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Novel catechin ester from the leaf extract of *Chromolaena Odorata* harvested from south eastern Nigeria

Irenus Chinonye IWU[1*], Augusta Adanma UKAOMA[1], Donald Mgbodum EMEZIEM[2], Nneamaka Alice CHIEGBOKA[2], Rita Nwanneamake OZE[1], Ifeoma OBIAGWU[1], Maryann Chioma IGBOMEZIE[1] and Maryjane UYANWA[1]

[1]*Department of Chemistry Federal University of Technology Owerri, Nigeria.*
[2]*Department of Biological Sciences Federal University of Technology Owerri, Nigeria.*
[*]*Corresponding author; E-mail: iwu.chinonye@yahoo.com*

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ABSTRACT

Nigeria is blessed with abundant number of medicinal plant and most of these plants though used locally, has not been fully characterized. There is a need to identify the active compounds responsible for the pharmacological activities of these plants extracts. One such plant is *Chromoleana odorata,* a plant that grows much in the wild of which every of its parts serves as medicine. Our work is aimed at isolating and characterizing bioactive compounds from the leaf extract of this plant. The dried leaf sample was extracted with ethanol, concentrated and re-extracted with chloroform. The extract was partitioned and subjected to column chromatography. The pure compound was obtained from diethyl ether/ chloroform (90;10) ml eluate and was subjected to thin layer chromatography which showed one spot (R_f 0.36), a yellow oily liquid with a mass of 0.58g. Infra-red absorption (cm^{-1}) showed peaks at 801.378, which is out of plane aromatic C-H bending, 1095.837, stretching vibration of alcohols and phenols C─O. 1159.202, 1461.116 vibrations for aromatic C=C , 1654.938 aromatic stretching C=C vibrations , 1744.394 for ester C= O and 2855.141, 2922.233, 3365.786 for alcohol OH vibrations . Proton nuclear magnetic resonance spectroscopy (^{1}HNMR) and carbon 13 nuclear magnetic spectroscopy (^{13}CNMR) spectra of compound were used to elucidate the structure, mass spectrometric analysis gave the mass to charge ratio (m/z) as 346 corresponding to molecular formula $C_{18}H_{18}O_7$. The compound was identified as Catechin -7- methoxy methyl ester.

Keywords: Catechins, Chromatography, Flavonoids, Anti-cancer, Aromatic Stretching, Ester.

INTRODUCTION

Chromolaena odorata (L) King and Robinson is a plant that grows well in the tropics. Though not well regarded by the locals, it persists abundantly in South Eastern Nigeria. It is an important composite of local traditional medicine used for the treatment of malaria, abdominal pain and cancer and as local antiseptic for wounds (Usunobon and Ewere, 2016). Also, it is usually applied to treat skins infection and inflammation (Nur et al., 2020), it has also been reported that the plant has anticancer properties monitored using ethanol leaf extract against cells (Hanifa et al., 2021). The Plant has homostatic and wound healing properties as suggested by Hataichanok et al.

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(2016). Phytochemical screening by Norlizawati et al. (2023) showed that the plant is rich in phytochemicals which showed activities against *S aureus, E. coli, and B. substilius.* The plant possesses antioxidant, antibacterial, molluscidal and larvicidal properties (Phan et al., 2001). Also, it has been reported that the methanolic leaf extract of the plant has activities against *P. aeruginosa* at high concentrations but when combined with certain antibiotics, its properties are enhanced (Hridhya and Kulandhaivel 2017; Odinakachukwu et al., 2019). Studies by Angita et al. (2021) revealed that various parts of the plant have good activities against inflammation, wounds, cancer, diabetics and as fungicides. Asomugha et al. (2013) demonstrated that the leaf extract of the plant is non-toxic at low doses when applied to albino rats. In their review, Nur et al. (2020) confirmed the antiinflammatory, antioxidant, analgesic propertie s and wound healing effects of the plant. They made mention of its usefulness in the treatment of skin infections and related stomach maladies. Similarly, fats obtained from the plant showed anti-inflammatory activities (Tran et al., 2011). Hataichanok et al. (2013) studied the antioxidant properties of the plant. Devi and Sri (2019) studied the anthelmintic properties, anti-malarial, analgesic, anti- inflammatory, antipyretic, anti-fungal properties as well as Vanita and Sana (2018).

With much research done on the plant, yet the constituents of the plant have not been fully characterized. Our research work is aimed at isolating and characterizing the bioactive compounds from the leaf of *Chromolaena odorata* leaf.

MATERIALS AND METHODS

Sample collection

Fresh leaves of *Chromolaena ordorata* were obtained from its natural habitat in Amakohia, Owerri, Imo State, Nigeria. The plant was identified by Dr F. Ibeawuchi of Crop Science Department Federal University of Technology Owerri. The samples were washed, air dried, and pulverized into uniform fine powder using an electric milling machine and stored in a new air tight clean sample container.

Extraction and Isolation of extract for IR, ^{I}H and ^{13}C NMR determination

800 g of the ground sample was percolated in 95% ethanol for 48 hrs and filtered. The filtrate was concentrated using rotary evaporator regulated at 40^{o}C to get a dark extract (23.2 g) of *Chromolaena Odorata* leaves. The extract was partitioned between chloroform and water to obtain chloroform soluble fractions of 10.4 g.

Column chromatography

The chloroform extracts of *C-odorata* was subjected to column chromatography. The column size used was 5 x 90 cm. The chloroform extract was used in a column packed with silica gel (Merck, G60) and eluted with Diethyl ether, 100 ml, followed by varying volume mix of diethyl ether/ chloroform mix and chloroform methanol mix at varying concentrations, the fractions were collected per 5 ml of the eluent vial. Fractions collected were grouped in series after monitoring with thin layer chromatography (TLC) and developed in an iodine tank. Each series was then treated separately to identify the pure samples.

Chromatographic conditions

Analytical TLC was performed on silica gel G 60 F254, 0.25 mm layer using chloroform as the mobile phase.

Detection

Visualized in daylight by viewing in an iodine tank. The retardation factor of pure fractions was calculated with the formula

$$\text{Retardation factor } R_f = \frac{\text{distance travelled by the solute front}}{\text{distance travelled by the solvent front}}$$

IR, ^{I}H and ^{13}C NMR mass spectrometry determination

The pure components obtained from the TLC were subjected to IR, ^{1}H and ^{13}C NMR analysis and mass spectrometry. IR Spectrum

was determined using a thermo Nicolet Nexus 470 FR-IR spectrometer. The ^{l}H and ^{13}C NMR spectra were obtained on a Bruker Avance 400 FT NMR Spectrometer using tetra methyl silane as internal standard. Chemical shifts are expressed in ð values. Liquid chromatography electrospray ionization mass spectrometer (LC – ESIMS) spectra where determined in the positive ion mode on a PE. Biosystem APl 156 single quadruple instrument. High resolution electrospray ionization mass spectrometr (HRESIMS) (positive ion mode) spectra were recorded on a thermo Finniga MAT 95 x L.

RESULTS

The Compound was isolated as a yellow oily liquid with a mass of.0.58g Thin layer chromatography (TLC) carried out on the compound showed one spot in iodine vapour (R_f 0.36)

Infrared (IR) spectrum shows absorption at 864.7cm^{-1} (C =CH) table 1, indicating an out of plane aromatic C—H bending. An absorption at 1095.8 cm^{-1} (C—O) indicates stretching vibrations in alcohol and phenol. Peaks at 1461cm^{-1} (C=C) showing the presence of aromatic, 1654.9 cm^{-1} (C=C) stretching vibrations in aromatic A peak at 1744.4cm^{-1} (C=O indicating. the presence of carbonyl due to ester was seen in the spectrum. The Infrared spectrum depicted the presence of methyl (CH_3) methylene (CH_2) groups with their peaks observed at 2855cm^{-1}and 2922cm^{-1}respectively. Peak showing the presence of alcohol was shown at 3365. 8cm^{-1} (OH)

The analysis of ^{1}H NMR spectrum shown in table 2 depicted a chemical shift at δ 2.077 indicating the presence of methylene (CH_2) group labeled H—H Peaks at methine protons in aromatic ring were seen at δ 4.500,δ 4.610, δ4.800 δ4.900 and δ5.00 and labeled H─6^{i},H─8’ H-2’ ,H-5’ and ,H ─6’, .Presence of methoxy was δ3.727 and labeled H─3’’,.Peaks showing alcohols were seen at δ5.400 and δ5.600 and that of ester was observed at11.600

Analysis of ^{13}C NMR spectrum table 2 depicted the presence of methylene carbon whose absorption was seen at δ27.253 Labeled C—4. Quaternary carbon were found at δ121.300,δ124.200,δ127.000 and δ164.00 labeled C--4a,C─7, C─8a and C-1’’ .The spectrum indicates the presence of methoxy carbon from the absorption at δ70.200 labeled C—3$^{''}$, .Quaternary carbon hydroxy (OH) groups showed their chemical shift at δ80.100,δ82.300, δ82.300 and δ85.000 and were labeled C—5,C—3’ and C—4’.Signals in ^{13}C NMR spectrum confirming substituted benzene were seen at δ114.000,δ117.000,δ118.500 and δ120.000 labeled C—6,C—8,C—2’,C—5’ and C—6’.The chemical shift carbonyl carbon confirming the presence ester seen at δ164.000 labeled C-1’’ .From the analysis of IR,^{1}HNMR and ^{13}CNMR spectra compound A was characterized and proposed as catechin, -7$^{'}$-methoxy,methyl ester with a molecular formula $C_{18}H_{18}O_7$,m/z 346. Figure 1.

Table 1 : Infrared analysis (cm^{-1}) of compound.

IR absorption(cm^{-1})	Functional group	Bond type
801.378	C=C─H	Out of Plane aromatic C-H bending
1095.837	C─O	Stretching vibration im alcohols and phenols
1159.202	C ─O	Stretching vibration im alcohols and phenols
1461,116	C=C	Aromatic
1654.938	C=C	Aromatic stretching
1744.394	C= O	Ester
2855.141	CH	Aliphatic
2922.233	CH	Aliphatic
3365.786	OH	Alcohol

Table 2 : ^{1}H NMR and ^{13}C NMR results.

Position	**^{13}C chemical shift (δ)**	**Types of carbon**	**^{1}HNMR shift (δ)**	**Types of proton**
1	─	─	─	─
2	110.000	O─C─H	2.753	O─C─H
3	112.000	H─C─O	2.500	H─C─O
4	27.255	CH_2	2.077	CH_2
4a	122,300	C	─	─
5	80.100	C─OH	5.700	C─OH
6	114.100	=CH	4.610	=CH
7	121.200	C	─	─
8	115.100	=CH	4.610	=CH
8a	124.00	C	—	—
1’	127.00	C	—	—
2’	117.00	=CH	4.800	=CH
3 ‘	82.300	C—OH	5.400	—C—OH
4’	85.000	C—OH	5.600	—C—OH
5’	118.500	=CH	4.900	=CH
6’	120.000	=CH	5.000	=CH
1’’	164.000	O ‖ O—C—CH_3	11.5000	O ‖ —O—C—CH_3
2’’	165.000	O ‖O—C—CH_3	10.300	O ‖O—C—CH_3
3’’	70.200	—OCH_3	3.727	—OCH_3

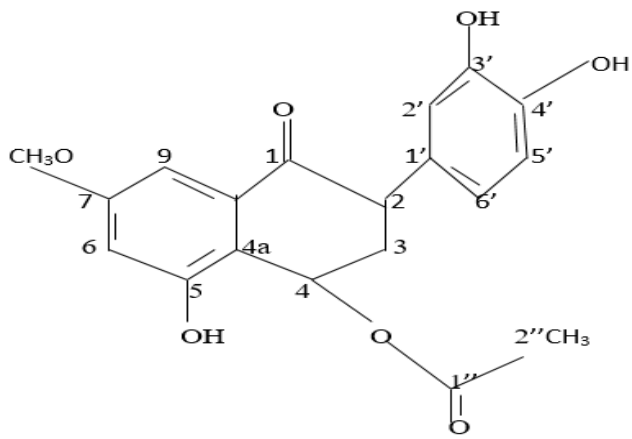

Figure 1 : Catechin -7-methoxy methyl ester.

DISCUSSION

The isolated compound, catechin,7-methoxy, methylester is derivative of catechin. Catechins are polyphenol compounds found in many plants, they are constituents of many tea leaves, especially green teas. Reports have shown that it can be extracted from different plants, and can be used to treat or prevent infectious diseases (Raygaert, 2018). It has antioxidant, antitumor, anti-inflammatory, antimicrobial, an tiviral and anti-obesity properties (Musical et al., 2020). In plant, Catechin acts as an infection-inhibition factor in leaves such as strawberry leaves. It may also prevent diseases in leaves (Yamemeto et al., 2000). Catechins and their isomers are effective as anti-inflammatory, anti-diabetic, anti-cancer, memory enhancers, bactericidal anti-arthritis and hepato protective (Aadrika et al., 2022). The major bioactive ingredients in green tea are the Catechins, thus the anti-carcinogenic and anti-mutagenic activities of green tea were attributed to the presence of Catechins in the leaves

The isolated compound is a flavonoid .Reports have shown that flavonoids help to reduce chronic diseases such as cardiovascular diseases .Flavonoids are phytochemical compounds found in various fruits vegetables ,leaves .They are known to possess medicinal benefits such as antiviral ,anticancer antioxidant and anti-inflammatory activities .Thousands of flavonoids have been isolated from different medicinal plants and this number increase steadily (Asad et al., 2020) *Chromolaena odorata* is widely known for its wound healing property It is used traditionally for the treatment of wounds burns and skin infection (Kavitha et al. 2017 ;Vijayaraghavan et al. 2017).

Conclusion

The isolated compound being a flavonoid must be part of the reason for the antioxidant, anti-microbial, anti-inflammatory cardio protective activities of the plant and could be the reasons for which the plant leaves are used in the treatment of such disease as cancer and inflammation among others.

CONPETING INTERESTS

The authors declare that there are no competing interests.

AUTHORS' CONTRIBUTIONS

Conceptualization/writing of original draft, ICI is the corresponding authors responsible for assembling the various findings into an article. DME and AAU established the methodology and worked on it. RNO conducted the formal analysis. Supervision was done by NAC. IO and MCI carried out the spectra analysis. Finally, MU carried out the editing of manuscript

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