Innovations in Science Education and Practice, 3(1), 2026, 12-29

# Assessment of the bioactive qualities of chemical substances (chromenes) present in Ageratum conyzoides

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**Article History**
Received: 21 November 2025
Revised: 26 December 2025
Accepted: 20 January 2026

**Keywords**
*Ageratum conyzoides*
Assessment
Characterization
Bioactive Components
Extraction
Separation

**Abstract**
*Ageratum conyzoides* is a medicinal plant widely utilized in traditional healthcare systems and recognized for its diverse bioactive compounds. This study assessed the bioactive qualities of chromene-based chemical substances present in *Ageratum conyzoides*. Fresh leaves, roots, and flowers were extracted using chloroform equilibrated with a basic aqueous solution to enhance the extraction of moderately polar metabolites. The resulting extracts were analyzed using Gas Chromatography Mass Spectrometry (GC–MS). Nineteen compounds were identified in the leaf extract, fifteen in the roots, and eight in the flowers, with considerable compositional similarities observed between the root and flower extracts. Prominent compounds identified in the leaves included N-2-fluorenyl acetamide and isoheptadecanol, alongside coumarin derivatives. Several chromene compounds were detected exclusively in the fresh leaves, including 7-methoxy-2,2-dimethylchromene, 6-(1-methoxyethyl)-7-methoxy-2,2-dimethylchromene, 6-vinyl-7-methoxy-2,2-dimethylchromene, and 6-(1-ethoxyethyl)-7-methoxy-2,2-dimethylchromene. Root and flower extracts contained lipid-related isomeric compounds such as butyric acid decyl ester. Additionally, n-butoxyretrocine and two previously reported isomeric pyrrolizidine alkaloids were identified, confirming these as the only alkaloids present in the plant. The predominance of chromenes and lipid compounds highlights the potential non-food and pharmacological applications of *Ageratum conyzoides* and provides valuable insight into its bioactive chemical profile.

## 1. Introduction

*Ageratum conyzoides L.* is a widely distributed herbaceous plant belonging to the family Asteraceae and is commonly found in tropical and subtropical regions of the world. The name *Ageratum* is derived from the Greek words *a* and *geras*, meaning "*non-aging*," a reference to the long-lasting nature of its flowers, while *conyzoides* originates from *konyz*, the Greek name for *Inula helenium*, which the plant is said to resemble morphologically. Despite its widespread occurrence and long-standing use in traditional medicine, *A. conyzoides* remains incompletely characterized in terms of its chemical composition and bioactive constituents.

According to Kamboj and Saluja (2008), the genus *Ageratum* comprises approximately thirty species, yet only a limited number have undergone comprehensive phytochemical investigation. Previous studies have reported that the essential oil of *A. conyzoides* possesses a strong, nauseating odor and exhibits toxicity in experimental animals, attributed largely to the presence of hydrogen cyanide and coumarin derivatives. Dalziel (1937) observed that the plant is generally not consumed by humans as food, although it may be eaten by domestic animals in some cultures. These early observations suggest that *A. conyzoides* occupies a unique position as a medicinal rather than dietary plant, warranting careful scientific evaluation of its chemical constituents.

In southeastern Nigeria, particularly in Orumba North Local Government Area of Anambra State, *A. conyzoides* is used exclusively for ethnomedicinal purposes. Traditional medicine practitioners administer the plant mainly through mastication of the fresh aerial parts. Anecdotal reports indicate that such usage may induce physiological responses such as vomiting, diarrhea, or relaxation, which are interpreted within the traditional context as part of the healing process (Onwudinjo & Nnoli, 2024). Similar medicinal applications have been documented by Igoli et al. (2005) and Kamboj and Saluja (2008), who reported its use in the treatment of wounds, fever, gastrointestinal disorders, and inflammatory conditions. These widespread traditional uses underscore the need for systematic

doi: 10.20961/n13wc209

ISSN: 3108-9097

scientific investigation aimed at identifying and characterizing the compounds responsible for its bioactivity.

Traditional medicine has historically served as a foundation for modern drug discovery, particularly through the isolation and characterization of bioactive natural products. Bioactivity-directed fractionation, which involves the separation of crude extracts into distinct chemical groups followed by chromatographic and spectroscopic analyses, remains a central strategy in natural product chemistry. In the case of *A. conyzoides*, however, significant gaps persist in the understanding of its phytochemical profile, especially with respect to oxygen-containing heterocycles such as chromenes and related compounds.

Several studies have begun to address these gaps. Mao et al (2010) reported that precocene I, a chromene derivative isolated from *A. conyzoides*, significantly reduced the proportion of termite soldiers in colonies, highlighting its potential ecological and pesticidal relevance. Hussien et al. (2010) isolated a novel compound, 5-ethoxy-1H-pyrrol-2(5H)-one, from the dichloromethane extract of the plant, alongside a known compound. The new isolate was characterized as a yellowish oily substance with distinct chromatographic and infrared spectral features, demonstrating that compounds previously thought to be synthetic may also occur naturally. These findings reinforce the chemical diversity of *A. conyzoides* and the need for further exploration.

More recently, Paul et al (2022) reviewed the broader applications of *A. conyzoides* in sustainable agriculture, environmental remediation, and biopharmaceutical development. They emphasized that although numerous studies have focused on phytochemical screening and biological activity assays, limited information is available on the molecular basis underlying the plant's diverse applications. This lack of detailed molecular characterization limits the effective translation of traditional knowledge into safe and standardized therapeutic or industrial products.

The presence of pyrrolizidine alkaloids (PAs) in *A. conyzoides* raises additional concerns regarding its safety. Dipti et al. (2022) identified the plant as a potential source of pharmaceutical raw materials, noting its use by pharmaceutical companies in Brazil under the guidance of the Brazilian Drug Centre. Conversely, Wiedenfeld (2011) cautioned that PAs containing a 1,2-unsaturated necine base are hepatotoxic, carcinogenic, genotoxic, teratogenic, and occasionally pneumotoxic. Such compounds are commonly found in the Asteraceae family, to which *A. conyzoides* belongs. Wiedenfeld further described a public health crisis in the Tigray region of Ethiopia, where contamination of millet by *A. conyzoides* seeds led to fatal PA poisoning. In one reported case, 87.1 mg of PAs were detected in 182 g of millet from a household in which all family members had died following prolonged consumption. These findings underscore the urgent need to clearly identify and quantify the alkaloid content of *A. conyzoides*, particularly in regions where traditional medicine and subsistence agriculture intersect.

Given the dual medicinal potential and toxicological risks associated with *A. conyzoides*, comprehensive chemical characterization is essential. In particular, the identities and distribution of chromenes, pyrrolizidine alkaloids, and related heterocyclic compounds remain incompletely resolved. This study therefore seeks to bridge these knowledge gaps through systematic extraction, separation, and characterization of oxygen-containing heterocycles using gas chromatography–mass spectrometry (GC–MS).

## 1.1. Scope of the Study

This study focused on:

a. Collection and botanical identification of *Ageratum conyzoides* as a source of oxygen-containing heterocycles;

b. Extraction and preliminary separation of these heterocycles using appropriate solvent systems;

c. Isolation of selected components through GC-based separation and cryogenic trapping;

d. Structural elucidation of isolated compounds using mass spectrometry and confirmation through established chemical databases.

# 2. Method

## 2.1. Major Equipment and Instrumentation

The major analytical instruments used in this study included a rotary evaporator fitted with a vacuum system for solvent removal and concentration of extracts. Chemical analysis was carried out using a Shimadzu GC–MS-QP2010 Plus (Japan), equipped with an electron impact mass selective detector (EIMS) operated at 70 eV. The mass spectrometer was set to scan a mass range of 45–400 Da at a rate of 4 scans per second. Infrared (IR) spectra of isolated and partially characterized precocene I were recorded using a JASCO FTIR-460 Plus spectrophotometer.

## 2.2. Study Design and Analytical Approach

The analytical strategy was designed to (i) minimize interference from tannins, polyphenols, and other water-soluble compounds through appropriate solvent selection; (ii) fractionate the crude extracts using efficient separation techniques; and (iii) identify individual components using GC–MS analysis supported by spectral matching with reference databases, including the NIST WebBook.

## 2.3. Sample Collection and Preparation

Fresh samples of *Ageratum conyzoides* were collected from farmland within the Government Reservation Area (GRA), Awka, Anambra State, Nigeria. The aerial parts and root systems were separated and thoroughly washed with deionized water to remove adhering soil and debris. Plant materials were air-dried; roots were subsequently ground into a fine powder prior to extraction.

## 2.4. Extraction Procedure

To ensure exhaustive extraction of plant constituents, approximately 2 kg of air-dried leaves or ground roots were separately extracted using 3 L of chloroform equilibrated with a mixture of 2 L deionized water and 50 mL of 15 M ammonia solution. Extraction was carried out under continuous agitation. Following extraction, the chloroform (organic) layers and aqueous layers for each plant part were pooled separately. The organic extracts were concentrated by solvent evaporation under atmospheric pressure using a water bath maintained below 70 °C. Recovered chloroform was reused for subsequent extraction cycles.

## 2.5. Preliminary Separation by Thin-Layer Chromatography (TLC)

Preliminary separation of extract components was performed using silica gel-coated TLC plates. Various solvent systems were employed, including n-hexane:ethanol (10:1), methanol:aqueous ammonia (200:3), and aqueous acetic acid:n-hexane:ethanol (1:10:1). Developed chromatograms were visualized using iodine vapour, and retention factor (Rf) values were recorded.

## 2.6. GC–MS Analysis

Due to the complexity of the extract mixtures, definitive separation and identification were achieved using GC–MS. Chromatograms were obtained for chloroform, aqueous, and ethanolic extracts of leaves, roots, and flowers. The number of detected components varied across extracts, with chloroform extracts yielding more identifiable peaks than aqueous or ethanolic extracts. These chromatographic profiles were summarized in tables and appendices.

## 2.7. Characterization of Identified Compounds

Mass spectral fragmentation patterns corresponding to each GC peak were analyzed individually. Structural elucidation was achieved through interpretation of molecular ion peaks ($M^+$), isotopic peaks (M+1, M+2), and base peaks, combined with spectral matching against reference libraries and literature data, following established EI-MS principles.

## 2.8. Physicochemical Characterization of Precocene I

Isolated precocene I was further characterized by determining its boiling point using a paraffin bath method, density using a 10 mL microdensity bottle, and refractive index using an Abbé

refractometer. The hydrogen deficiency index was calculated from the molecular formula obtained from mass spectral data, while functional group analysis was confirmed by FTIR spectroscopy.

# 3. Results and Discussion

## 3.1. Distribution of GC-Detectable Components in Ageratum conyzoides Extracts

The number of GC-resolved components obtained from the different solvent extracts of *Ageratum conyzoides* is summarized in Table 1.

**Table 1. Summary of GC peaks obtained from extractions**

| Plant species / Part extracted | Extracting solvent | Number of GC peaks (components) |
|---|---|---|
| AC / leaves | $CHCl_3$ | 16 |
| | Aqueous medium | 5 |
| AC / root | $CHCl_3$ | 9 |
| | Aqueous medium | 6 |

The results clearly indicate that chloroform extracts yielded a higher number of GC-resolvable components than aqueous extracts for both leaves and roots. This observation reflects the effectiveness of chloroform, particularly under basic conditions, in extracting moderately polar and non-polar constituents such as chromenes, aromatic hydrocarbons, and lipid-related compounds. In contrast, aqueous extracts contained fewer components, suggesting that highly polar constituents, including tannins and polyphenols, were largely excluded. This validates the extraction strategy adopted in this study, which aimed to reduce analytical interference and enhance chromatographic resolution.

## 3.2. GC–MS Profile of the Chloroform Extract of Aerial Parts

Among all samples analyzed, the chloroform extract of the aerial parts (Sample C) exhibited the most complex chromatographic profile. The combined GC–MS data for this extract are presented in Table 2.

**Table 2. GC–MS combined data for chloroform extract of aerial parts of *Ageratum conyzoides* (Sample C)**

| GC Component No. | Retention time (min) | $M^+$ | M+1 | M+2 | Base peak |
|---|---|---|---|---|---|
| 1 | 5.467 | 106 | – | – | 91 |
| 2 | 19.933 | 190 | 191 | – | 175 |
| 3 | 21.825 | 189 (est.) | – | – | 43 |
| 4 | 22.483 | 136 | – | – | 41 |
| 5 | 22.758 | 216 | – | – | 201 |
| 6 | 23.225 | 248 | – | – | 233 |
| 7 | 23.642 | 262 | – | – | 247 |
| 8 | 24.342 | 224 | 225 | – | 153 |
| 9 | 24.767 | 223 | – | – | 57 |
| 10 | 25.542 | 270 | – | – | 74 |
| 11 | 25.933 | 256 | 257 | – | 43 |
| 12 | 27.625 | 264 | – | – | 55 |
| 13 | 27.875 | 284 | – | – | 59 |
| 14 | 29.283 | 269 | – | – | 57 |
| 15 | 31.567 | 149 | – | – | 105 |
| 16 | 32.617 | 239 | – | – | 43 |

The retention time range (5.467–32.617 min) reflects the wide chemical diversity of the aerial part extract, encompassing low-molecular-weight aromatic hydrocarbons, substituted benzene derivatives, chromenes, and higher molecular weight lipid-like compounds. The predominance of components with molecular ions above m/z 190 indicates a high abundance of oxygenated aromatic compounds, consistent with earlier phytochemical reports on *A. conyzoides*.

## 3.3. Structural Assignment of GC Component Peak C1

GC component peak C1, eluting at 5.467 minutes, represents a low-molecular-weight and relatively volatile constituent. The mass spectrum exhibited a molecular ion at m/z 106, an even-

numbered mass indicating either the absence of nitrogen or the presence of an even number of nitrogen atoms. Fragmentation of this ion produced a dominant base peak at m/z 91, corresponding to the tropylium ion ($C_7H_7^+$•), which is characteristic of alkyl-substituted benzene rings (McLafferty & Turecek, 1993). Further fragmentation yielded ions at m/z 77 ($C_6H_5^+$) and *m/z* 65 ($C_5H_5^+$), consistent with progressive aromatic ring cleavage. The presence of an (M − 1) ion at *m/z* 105 supports the existence of a benzylic hydrogen capable of rearrangement. Using the relative abundance of the M+1 peak (8.81%), the molecular formula was calculated as $C_8H_{10}$. The short retention time corresponds well with reported values for monoethylbenzenes (Ho, 1990), leading to the identification of peak C1 as ethylbenzene. Although ethylbenzene itself is not a major bioactive compound, its detection highlights the complexity of volatile constituents present in the aerial parts.

## 3.4. Identification and Characterization of GC Component Peak C2 (Precocene I)

GC component peak C2, eluting at 19.933 minutes, was identified as precocene I, a chromene derivative of particular biological significance. The key mass spectral data are presented in Table 3.

**Table 3. Mass spectral data for GC component peak C2**

| Species | m/z | Abundance | Relative abundance to $M^+$ |
|---|---|---|---|
| $M^+$ | 190 | 1.9 | 100 |
| M+1 | 191 | 0.25 | 13.158 |
| M+2 | – | – | – |

The relative abundance of the M+1 peak indicated the presence of approximately twelve carbon atoms, while the absence of an M+2 peak ruled out the presence of heavy heteroatoms such as chlorine or bromine. The fragmentation pattern revealed four major ions at m/z 190, 175, 160, and 132, corresponding to the molecular ion, sequential losses of methyl groups, and loss of a carbonyl group. The base peak at m/z 175 indicates a particularly stable fragment, characteristic of methoxy-substituted aromatic systems.

The predominance of high-mass fragments and the presence of a weak ion at m/z 77 suggest an aromatic heterocyclic structure. Fragment assembly and accurate mass considerations yielded a molecular formula of $C_{12}H_{14}O_2$. The hydrogen deficiency index was calculated as four, consistent with an aromatic ring system. Confirmation of the compound as precocene I was achieved through comparison with the NIST Chemistry WebBook and literature data, notably those reported by Hiramatsu et al (2013), which showed excellent agreement with the experimental spectral features. The identification of precocene I is particularly significant due to its documented insecticidal and anti-juvenile hormone activities, as well as its role in plant defense mechanisms. Its presence in the aerial parts supports traditional medicinal uses of *A. conyzoides* and highlights chromenes as key contributors to the plant's bioactivity.

## 3.5. Recurrent Detection and Pooling of Precocene I

The characterization procedure applied to GC peak C2 was also used for other peaks with similar fragmentation patterns. Notably, GC peak E4 (not shown) yielded identical mass spectral characteristics, confirming it as precocene I. These components were pooled after isolation, and the combined sample produced an identical fragmentation pattern, further validating the structural assignment. Such duplication of peaks may arise from minor chromatographic variations or conformational effects during separation. Several GC peaks displayed lower molecular ion masses at higher retention times than adjacent peaks with higher molecular masses. This phenomenon was observed for peaks C3 and C4 following C2, as well as peaks C8 and C9 following C7. Such inversions have been attributed to hydrolysis of adducts or rearrangements involving hydrogen migration during chromatographic separation or electron ionization (Hites, 1992; McLafferty & Turecek, 1993).

The use of chloroform under basic conditions may facilitate partial hydrolysis reactions. Margolin and Long (1973) proposed that chloroform hydrolysis proceeds through formation of dichlorocarbene, a highly reactive intermediate capable of inducing rearrangements in co-eluting compounds. The positive activation volume reported by LeNoble (1978) supports charge delocalization effects that could contribute to these observations. These mechanistic considerations

provide a plausible explanation for the observed anomalies and reinforce the need for careful interpretation of GC–MS data. The hydrolysis scheme was presented (LeNoble, 1978) in Figure 1.

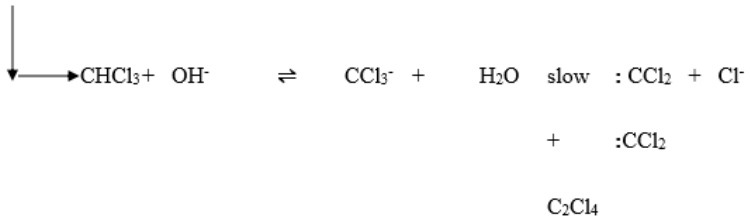


**Figure 1. Hydrolysis scheme**

As the case in D1 extract. All the lipophilic components were extracted into chloroform ($CHCl_3$) solvent from the aerial parts of the plant Ageratum conyzoides and includes sixteen (16) component peaks in the GC. Component peak No. 2 (C2) was identified as 2,2 – dimethyl – 7 – methoxy chromene. The alternative structure, $C_6H_5(CH3)_2$ is ruled out by the (M – 1) ion mass at m/z 105. GC Component peak C2 (retention time 19. 933 min.). The component under this peak was isolated using liquid nitrogen, characterized by several physical methods.

Component C2 (Figure 2)

Molecular mass: 190

Molecular formula: $C_{12}H_{14}O_2$

Compound name: 7-methoxy-2,2-dimethyl-1-benzopyran

CAS number: 17598-02-6

The usual processing of data results in the identification of this heavily fragmented molecule yields results as follows:

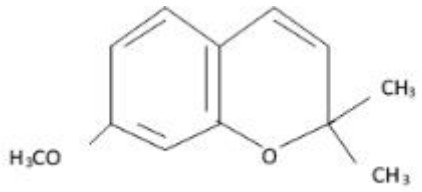


**Figure 2. Structure formula of 7-methoxy-2,2-dimethyl-1-benzopyran**

Other GC component peaks in Extract C (Plant species, Ageratum conyzoides; plant part, aerial parts; solvent, chloroform); from peak C3 to peak C16, appear as a cluster of separate peaks at individual retention times that are very close to each other (peaks C4 to C7) or as unresolved doublets (C8 to C16), the latter within a retention time window where interferences do occur. Considerations of the peaks follow, but assignments would be tentative until supporting evidence is available in all cases so indicated.

## 3.6. GC Component Peak No. C3 (retention time 21.825 min.)

This GC peak is weak. Concentration considerations indicate that the component is a C11 or C12 compound, with one of three possible molecular formulae, namely, $C_{11}H_{11}NO_2$, $C_{11}H_9O_3$, and $C_{12}H_{11}O_2$. The monomolecular ion mass corresponding to each of these three formulae and assigned to m/z 189 is odd and, according to the nitrogen rule, would include at least one N atom. This rule out the other two molecular formulae, leaving one formula, C11H11NO2 for consideration. The loss of 70 Da from the monomolecular ion mass to produce the next lower ion mass at m/z 121 which rearranges to m/z 119 indicates the loss of mass $C_2H_2N$ (43Da) and another mass $C_2H_3$ (27 Da). Both these ion mass peaks appear in the mass spectrum. Characteristic ion peaks at m/z 55 and m/z 109 indicate that the component is an alicylic/olefinic molecule, while peaks at m/z 91 and m/z 119 place it

among monoalkyl benzenes. Thus, the component is complex, consisting of the two. The loss of oxygen indicates the presence of a keto group. Since in alkyl-substituted aromatic compounds, cleavage is very probable at the bond beta to the ring, resulting in a stabilized ion, a base peak is more likely to result from such cleavage, and noting that the component is unsaturated, the molecular structure can properly be represented as a substituted coumarin as shown in Figure 3.

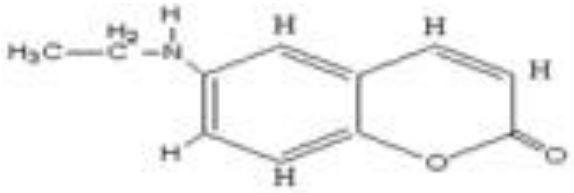

**Figure 3. Structure of N-ethylaminocoumarin (189)**

The 6-amino and 6-acetamido derivatives of chromenes have been reported to have anti-depressant and antipyretic properties (Miller, J. A., and Wood, H. C., US Patent 1968; Kamboj and Saluja, 2008).

## 3.7. GC Component peak C4 (retention time 22.483 min.)

This weak GC peak appeared as an unresolved doublet with a longer retention time of 22.483 min. compared with 21.825 min. for GC peak C3, but with a lower value of ion mass at m/z 136 assignable to the monoisotopic ion mass. The ion mass peak at m/z 136 is indicative of an alicyclic compound with molecular formula $C_{10}H_{16}$. All the characteristic ion mass peaks for acyclics are present in this mass spectrum. These appear at m/z 55, 69, 83, and the cluster of ion masses around m/z 91. This cluster represents rearrangements for stable ion formation in the fragmentation of this highly unsaturated component. The ion masses at m/z 93 and 136 are characteristic of acyclic monoterpenes. The mass at m/z 136 is weak, while those at m/z 69 (strong) and m/z 67 (weak) are both characteristics of ions of monoterpenoids. The base peak is at m/z 41, assignable to the $C_3 H_5^+$ ion. The two pairs of peaks at m/z 105 and 91 as well as those at m/z 69 and 55 indicate the loss of 14 Da each, signifying the presence of two CH2 groups in the molecule. A literature search indicated that these characteristics fit the monoterpene myrcene, a substance that occurs in some natural oils and can form adducts with other compounds such as oxygenated substances (Finar, 2004). The structural formula is in Figure 4.

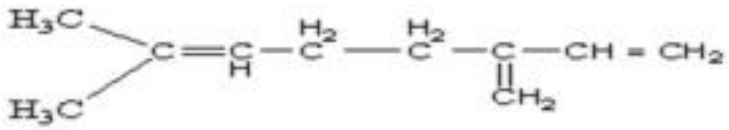

**Figure 4. Sketch of terpenoid.**

This is represented in terpenoid practice as:

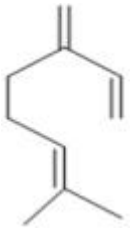

**Figure 5. Systematic name: 7-methyl-3-methyleneocta-1,6-diene**

The component is most likely a monoterpene aglycon of an addict.

## 3.8. GC Component Peak No. C5 (retention time 22.758 min.)

The presence of an M – 15 peaks (loss of CH3) in the fragmentation pattern of this component confirms the assignment of the molecular ion peak to the peak at m/z 216, while concentration values indicate that the component is a C14 compound. The base peak is at m/z 201 which loses 16 Da to give the next lower ion peak at m/z 185, signifying the presence of an oxygen atom in the base peak.

Further fragmentation of the ion mass at m/z 185 indicates a loss of 57 Da to give an ion peak at m/z 128, an ion mass that is characteristic of alkyl-substituted naphthalene or naphthalene-like moieties (Ho, 1990). This ion mass peak at m/z 128 can be assigned to the chromene skeleton that has lost its attachments (three), namely, $CH_3$, O, mass of 57 ($(CH_3)3C$), and thus has the form Figure 6.

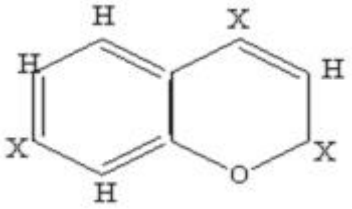


**Figure 6. Chromene skeleton**

The circled xs indicate the three points from which attachments were lost.

The rest of the fragmentation pattern justifies the molecular formula $C_{14}H_{16}O_2$, and the most likely molecular structure in Figure 7

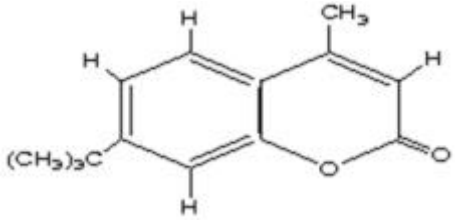


**Figure 7. Structural formula of 4-methyl-7-trimethyl butyl coumarin (216)**

## 3.9. GC Component Peak No. C6 (retention time 23.225 min.)

The ion mass at m/z 248 selected as the monoisotopic ion mass is accompanied by an (M -15) mass loss to give a peak ion at m/z 233. This peak at m/z 233, is the base peak which has also lost an additional 16 Da to give the peak at m/z 217. This would also be interpreted to mean that the molecular ion mass lost a total mass of 31 Da to give the ion peak at m/z 271. Concentration measurements and considerations suggest that the component has a C14 molecular formula which, on treatment using the method of Dromey and Foyster, (1979), yielded the molecular formula $C_{14}H_{16}O_4$. Ion peaks at m/z 248, 233,128, and 91 were selected for monitoring in the retention time window between 22.8 min. and 23.4 min. enabled the assignment of the fragmentation ion which appeared at peaks m/z 128, 91, and 77 as those associated with the fragmentation of chromenes, The two peaks at m/z 59 ($CH_3COO–$) and 43 ($CH_3CO$) denote as attached acetate ($CH_3COO$) group. The structural formula of the chromene ($C_{14}H_{16}O_4$) is:

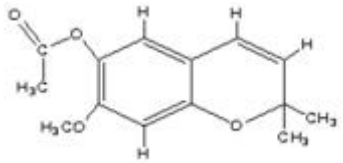


**Figure 8. Structure of 6-acetoxy-7-methoxy-2, 2-dimethyl-2H-chromene (248)**

Some 6-acetyl chromene derivatives were listed by Kamboj and Saluja (2008) as components of the essential oils obtained from Ageratum conyzoides in various parts of the world including Vietnamese oil, Congo oil, and Brazil oil but none was or has been reported for Nigerian oil.

## 3.10. GC Component Peak No. C7 (retention time, 23.642 min.)

The ion mass peak at m/z 262 (monoisotopic ion mass) has lost 15 Da ($CH_3$) to give the next lower ion peak at m/z 247 (base peak). Further fragmentations result in ion mass peaks at m/z 115 and 77 characteristic of chromenes. The loss of 14 Da ($CH_2$) from the ion peak at m/z 217 to give a peak at m/z 203 indicates that the component under the peak is a homologue of component C6, with the molecular formula $C_{15}H_{18}O_4$, and structural formula in Figure 9.

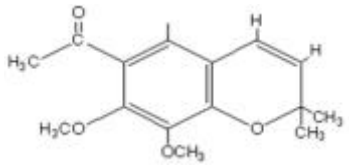

**Figure 9. Structural formula of methylripariochromene (262)**

Pratap and Ram, (2014) have quoted Anthonsen's work (Anthonsen, 1969) in which a component methylripariochromene was extracted and characterized as a chromene from an Australian plant, E. riparium Regel, a member of the Tribe Eupatorieae (same Tribe as Ageratum conyzoides). The same chromene component was also extracted and identified by Taylor and Wright, (1971) from the Jamaican weed Eupatorium riparium Regel. Both groups of researchers used chloroform ($CHCl_3$) as the extract solvent. Methylripariochromene is a derivative of precocene III which has not been identified as such in any of the chloroform extracts obtained in these experiments.

## 3.11. GC Component Peak No. 8 (retention time 24.342 min.) to GC peak C16 (retention time 32.617 min.)

Component peak No. C8 is an unresolved doublet and is the first of the series of peaks C8 to C16 recorded within a retention time window additionally complicated by background noise. The fragmentation pattern of the component under peak C8 is complex, with several ion peaks accompanied by clusters of weak peaks which may be regarded as H-rearrangements but also as fragmentation resulting from the unresolved GC peak. These clusters of peaks which have been notably absent from the GC peaks earlier considered have been a feature in all the GC peaks from C8 to C16, with the sole exception of GC peak No. C15 which is considered as arising from an impurity peak. For instance, the mass spectrum of the component under GC peak C15 includes an ion mass peak at m/z 149 which ordinarily may be assigned to the monomolecular mass of the component. The appearance of this monomolecular mass at a retention time of 31.567 min. is one of the reversals in the mass spectrum that has been noticeable in these spectra. It has occurred long after component peak No. 8 (retention time 24.342 min.; $M^+$ 224); peak No. 9 (retention time 24.767 min.; $M^+$ 223); peak No. 10 (retention time 25.542 min.; $M^+$ 270); peak No. 11 (retention time 25.993 min.; $M^+$ 256); peak No. 12 (retention time 27.625 min.; $M^+$ 264); peak No. 13 (retention time 27.875 min.; $M^+$ 284); peak No. 14 (retention time 29.283 min.; $M^+$ 269).

The loss of 44 Da (COO) from this ion mass at m/z 149 to give a fragment ion at m/z 105 (base peak) indicates that the component under this peak is a phthalate. There is however no reason to expect a phthalate (synthetic product) in a chloroform extract obtained from a natural source. Thus, the appearance of this GC peak No. C8 is implausible unless it is considered as a contaminant peak. More importantly, Hites, (1992) has documented three important ions at m/z 149, 167, and 279 and warned that they individually or collectively indicate a very common contaminant in MS, namely, di (2-ethylhexyl) phthalate, which is present in several polyvinylchloride – based plastic products such as Tygon tubing and or septa. The appearance therefore of these three mass ion peaks at m/z 149, at m/z 167and at m/z 279, in the fragmentation pattern of component peak, the presence of the two mass peaks at m/z 149 and m/z 167 in the fragmentation pattern of the GC component peak C9 where the base peak is at m/z 149, as well the mass ion peak at m/z 149 in the fragmentation pattern of GC peak C15, make assignments of the GC peaks C8 to C16 ambiguous. Further assignments will therefore not be pursued any further in these studies until complementary instrumentation is available for relevant information.

Table 4 lists secondary GC-MS data for the five component peaks obtained in the GC from the aqueous extract of the leaves of Ageratum conyzoides from which some observations can be made. These include the total absence of any retention and fragmentation of mass data that could be linked to precocenes I and II. Three of the five component peaks in Table 4 have prominent fragmentation lines to high masses, indicating either aromatic characteristics or reasonably heavy atom presence in the molecules, while only two have measurable associated M + 1 values.

**Table 4. GC-MS Combined data for Sample D (Aqueous extract of aerial parts of AC**

| GC component no. | GC Retention time (min) | $M^+$ | M+1 | M+2 | Base peak | |
|---|---|---|---|---|---|---|
| 1 | 4.06 | | 164 | 166 | 168 | 94 |
| 2 | 5.467 | | 106 | 107 | - | 91 |
| 3 | 20.775 | | 206 | - | - | 191 |
| 4 | 25.925 | | 256 | - | - | 43 |
| 5 | 27.608 | | 264 | - | - | 55 |

Component D1

This component was extracted in water after equilibration with chloroform, $CHCl_3$. Its monoisotopic ion mass of 164 is even. This is the only case that has a full complement of the characteristic isotopic pattern, namely $M^+$ (164), M+1(166), and M+2(168). All three peaks are spaced 2 mass units apart and occur in ratios characteristic of multi-chloro atoms in the molecule.

Molecular mass: 164
Molecular formula: $C_2Cl_4$
CAS number: 127-18-4
RetIndex: 887
Compound name: tetrachlorothvlene

Details for this non-aromatic compound are (Figure 10).

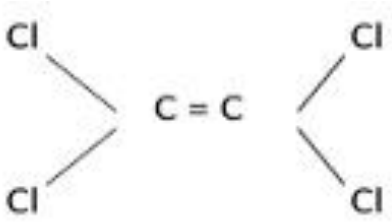


**Figure 10. Tetrachloroethylene**

This is another case of reversal of the retention time/monoisotopic ion mass order, this time between D1 and D2 in this aqueous medium extraction of Ageratum conyzoides. More important is the occurrence of the compound tetrachloroethylene in an aquatic solvent medium (it is known to be immiscible with water in moderate concentrations). A likely explanation for its presence is that it is an artifact of the extraction process.

## 3.12. $CHCl_3$ extract of the root system of Ageratum conyzoides, Sample E (Table 6)

Components E1and E2. These two compounds are either isomers of the same substance or two different substances having the same monoisotopic ion mass 136 but different base peak m/z values, 93 and 95 respectively. The GC retention times are 6.375 min., and 13.892 min., respectively. The degree of unsaturation in the two components is different; the component under peak E1 is more unsaturated than the component under peak E2. These two peaks are the weakest peaks in the GC. The fragmentation patterns of both components under the two peaks exhibit ion mass peak at m/z 93 and at m/z 136, both of which are ions characteristic of monoterpenoid hydrocarbons. That GC peak E1 exhibits a molecular ion M+ 136, has a base peak at m/z 93, as well as a peak at m/z 91 makes the assignment of the component under it as ?-pinene most probable. Thus ?-pinene would be the major component under GC peak E1.

GC peak E2, with its longer retention time of 13.892 min., but the same molecular ion peak M+ at 136, appears to be a monoterpenoid (isoprene) adduct from a prenylated chromene. Such adducts have been reportedly isolated from the genus Piper, including Piper gaudichaudianum (Lago et al., 2004), who gave the structure below to the biologically active chromene which they did not name (Figure 11).

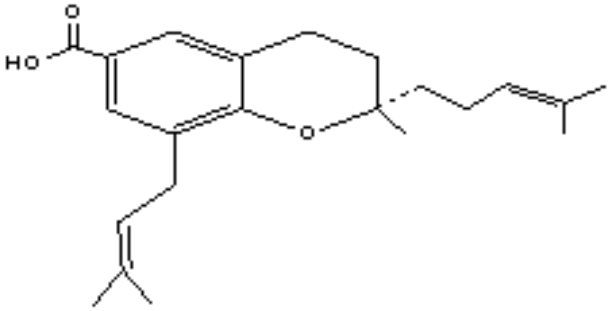

**Figure 11. Structure of biologically active chromene (Lago *et al*., 2004)**

A similar adduct is 5-hydroxy-8- (3I, 7I-dimethylocta-2I, 6I-dienyl)-2, 2, 7-trimethyl -2H -1-chromene which Kitomura et al. (2006) isolated from the methylene chloride extract of the leaves of *Piperonia serpens* (Figure 12).

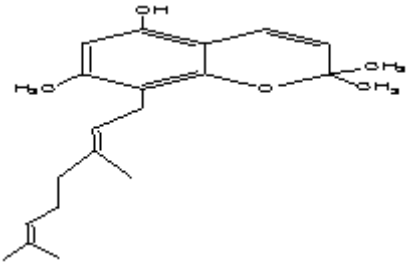

**Figure 12. Structure of 5-hydroxy-8- ($3^{I}$, $7^{I}$-dimethylocta-$2^{I}$, $6^{I}$-dienyl) - 2, 2, 7-trimethyl -2H -1- chromene (Kitomura *et al*., 2006)**

The structures of both compounds were reportedly elucidated by spectroscopic analyses but no names were given. The pinene isomer most likely to be involved in the proposed adduct formation would be the beta isomer with the unsaturation point at the 1, 7 position through which it would form the adduct.


**Figure 13. α- pinene and β- pinene (both isomers occur naturally)**

## 3.13. Component E3 and E5

GC component peak No. E3 (retention time 17.183 min.) and peak No. E5 (retention time 17.750 min.). The components under the two peaks exhibit two different retention times in between which GC peak No. 4 (retention time 17.583 min.) appeared. Both component E3 and component E5 exhibit the same monomolecular ion mass of 204. These two components have two different fragmentation patterns. The ion mass for the two components assigned to m/z 204 as the monoisotopic mass loses 15 Da ($CH_3$) in both cases to give the next ion mass peak at 189. However, the base peak has different values. The component under GC peak No.3 has a base peak at m/z 41 which shows that the unsaturation group $C_3H_5$ is the most stable ion. GC peak component 5 has a base peak at m/z 69. By the nitrogen rule, there is no possibility in either case that the formula (molecular) has any nitrogen (N) content. Additionally, one component, with m/z 41 as the base peak, has no oxygen content, while the other, at m/z 69, contains at least one oxygen (O) atom in its molecular formula. Concentration determinations give the component of the peak at E3 as a sesquiterpenoid, $C_{15}$ hydrocarbon compound, while the E5 component has $C_{13}$ composition.

Appropriate molecular formulae were obtained as:

| | |
|---|---|
| E3: | $C_{15}H_{24}$ |
| E5: | $C_{13}H_{16}O_2$ |
| Compound formula: | $C_{15}H_{24}$ |
| Molecular mass: | 204 |
| Compound name: | caryophyllene (exist in the dl form) |
| CAS number: | 87-44-5 |
| RetIndex: | 1494 |

The corresponding structures are shown in Figure 14 and Figure 15.

E3:

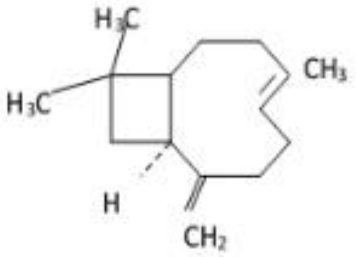


**Figure 14. 4, 11, 11-trimethyl-8-methylenebicycloundec-4-ene**

(α- and β- caryophyllene)

E5:

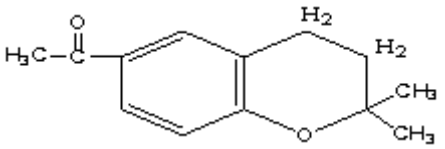


**Figure 15. 6- acetyl- 2, 2- dimethyl- 3, 4- dihydrochroman**

The difference in mass of the base peak in each of the two cases may be rationalized on the basis that component E3 would have an unsaturated moiety ($CH_2$=$CHCH_2$. = 41) as base peak while component E5 would have a branched unit ($CH_3$ – $CH(CH_3)$ – CH = $CH_2$. = 69) as base peak following their structural differences. Kamboj and Saluja (2008) reported that caryophyllene, a macrocyclic sesquiterpenoid, is a major component of the essential oil of *Ageratum conyzoides* found in Cameroun oil and in Pakistani oil. Ekundayo et al (1988) have also reported that 6- acetyl -2, 2-dimethyl-3, 4-dihydrochromene was among the component chromene derivatives isolated from the hexane extract of the aerial parts of Ageratum *conyzoides*.

## 3.14. GC Component Peak No. E4

The component under GC Peak No. E4 was identified as precocene I. The inversion in the order of increasing molecular mass with increasing retention time between GC peak No. E3 and GC peak No. E4 may thus be interpreted as arising from a difference in functional groups present in the components under each of the two peaks. GC peak No. E4 is the highest concentration in the chloroform ($CHCl_3$) extract of the root system of *Ageratum conyzoides*.

## 3.15. GC Components E6 – E9

These four GC peaks have occurred within a retention time window in which interferences have been pointed out as occurring. Identification within this window is therefore unreliable and will not be presented. GC peak E6 has a retention time of 23.325 min. within a time range that is congested in spectrum C, and again "clear" in spectrum E. GC peak E6 occurs as the lowest concentration of these two GC peaks, namely, C9 and E6. The fragmentation patterns in the mass spectra of each of

the two GC peaks show the same two prominent peaks, namely m/z 57 and m/z 149; all other MS peaks are 50% (m/z 41) abundant or less. Peaks at m/z 27 and m/z 29, present in the GC C9 chromatogram, are both absent in the E6 mass spectrum which has an additional peak at m/z 175 not present in the C9.

A check on two databases produced a search result of at least 135 matching species, and the concentrations of both the monoisotopic ion peak and the M+1 ion peak were so weak that no measure of reliability could be placed on their concentration measurements. Approximate measurements, however, gave a value of ten carbon (C10) in the molecule. Since no halogen was detected in a preliminary test of the chloroform extract, C9, as well as the ethanol extract, E6, all the search results that contained halogens were eliminated, and attention was focused on those that had associated amide group, resulting in the choice of the compound N-2-fluorenyl acetamide of structural formula C15H13ON (223) can be seen in Figure 16.

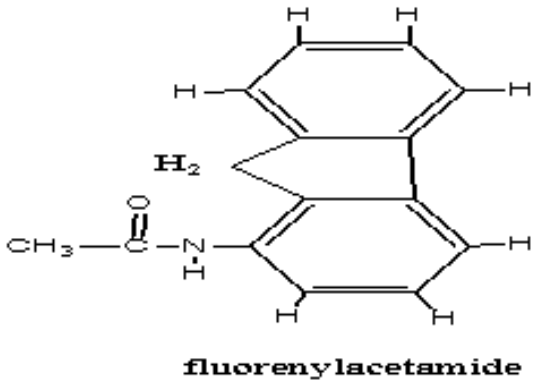


**Figure 16. Thus, C9 is the compound, N-2-florenylacetamide.**

Compound E6 is slightly different. It has an additional peak corresponding to the (M-48) ion at m/z 175, (not present in the C9), and no peaks at m/z 27 and m/z 29, the latter being significant in indicating the presence of an oxygen-containing ion associated with a carbon atom. GC-MS Combined data for CHCl3 extract of the root system of Ageratum conyzoides (Sample E) can be seen in Table 5.

**Table 5. GC-MS Combined data for $CHCl_3$ extract of the root system of Ageratum conyzoides (Sample E)**

| GC component Peak no | GC Retention time (min) | $M^+$ | M+1 | M+2 | Base Peak |
|---|---|---|---|---|---|
| 1 | 6.375 | 136 | - | - | 93 |
| 2 | 13.892 | 136 | - | - | 95 |
| 3 | 17.183 | 204 | 191 | - | - |
| 4 | 17.583 | 190 | - | - | 175 |
| 5 | 17.750 | 204 | - | - | 69 |
| 6 | 23.325 | 223 | - | - | 57 |
| 7 | 24.583 | 256 | - | - | 73 |
| 8 | 26.125 | 316 | - | - | 301 |
| 9 | 29.075 | 295 | - | - | 55 |

Comparison of the data in Table 6 with data in Table 5 in which data for the CHCl3 extract have been presented, shows clearly that component peaks Nos. 1 – 6 in Table 5 are completely absent from Table 6, indicating the differences in solubilities of components in the two solvents used. Component peak No.7 in Table 5 has the same value of retention time (24.583 min.) as component peak No. 1 in Table 7, with the same value of m/z 256 (M+) but different base peak values. This would indicate the same molecular ion mass values but different stable ions and hence different molecular forms and solubilities. The same inference can be made for component peak No.9 in Table 5 and component peak No. 5 in Table 6.

Both peaks recorded the same retention time of 29.075 min., the same m/z 295 for M+, and the same base peak value of m/z 55. Whereas however Table 6 records all data in the formal ascending order in both retention time and molecular ion mass, two inversions of the order are noticeable in the data in Table 5 including that involving peak Nos. 7, 8, and 9. As already remarked, such reverses

of elution order are traceable to several factors such as migration of atoms (for example H) or group of atoms (for example methyl) to form more entities, or interaction with the material of the separating column resulting in bleeding of adsorbents, or the splitting of adducts of, say, sugars with other interacting substances (McLafferty and Turecek, 1993). Hites, (1992) has detailed matters that can go wrong in GC-MS experiments and offered suggestions for amelioration some of which have been observed in these Table 6.

**Table 6. GC-MS Combined data for EtOH extract of the root system of (AC) (Sample F)**

| GC component Peak no | GC Retention time (min) | $M^+$ | M+1 | M+2 | Base Peak |
|---|---|---|---|---|---|
| 1 | 24.583 | 256 | 257 | - | 43 |
| 2 | 26.308 | 282 | - | - | 55 |
| 3 | 26.533 | 284 | 285 | - | 43 |
| 4 | 27.600 | 269 | - | - | 43 |
| 5 | 29.075 | 295 | - | - | 55 |
| 6 | 29.283 | 297 | - | - | 43 |

Data in Table 7 have been presented to enable confirmation of chloroform (CHCl3) as the only solvent among others used in these experiments that has been able to extract chromenes.

Aside from two GC peaks, namely, that at peak No. 1 (retention time 14.275 min; M+ 150) and at peak No. 8 (retention time 30.783 min, M+ 265), the other six (6) GC peaks in Table 7 occur as a replica of those in Table 6.

**Table 7. GC-MS Combined data for EtOH extract of fresh flower of Ageratum conyzoides (sample G)**

| GC component Peak no | GC Retention time (min) | $M^+$ | M+1 | M+2 | Base Peak |
|---|---|---|---|---|---|
| 1 | 14.275 | 150 | 151 | - | 77 |
| 2 | 24.592 | 256 | - | - | 43 |
| 3 | 26.308 | 282 | - | - | 55 |
| 4 | 26.533 | 284 | - | - | 43 |
| 5 | 27.600 | 269 | - | - | 57 |
| 6 | 29.075 | 295 | - | - | 55 |
| 7 | 29.283 | 297 | - | - | 57 |
| 8 | 30.783 | 265 | - | - | 55 |

Component G1. Identification details are as follows (Figure 17)

Molecular mass: 150
Molecular formula: $C_9H_{10}O_2$
CAS number: 7786-61-0
RetIndex: 1293

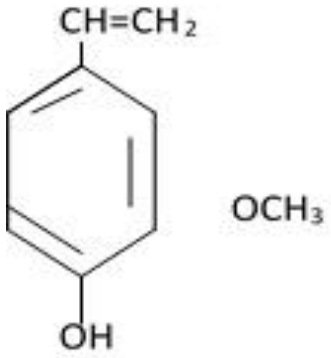


**Figure 17. 2-methoxy-4-vinyl phenol or 4-hydroxy-3-methoxy styrene**

The G2 component appears in the GC as a doublet that is not fully resolved. The component may consist of two isomers, with two slightly different retention times. One isomeric form of the substance with a C16 content is thus: 2-ethylbutyric acid, decyl ester, with base peak at m/z 73, and

molecular formula, $C_{16}H_{32}O_2$; the other isomer is isoheptadecanol, formula $C_{17}H_{36}O$. This is the isomer with a base peak of m/z 43.

**Table 8. Partially resolved GC component peaks show the retention time range in which each occurs.**

| Sample extract | Details | GC Peak No. | Retention time range (min) |
|---|---|---|---|
| A | $CHCl_3$ extract of A.C. leaves | 12 (m) | 27-28; Peaks skewed at lower time side |
| B | Aqueous extract of A.C. leaves | 5 (m) | 27-28; Peaks skewed at lower time side |
| C | $CHCl_3$ extract of root of A.C. | 9 (w) | 28-29; Peaks skewed at lower time side |
| D | EtOH extract of root of A.C. | 2 (s) | 26-26.5; Peaks skewed at lower time side |
| | Ditto | 5 (s) | 29-29.5; Peaks skewed at lower time side |
| E | EtOH extract of flower of A.C. | 2 (s) | 24-25; Peaks skewed at lower time side |
| | Ditto | 3 (s) | 26-26.5; Peaks skewed at lower time side |
| | Ditto | 6 (m) | 29-29.5; Peaks skew at lower time side |

Peak concentrations: s, strong; m, medium; w, weak

For each of these peaks therefore any given time must necessarily refer to the major component. The partially resolved GC components were emphasized for several reasons, in particular, for the fact of the history of as well as the considerable stir caused by the sensation, thalidomide, once touted as "the baby-sitter's friend". Quoted as follows:

> "in 1958, this drug (thalidomide) was launched in Germany in a blaze of publicity by the company Chemie Grunenthal, who even felt it was safe enough to be sold without prescription under the brand name Contergan. Within a few years, it was available in over 40 countries. The liquid form of thalidomide gained a reputation I Germany as the baby-sitter's friend because it was superb at calming young children and sending them to sleep. Chemie Grunenthal was so confident that Contergan was safe that it was advertised with a picture of a child taking a bottle from a shelf with the claim: 'completely harmless, even for infants'. Doctors and patients were unaware of the dreadful side-effect of this drug- it was a teratogenic compound. This is the technical term for a substance that causes a developing foetus to become deformed. Tet Chemie Grunenthal had a superb product in thalidomide, and what they did not realize was that while half the molecules in thalidomide were safe, the other half were anything but (safe). The two forms of thalidomide are identical except for one minor detail: they come as left-(L, from the Latin for left) and right-handed (D, dexter) pairs, Chemists talk of such pairs of molecules in terms of one being the mirror image of the other. They label them not as left or right but as S (from the Latin sinister) or R (rectus). R- laevo thalidomide was fine, but the S-thalidomide could interfere with the development of the foetus and deform it. In the form in which it was manufactured, thalidomide consisted of both R and S molecules in equal numbers, and back on a commercial scale, even if they had known that this was necessary. Advances in chemical research over the past 40 years have brought such achievements about".

Is deemed necessary to have recourse to these advances in chemical research which the chemist can muster in order to fully exploit these partial resolutions and to further extend the frontiers presented by these components. Who knows? Anecdotal stories of possible alleviation of the AIDS pandemic to which we alluded in the literature review may have their origins in these unresolved or partially resolved components to which no references have previously been made in the literature, to the best of our review knowledge. A fractionator in the GC-MS equipment is expected to aid in this quest whenever available. Thalidomide has the chemical formula $C_{13}H_{10}N_2O_4$. Its molecule mass of 258 Da is only 2 Da away from, say, the monoisotopic ion mass for GC components C11, D4, E7, F1, G2 (M+, m/z 256)!

## 3.16. Contributions to Knowledge

a. The solvent used for extraction in this experiment has enabled the extraction reasonable quantity of pure precocene I and not as an addict. This method of extraction would certainly be of help in future attempts to further study the chemistry of simple chromenes.

b. The retention time parameter has not been available for these chromenes in the literature available to us. precocene I have been characterized in terms of physical parameters such as the

retention time and this is a development that will ease the extraction and isolation of this substance whenever it is required for further studies.

c. The extraction procedure adopted in these studies has resulted in the identification of a chloroform-extractable metabolite from AC. The Metabolite in question is N-2-fluorenylacetamide which is believed to be a potential carcinogenic and mutagenic derivative.

## 3.17. Recommendation for Further Work

a. increased quantities of the component should be run to ameliorate the difficulty in the identification of monoisotopic ion peaks.

b. The instrument should be run in the select-ion monitoring (SIM) mode to provide much greater sensitivity than the scan mode, or even apply the fast-automated scan/SIM type (FASST) data acquisition technique which the SHIMADZU PLUS accommodates in order to ameliorate the difficulty in the identification of monoisotopic ion peak.

## 4. Conclusion

The study enables us to advocate that natural product mixtures intended for screening purposes can and ought as much as possible to be separated from pure substances and, whenever possible, be associated with specific structures for ease of determination of structure-activity relationships. Of the three solvents used in our extraction experiments, namely, chloroform ($CHCl_3$), ethanol, and water, the greatest number of components have been obtained from AC using chloroform as extracting solvent. This has been the case whether extraction has been affected using pure solvent, or whether equilibration has accompanied each extraction. The use of basic aqueous medium in the extractions has enabled the suppression of components such as sugar as well as tannins which would have complicated further analysis of components obtained from the extractions had they been co-extracted. Such basic media has led to partial hydrolysis of components such as terpene phenolic compounds, certain glycoside linkages, or even some alkaloid side chains. Such fall-outs are not unexpected.

Ageratum conyzoides, on the other hand, gave sixteen (16) chloroform-extractable components in the basic aqueous medium. Using the retention time as an index, this resulted in a total of nineteen (19) chemical entities from the original twenty-one, with fourteen (14) extracted solely into Chloroform, three (3) into the basic aqueous medium, and two (2) extracted partly into chloroform and partly into basic aqueous medium. A total of eighteen (18) extracted using EtOH. The only reported use of a solvent similar to chloroform, namely, $CH_2Cl_2$, was for reinvestigation and not a total extraction as has been done here. All the major GC peaks occurring in the various extracts appear within the retention time range of 20-30 min., with a considerable number, showing evidence of partial resolution. This points to the possible existence of the chemical entities concerned with isomeric species. Whether structural or stereochemical. Such speaks are listed in Table 8.

One of the suspected components, extracted from the leaf system of AC, was identified as N-2-fluorenylacetamide. It was extracted using chloroform but was not extracted in a basic aqueous medium. An isomer of this compound, naphthalene sulphonamide, was extracted from the root of AC, also using chloroform as extracting solvent, and present as a minor component. The second major component present in the plant has been identified as isoheptadecanol. This compound was extracted also from the leaves of AC using both chloroform and essential aqueous medium, including EtOH. An isomeric form of the compound was also identified in the root extract of AC and extractable only in $CHCl_3$. It is 2-ethylbutyric acid, decyl ester ($C_{16}H_{32}0_2$). A substituted isoheptadecanol, namely, isoheptadecanol methyl ether, was also identified in the AC leaves but has thus far not been characterized in terms of its Chemical Abstract Service (CAS) number or Ret (Retention) Index. The search has so far not turned out any similar compound in any of the libraries consulted. This is the same with the compounds N-2-fluorenyl acetamide and n-butyl ethyl ketone.

## Author Contributions

All authors have equal contributions to the paper. All the authors have read and approved the final manuscript.

## Funding

No funding support was received.

## Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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