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CHROMATOGRAPHIC EXAMINATION AND ANTI-OXIDANT ACTIVITY OF THE LEAF EXTRACT OF *COLA ACUMINATA* (STERCULIACEAE)

H. B. Yesufu*[1], G. T. Mohammed[1], A. B. Bababe[1], M. B. Fugu[2]

[1*]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Maiduguri, P.M.B 1069, Maiduguri, Nigeria.

[2]Department of Chemistry, Faculty of Science, University of Maiduguri, P.M.B 1069, Maiduguri, Nigeria.

ABSTRACT

The Plant material *C. acuminata* is prominent for its fruit (kolanut) which is widely consumed amongst many cultures in West Africa. The preliminary phytochemical screening of the leaf extract of *cola acuminate* revealed the presence of cardiac glycoside, flavonoid, tannins, carbohydrate, anthraquinone glycoside, steroid and alkaloid. Column chromatography of the aqueous ethanol extract was conducted using prepared column set-up, the aqueous ethanol extract was ran with n-hexane, chloroform and methanol at different ratios. The TLC profile of the leaves extract using the above stated solvent system showed characteristic distinct spots as obtained from TLC plate 1 and 2 using different spraying agent with characteristic R_f values. The above mentioned extract were evaluated for their antioxidant/free radical scavenging activities at concentration 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml using DPPH assay method and vitamin C as a standard. The activity was more prominent in the non-polar component (n-hexane extract) than the polar component (ethanol extract). The DPPH/TLC assay conducted on the column fractions confirmed the presence of antioxidant principles on the leaf extract of *C. acuminata*.

KEYWORDS

Phytochemicals, Thin layer and column chromatography, DPPH radical scavenging assay and *Cola Acuminata*.

Author for Correspondence:

Yesufu H B,
Department of Pharmaceutical Chemistry,
Faculty of Pharmacy, University of Maiduguri,
P.M.B 1069, Maiduguri, Nigeria.

Email: mallamasan@yahoo.com

INTRODUCTON

Free radicals are products of cellular metabolism and transition-metal ions, and they seem to play an important role in causing bio-macromolecule damage in vivo. Increasing oxidative stress and disorders in energy metabolism may lead to mutations and eventually to many severe diseases[1-3]. The human body has several mechanisms to reduced oxidative stress, such as produced antioxidants, which are either naturally produced inside the body, or externally supplied through

foods and/or supplements. The mechanism of antioxidant is suppression of Reactive oxygen species (ROS) formation by inhibition of enzymes or by chelating the trace elements which are involved in ROS production, scavenging ROS, and thereby protect the antioxidant defense system[4,5]. Antioxidants may play an essential role in protecting the body from various oxidative damages that are linked to obesity, diabetes, cancer, cardiovascular diseases and neurode generative ones, including Parkinson's and Alzheimer's disease[6]. The aetiology of these several health disorders implicates free radicals or reactive oxygen species generated by normal physiological processes and various exogenous factors[7]. The medicinal importance of the fruit of *C. acuminata* has been frequently discussed in literatures, but there is dearth of information regarding the leaves.

MATERIAL AND METHODS

Sample Collection and Preparation

The fresh leaves of *Cola acuminata* were collected in the month of February, 2015, from Agbede in Etsako west local government of Edo state, Nigeria. The plant was identified and authenticated by a taxonomist at department of biological science, faculty of science, University of Maiduguri.

600 g of the dried plant material (leaves) was extracted with two solvents in an increasing order of polarity using maceration method. The solvent n-hexane was first used for extraction twice. The extract was filtered and collected in a single beaker and evaporated to dryness. The marc was then removed from the maceration bottle, dried and weighed. The resultant marc was extracted with ethanol (2×2L). The percentage yield for each extract was calculated.

Analytical procedure

Column chromatography of the aqueous ethanol extract was conducted using prepared column set-up, the aqueous ethanol extract was run with *n*-hexane, chloroform and methanol in the following ratio: NH/CF (3:2), NH/CF (1:1), NH/CF (2:3), NH/CF (1:4). Also, CF/M (3:2) and CF/M (1:1) were among the fractions obtained. The thin layer chromatography of different fraction obtained from the column chromatography was conducted using prepared TLC plates and two solvent was used to run the TLC: Pet- ether and ethyl acetate at the ratio 4:1 respectively. The extract was spotted using a capillary tube the plates using a carefully ruled line as the starting point. The plates were then developed using the two solvent systems above. The developed chromatograms were dried using a hot air drier and spray using three spraying agent; 5% sulphuric acid, 0.1% sulphuric acid in 99% ethanol and DPPH respectively. The length of distance travelled by the solvent front of each of the spots of each plates were recorded as x cm. thus number of spots obtained in each case was noted and the R_f values calculated in all instances.

Evaluation of Antioxidant Activity

2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) (Sigma-Aldrich) was used as the stable free radical, while the standard drug used for comparison was Ascorbic acid (vitamin C). Solvent used for dissolving the extracts, DPPH and the vitamin C was methanol (sigma- Aldrich).0.01g each of the extract and the vitamin C was dissolved in 10ml of methanol in individual test tubes. Using serial dilution technique, concentrations of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50µg/ml of the extract and the vitamin C were prepared in individual test tubes. Using the same methanol mentioned above, 0.001% of DPPH solution was prepared. To the prepared concentrations of the extracts and vitamin C, 1ml of the 0.001% DPPH solution was added each and mixed thoroughly. The above prepared samples were incubated in an oven for 30 min. The absorbance was taken at 517nm using an UV Spectrophotometer. Similarly, the absorbance of 0.001% DPPH solution was taken at the same wavelength[8].

RESULTS AND DISCUSSION

500g of the crude sample extracted with n-hexane and methanol gave higher yield of the n-hexane portion (3.8%) than that obtained for methanol portion (2.6%). It is expected that fats and other lipid-soluble substances will extract in the n-hexane portion suggesting that the plant contains more non polar component than polar. However, the preliminary phytochemical screening showed that all phytochemicals tested for were present in both the n-hexane and methanol extract (Table No.2). A non polar solvent system (Pet-ether/ethyl acetate at

ratio 4:1) effectively separated the bands in the leaf extract (Figure No.1). On the contrary, a previous study by the retention time for the bands obtained is evident of the different chemical components that present in the sample material (Table No.3). The presence of antioxidant principle in the sample was shown when prominent yellow spots were observed on purple background on prepared TLC plates that was run on Pet-ether- ethyl a-cetate solvent (Table No.4) and sprayed with a free radical, DPPH/ in methanol (Figure No.2). The n-hexane/ methanol solvent extracted samples were comparatively examined for their antioxidant potential, the findings revealed that the n-hexane extract showed better free radical scavenging activity than the ethanol extract which was reflected across the different concentrations (10µg- 50µg) in which the scavenging activity was measured. Previous studies have revealed the antibacterial properties of the two specie of Colanitida fruit[9] Also, the antimicrobial activity of the leaves, stem and bark; of *C. gigantea* has also been documented[10].

Except for anthraquinones, all the phytochemicals reported in this study were earlier reported in *C nitida and C gigantea*[11,12]. A previous study conducted by Odebode[13] identified the presence of phenolic chemical constituents such as catechin, quinic acid, tannic acid and chlorogenic acid in the fruit of *Cola nitida* and *Cola accuminata* using the solvent system ⁄ ratio: *n*-butanol, acetic acid, water; 4:1:5. However, the solvent ratio did not give a good resolution of the TLC band when applied in this study. The antioxidant potential of plant materials have been associated with the availability of Phenolics[14,15]. Epidermologists have observed that diet rich in polyphenolic compounds may result in a positive health effect attributed to their antioxidant properties[16,17]. The study have shown that the leaves of *Cola accuminata* contain chemically active principles which have radical scavenging properties deduced from the yellow spots on purple background in Figure No.2 above.

Table No.1: Weights of Extracts and Percentage Yield

S.No	Extracts	Weight of powder	Weight of extract	Percentage yield
1	*n*-Hexane	500g	18g	3.6%
2	Ethanol	500g	11.5g	2.5%

Table No.2: Results for Preliminary Qualitative Phytochemical Screening

S.No	Tests	*n*-Hexane	Ethanol
1	Carbohydrate	+	+
2	Flavonoid	+	+
3	Cardiac glycoside	+	+
4	Saponins	+	+
5	Tannins	+	+
6	Anthraquinone glycoside	+	+
7	Steroid	+	+
8	Alkaloid	+	+

Key: + Present - Absent

Table No.3: T.L.C of *Cola accuminata*

S.No	N/C	Spraying agent	No of spots	Rf Values
Fraction 1	40:10	5% H_2S0_4	5	0.54, 0.66, 0.79, 0.89, 0.9
Fraction 2	25:25	5% H_2S0_4	4	0.61, 0.80, 0.93, 0.98
Fraction 3	20:30	5% H_2S0_4	2	0.68, 0.98
Fraction 4	10:40	5% H_2S0_4	3	0.42, 0.64, 0.89
Fraction 5	00:50	5% H_2S0_4	4	0.13, 0.55, 0.77, 0.96

N/C: n-hexane/ chloroform, H_2S0_4- Sulphuric acid

Table No.4: TLC-DPPH Assay of *Cola accuminata*

S.No	N/C	Spraying agent	No of Spots	RF Values

Fraction 1	30:20	DPPH	3	0.78b, 0.88b, 0.97b
Fraction 2	25:25	DPPH	2	0.91b, 0.97b
Fraction 3	20:30	DPPH	--	--
Fraction 4	10:40	DPPH	--	--
Fraction 5	00:50	DPPH	--	--
Fraction 6	25:25	DPPH	6	0.67b, 0.86b, 0.95b 0.23b, 0.39, 0.53b

Key: N= N-hexane, C= Chloroform, M= Methanol, b= yellow spot,

R_f value = $\frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$

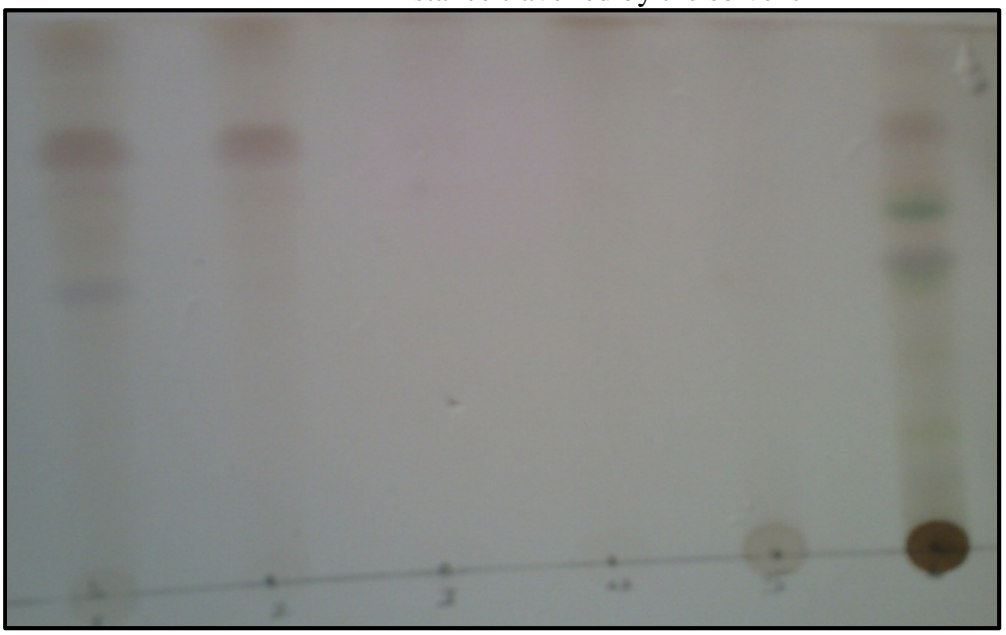

Figure No.1: TLC profile of the leaf extracts of *c. acuminata* sprayed with 5% sulphuric acid

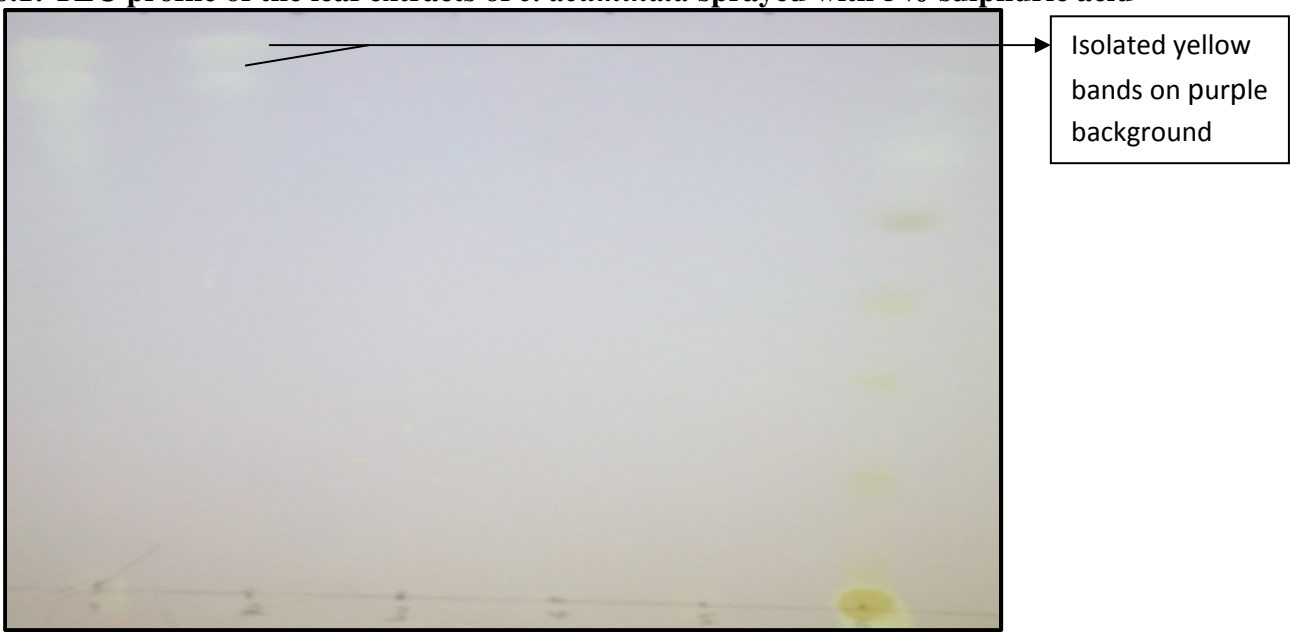

Figure No.2: T.L.C Profile of DPPH/ methanol sprayed Prepared TLC Plate

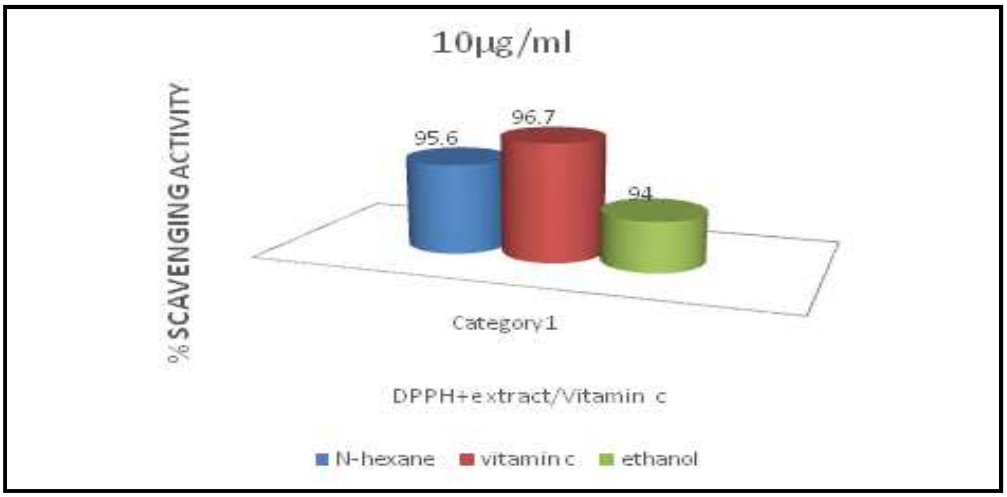

Figure No.3: *In-vitro* evaluation of radical scavenging potential of different extract of *C. acuminata* leaves at10µg/ml using DPPH assay method

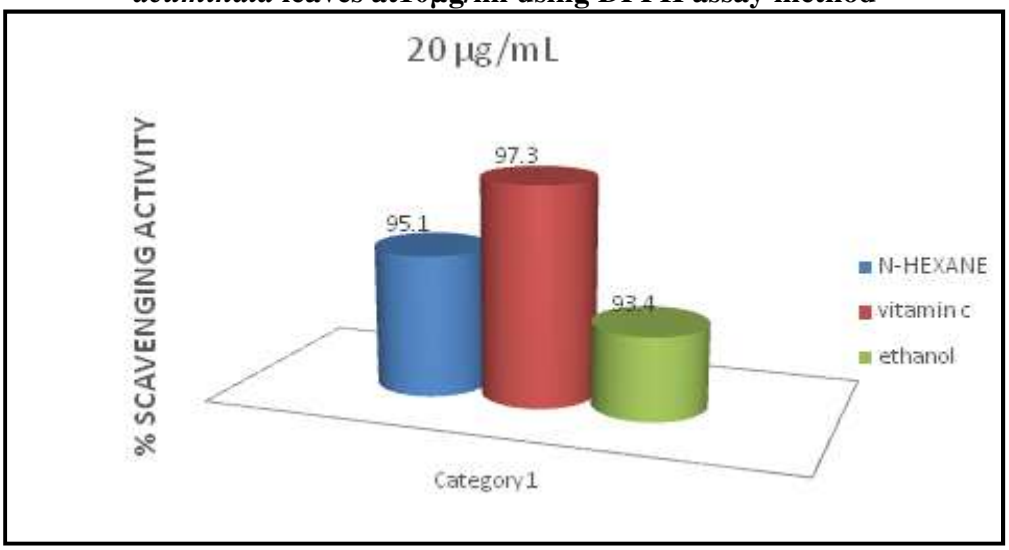

Figure No.4: *In-vitro* evaluation of radical scavenging potential of different extract of *C. acuminata* leaves at 20µg/ml using DPPH assay method

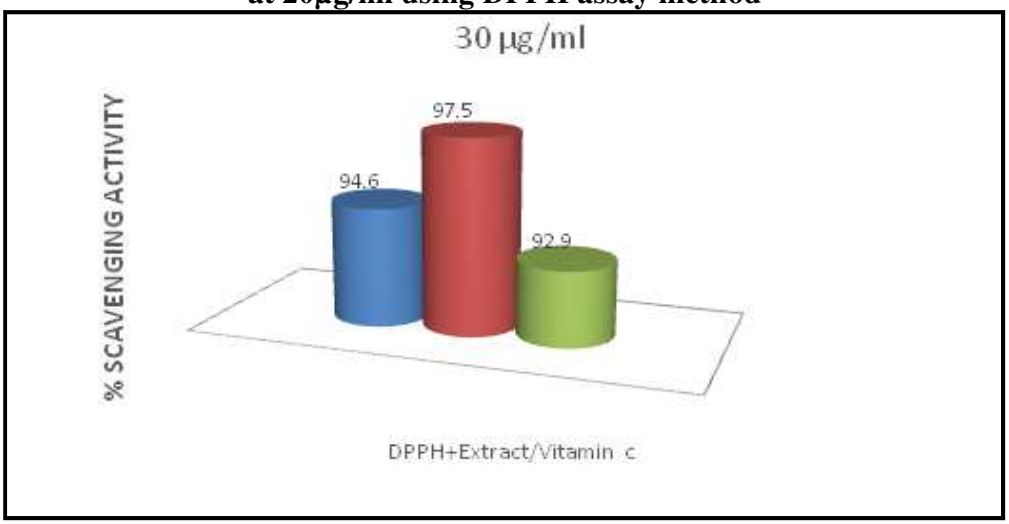

Figure No.4: *In-vitro* evaluation of radical scavenging potential of different extract of *C. accuminata* leaves at 30µg/ml using DPPH assay method

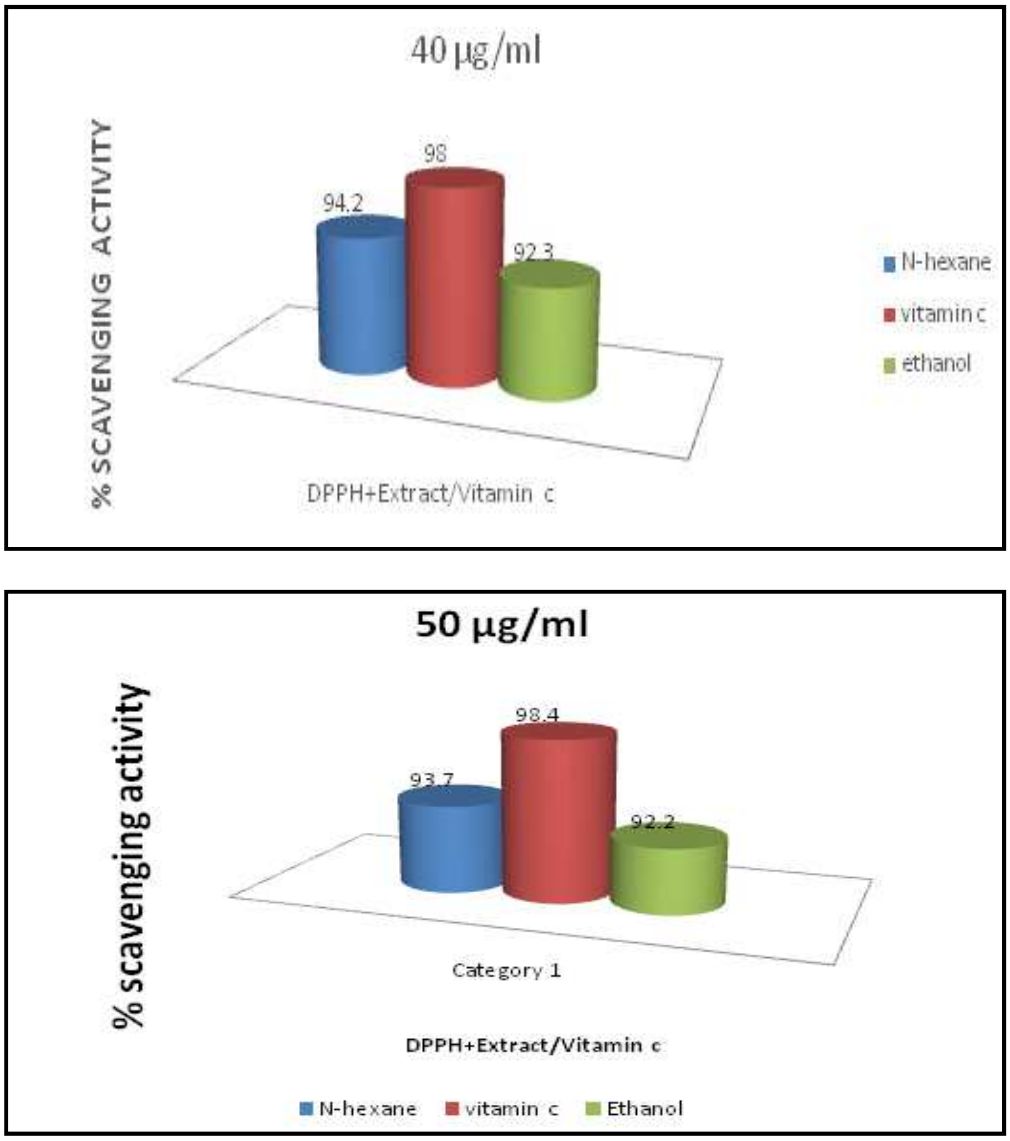

Figure No.5: *In-vitro* evaluation of radical scavenging potential of different extract of *C. acuminata* leaves at 50µg/ml using DPPH assay method

CONCLUSION

The study recorded the presence of vital phytochemicals that can elicit potent physiological responses. Also, the TLC showed that active ingredients could be isolated from the leaves of C. *accuminata*.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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