temperature. The powdered leaves (154g) were extracted with methanol using maceration method for 3 days. The extract was evaporated in-vacuo using rotary evaporator at 40^{o}C to yield a gummy greenish residue (25.6g) subsequently referred to as the crude methanol extract (ME). Twenty gram (20g) of ME was suspended in distilled water, filtered and partitioned successively into hexane (3.14g, HF), chloroform (0.22g, CF), ethylacetate (2.43g, EF) and n-butanol (4.40g, BF) fractions respectively. The fractions were concentrated and kept in a refrigerator prior to use.

Preliminary Phytochemical Screening

Portion of the fractions each was subjected to phytochemical screening for the presence of secondary metabolites including, flavonoids, saponins, tannins and steroids/triterpenes and alkaloids using standard procedures [7, 8].

Test for Alkaloids

0.5g of the extract was stirred with 5ml of 1% aqueous hydrochloric acid on a water bath and filtered. 3ml of the filtrate was divided into three. To the first 1ml few drops of freshly prepared Dragendoff's reagent was added. To the second, 1 drop of Meyer's reagent was added. To the third, 1ml of Wagner's reagent was added and observed.

Test for Flavonoids

Ferric chloride test:

To a small portion of the extract, distilled water was added. A drop of ferric chloride was added to a solution of the extract and observed.

NaOH test. Some portion of the extract was dissolved in 10% aqueous NaOH solution, dilute HCl was added and observed.

Test for Anthraquinones

0.5g of the extract was shaken with 5ml carbon tetrachloride, this was filtered and 10% dilute ammonia solution was added. The mixture was shaken and observed.

Test for Saponins

0.5g of the extract was shaken with distilled water in a test tube. It was allowed to stand for 10 minutes and observed.

Test for Steroids and Triterpenes

Liebermann-Buchard test:

A small portion of the extract was dissolved in chloroform. Equal volume of acetic anhydride and concentrated H_2SO_4 were added down the test tube and observed.

Salkowski test:

A small quantity of the extract was dissolved in 1ml chloroform and to it 1ml of concentrated H_2SO_4 was added down the test tube and observed.

Test for Tannins

Lead Sub-acetate Test:

To a small portion of the extract, distilled water was added. 3-5 drops of lead acetate solution was added and observed.

Test for Carbohydrates

Molisch test:

To a small portion of the extract, distilled water was added and mixed with a few drops of Molisch reagent. 1ml of concentrated H_2SO_4 was carefully added down the side of the inclined tube and observed.

Fehling's Test:

To a small portion of the extract, distilled water was added. 2ml Fehling's solution was added and boiled for 5 minutes and observed.

Test for Phenols

Ferric chloride test:

To a small portion of the extract, distilled water was added. A drop of ferric chloride was added to a solution of the extract and observed.

Test for Glycosides

Legal's test:

To a small portion of the extracts, sodium nitropruside in pyridine and sodium hydroxide was added and observed.

Ferric chloride test:

To a small portion of the extract, distilled water was added. A drop of ferric chloride was added to a solution of the extract and observed.

Thin-layer Chromatographic Studies (TLC)

Thin-layer chromatography was carried out on all the fractions using TLC pre-coated plates (silica gel $60F_{254}$) by using one way ascending technique. The plates were cut with scissors and marked with pencil about 1cm from the bottom of the plate. Each sample was faintly dissolved in methanol and capillary tubes were used to uniformly apply the dissolved samples on the plates and allowed to dry. The plates were developed in a chromatographic tank using the different solvent systems including; **(1)**hexane (100%), **(2)**hexane: ethylacetate (9:1; 8:2, 7:3), **(3)** chloroform: methanol (30:1, 15:1), **(4)** chloroform: ethylacetate: methanol: water (15: 8: 4: 1), **(5)** n-butanol: acetic acid: water (4: 1: 5). The plates were dried and visualized under normal day light, ultraviolet light (254nm & 366nm) and by spraying with 10% sulfuric acid followed by heating at 105^{o}C for 5-10minutes in an oven [9, 10].

The retention factor R_f for each active compound was calculated for each fraction using the following formula;

$$R_f = \frac{\text{Distance moved by the solute/compound}}{\text{Distanced moved by the solvent (solvent front)}}$$

UV spectroscopic analysis

The fractions were subjected to UV analysis [11]. Each sample was diluted to 1:10 with methanol and scanned in the wavelength ranging from 200-750nm using Optima Tokyo Japan (SP/3000PLUS) spectrophotometer and characteristic peaks were detected.

RESULTS

Percentage Yield

The percentage yield of each fraction is shown in (Table 1). Crude methanol extract (25.6g), hexane fraction (3.14g), chloroform fraction (0.22g), ethylacetate fraction (2.43g) and n-butanol fraction (4.40g).

Phytochemical Screening

The general preliminary phytochemical screening conducted on the fractions revealed the presence of various secondary metabolites as shown in (Table 2).

Thin layer Chromatographic Studies

TLC analysis of all the fractions using different solvent systems revealed the presence of promising spots as shown in (Table 3).

UV analysis

The qualitative UV profile *Citrullus lanatus* fractions was selected from 220-750nm and the absorbance ranges from 0.000 – 2.549 which as shown in (Table 4).

Table 1: Percentage Yield of Extract

S/ no	Fractions	Color of extract	Yield of extract (g)	Yield of extract (%)
1	Hexane	Green	3.14	15.70
2	Chloroform	Yellowish green	0.22	1.10
3	Ethylacetate	Brown	2.34	12.15
4	n-Butanol	Brown	4.40	22.00
5	Methanol	Green	25.60	16.62

$$\% \text{ Yield} = \frac{\text{Final weight of extract} \times 100}{\text{Initial weight of extract}}$$

Table 2: Phytochemical Constituents of Methanolic Leaf Extract of *Citrullus lanatus*

Constituents	Test	Observation	Inference				
			ME	HF	CF	EF	BF
Saponins	Frothing	Frothing persist for 15mins	+	+	+	- -	
Alkaloids	Mayer's	White-cream ppt	+	-	+	+ +	
	Draggondorf's	Orange ppt	+	-	+	- -	
	Wagner's	Reddish-brown ppt	+	-	+	+ +	
Flavonoids	$FeCl_2$	Green or violet ppt	+	-	-	+ +	
	Shinoda	Orange-red ppt	+	- -	+	+	
Tannins	Lead subacetate	Cream ppt	+	-	-	+ +	
Steroids & Terpenes	Lieberman-Buchard	Blue-green color at interphase	+	+	+	- -	
	Salkowski	Reddish color	+	+	+	- -	
Anthraquinones	Borntragers	Pink or violet	-	-	-	-	-
Carbohydrates	Molisch's	Reddish ring	+	+	+	- -	
	Fehling's	Red	+	+	-	-	-
Phenols	$FeCl_2$	Bluish black color	+	-	-	+	+
Glycosides	Legal's	Pink-blood red ppt	+	-	-	+	+
	Fehling's	Red ppt	+	-	-	+	+

Table 3: R_f values of TLC solvent systems for different fractions of *Citrullus lanatus*

S/no	Fractions	SS 1		SS2		SS3		SS4		SS5	
		No. of spots	R_f values	No. of spots	R_f values	No. of spots	R_f values	No. of spots	R_f values	No. of spots	R_f values
1	Hexane	1	0.02	3	0.65 0.84 0.87	4	0.04 0.10 0.57 0.39	4	0.02 0.48 0.56 0.82	-	-
2	Chloroform	-	-	1	0.87	3	0.06 0.70 0.13	2	0.76 0.88	-	-
3	Ethylacetate	-	-	-	-	-	-	1	0.76	-	-
4	n-Butanol	-	-	-	-	-	-	2	0.72 0.82	-	-
5	Methanol	-	-	3	0.65 0.84 0.87	4	0.04 0.39 0.57 0.10	5	0.20 0.48 0.56 0.82 0.92	-	-

SS=Solvent system

Table 4: UV Analysis of fractions of *Citrullus lanatus*

	Hexane	Chloroform	Ethylacetate	n-Butanol	Methanol
λ max (nm)	220	410	375	390	220
Absorbance	2.549	1.596	1.833	2.248	1.794
λmin (nm)	750	750	750	750	750
Absorbance	0.035	0.049	0.008	0.003	0.000

DISCUSSION

The result of preliminary phytochemical screening of the crude methanol extract revealed the presence of all the constituents tested including; alkaloids, flavonoids, saponins, tannins, carbohydrates, phenols, glycosides, except anthraquinones which varies in the fractions. Hexane fraction revealed the presence of saponins, steroids, terpenes carbohydrates and chloroform fraction revealed the presence of saponins, steroids and terpenes. Ethylacetate fraction indicated the presence of all the constituents tested except for saponins, steroids, terpenes, carbohydrates, anthraquinones and carbohydrates and n-butanol fraction revealed the presence of all the constituents except steroids, terpenes and carbohydrates. These constituents are responsible for most pharmacological activities of plants [12, 13]. Phytochemical constituents give different R_f values in different solvent system. This variation in R_f values provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds incorporated in different fractions by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extract can only be achieved by analyzing the R_f values of compounds in different solvent system [8, 9]. Thin-layer chromatographic analysis carried out on all the

fractions revealed homogenous spots which is not the case using some solvent systems. Hence, there is still a need to find another suitable mobile phase where the spots migration was not visible. Ethylacetate and n-butanol fractions seem to have complex spots as there was no separation in all the solvent systems used. Hexane fraction revealed homogenous spots in all the solvent systems used which is an indication that the mobile phase may show a good resolution if adopted for the fraction. The UV analysis showed wavelength range from 220nm-750nm. The ultraviolet spectroscopy is very useful method for identification of unsaturated bonds present in plant components, which can be used to distinguish between conjugated and non-conjugated system. Using the principle of absorption maxima, the structure of compounds can be deduced [11]. The result of the UV analysis of the fractions gave absorption peaks at 220nm (n-hexane), 410nm (chloroform), 375nm (ethylacetate), 390 (n-butanol) and 220nm (methanol). It can be inferred that the compounds present in the fractions have chromophores and hence, absorption takes place to allow transition [11].

CONCLUSION

In conclusion, the leaf of *Citrullus lanatus* contains secondary metabolites that can be used in traditional medicine to treat ailments. Further detailed isolation and characterization of active constituents of *Citrullus lanatus* is on-going.

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CONFLICT OF INTEREST STATEMENT

None Declared

REFERENCES

1. World Health Organization (WHO). Traditional Medicine. 2008, WHO Fact Sheet No.134. Geneva: WHO revised 2003. Available at: http://tinyurl.com/5mrd5 (accessed 11 December 2008).
2. WHO News; Traditional medicine strategy launched, 2002; 80, 610.
3. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants.African Journal of Biotechnology 1984, 4(7): 685-688.
4. Cowan MM. Plant products as antimicrobial agents.Clinical Microbiology Review, 1999, 12(4): 564-582.
5. Jeffrey C. Further notes on *Cucurbitaceae*. III. Some African taxa. Kew Bull 1975; 30: 475-493.
6. Robinson RW, Decker-Walters RDS. Cucurbits. New York Cab International (Crop Production Science in, Horticulture nE.6) 1997; 226.
7. Silva GL, Lee I, Douglas KA. Special problems with extraction of plants. In: Cannell J.P.R (eds). New Jersey USA: Natural Products Isolation, Human Publishers; 1998, p 251.
8. Trease K, Evans WC. Textbook of Pharmacognosy, 14th Edition, London: Balliere, Tindall; 1996.
9. Geiss F. Fundamentals of thin layer chromatography planar chromatography. Heidelberg: A. Hüthig; 1987.
10. Touchstone JC. Practice of thin layer chromatography. 3rd ed. New York: Wiley; 1992.
11. Omodara NB, Amoko JS, Obijole OA, Ojo BM. Infrared and ultraviolet spectroscopic analysis of methanol extract of *Phyllanthus muellerianus* root. Greener J Physical Sci 2013; 3 (4): 159-164.

12. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum books Ltd. Nigeria: Ibadan; 1993, p 191-289.
13. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Review 1999; 12(4): 564-582.

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