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# Gas Chromatography-Mass Spectrometry Determination of Bioactive Phytocompounds in Chromolaena Odorata Leaf Extract

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***Abstract*— Gas chromatography-mass spectrometry method was used in the analysis of Chromolaena odorata (Siam weed). The extract was prepared using soxhlet extraction method and concentrated at 40ºC in an oven. The concentrated extract was analyzed using GC-MS. The gas chromatogram showed the presence of ten compounds. The compounds are histamine (1.37%), 2-ethyl-2-hexen-1-al (3.91%), 1H-indole-4-carboxaldehyde (11.38%), p-cresidine (4.71%), hexadecanoic acid (17.37%), hexadecanoic acid, ethyl ester (14.17%), 9,12-octadecadienoic acid, methyl ester (13.53%), 2-ethylhex-3-enal (9.35%), 9,12,15-octadecatrien-1-ol (19.52%) and sarcosine, N-(2-methoxybenzoyl)-, octyl ester (7.32%). The result showed that Chromolaena odorata contains pharmacological active compounds that may enhance its use as a traditional drug. Isolation and synthesis of these bioactive compounds is recommended.**

***Keywords***— Chromolaena odorata, gas chromatography, mass spectrometry, bioactive, extraction.

## I. INTRODUCTION

Chromolaena odorata (Siam weed) is an invasive weed of field crop in rainforest zone of Africa [1]. It has been reported to be the most problematic weed in Africa [2]. It has a fast growth rate of about 20 mm per day. It is a major pest to crops such as coconut, rubber, tobacco and sugarcane. It has a prolific seed production and its minimum life span is ten years. It grows up to 2m tall [2]. The leaves of Chromolaena odorata are arrowhead shaped, 50 – 120 mm in length with a width of 30 -70 mm [2]. Chromolaena odorata is a herbaceous perennial plant with an aromatic smell [3]. Igbo's in Nigeria call it ''Obuinenawa'' while the Yoruba's Ijebu call it ''Ewe Akintola". The picture of Chromolaena odorata is shown in Figure 1.

**Fig. 1:** ***Chromolaena odorata plant***

Traditionally, Chromolaena odorata extract has been used in the treatment of ailments such as malaria, dysentery, toothache, diarrhea, diabetes, skin diseases, fever and wound dressing [3 – 5]. Most Nigerian populace depends on natural products in treatment of ailment because of the high cost of orthodox drugs. The healing ability of plant extract depends on their secondary metabolites. Studies have shown that Chromolaena odorata contains reasonable amount of flavonoids, tannis, steroids, phenolics and saponins [6]. In Thailand, the leaf extracts of Chromolaena odorata is used in the treatment of wounds, rashes, diabetes and as an insect repellant [7]. The nematicidal, fungicidal, ethno-pharmacological activity and soil fertility of Chromolaena odorata has been reported [8,9]. The medicinal properties of Chromolaena odorata extracts cannot be unnoticed in Nigeria. Its leaf extract helps in the prevention of blood loss from wounds [10]. Anthemintic properties of aqueous extract of Chromolaena odorata has also been reported [10]. The potentials of Chromolaena odorata leaf meal has been evaluated [11]. From the results, the egg quality characteristics of dietary inclusion of Cromolaena odorata leaf meal, would not compromise egg quality characteristics like egg weight, shell thickness, albumen height and Haugh's unit [11].

In continuation with the ongoing research on Chromolaena odorata extract, we have decided to report the gas chromatography-mass spectrometry determination of

bioactive phytocompounds in Chromolaena odorata leaf extract.

## II. METHODOLOGY

*Plant Materials*

Fresh leaves of Chromolaena odorata was harvested at Ohafia town in Abia State, Nigeria. The plant leaves were identified at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

*Preparation of Plant Extract*

Chromolaena odorata was dried in a shady place for 15 days and pulverized to powder using electrical grinder. Extraction was performed using soxhlet method [12]. Thirty five grams (36 g) of powdered sample was introduced into the extraction chamber of the soxhlet extractor using ethanol as solvent at a temperature of 70o C for 48 hrs. At the end of the extraction, the extract was concentrated in an oven at 35oC. Dried extract was sent for GC-MS analysis.

*GCMS analysis of Chromolaena odorata*

GC-MS QP2010 Plus (Shimadzu, Japan) was used in the characterization. The identification of the photochemical in the sample was carried out using a QP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was 70eV. Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60m, XTI-5).The initial column temperature was 80oC for 1min, and then increased linearly at 70oC min-1 to 220oC, held for 3 min followed by linear increased temperature 10oC min-1 to 290oC for 10 min. The temperature of the injection port was 290oC and the GC-MS interface was maintained at 290oC .The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min-1.

*Identification of phytocompoments in Chromolaena odorata*

The GC-MS chromatogram of ethanol extract of Chromolaena odorata was compared with the database of National Institute of Standards and Technology (NIST), NIST08.LIB [13], WILEY8.LIB [14] and with published literature. The name, molecular weight, formula, structure and bioactivities of the compounds were ascertained.easy way to comply with the paper formatting requirements is to use this document as a template and simply type your text into it.

## III. RESULTS

Chromolaena odorata gas chromatogram is presented in Figure 2. The mass spectra data of Chromolaena odorata leaf is show in Figure 3.

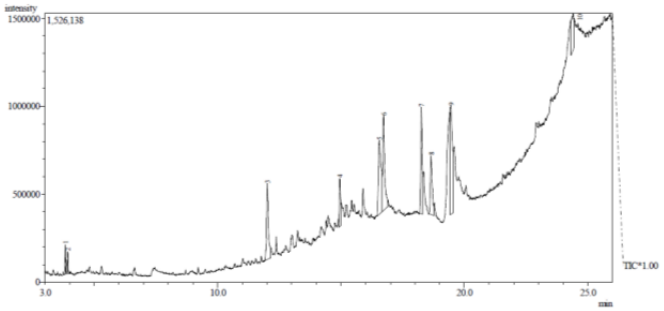


**Fig. 2: Gas chromatogram of ethanol extract of *Chromolaena odorata* leaf**

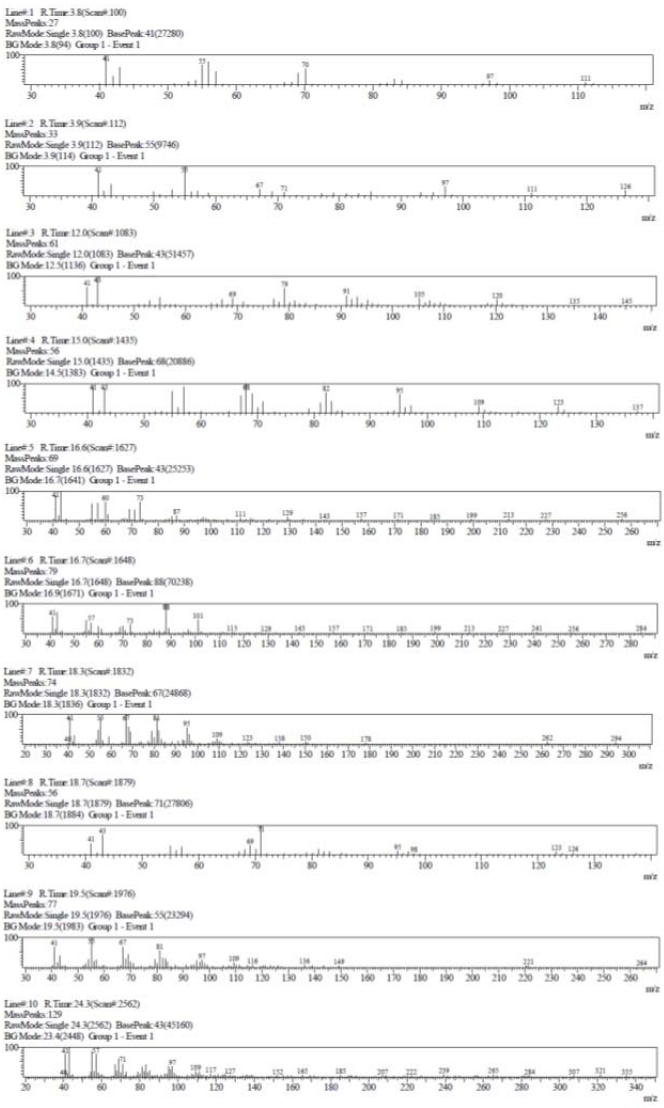

**Fig. 3: Mass spectra of *Chromolaena odorata* leaf extract**

## IV. DISCUSSION

Ten peaks were shown in the GC result. This implies that ten phytocompounds are present in the ethanol extract of Chromolaena odorata leaf. The suggested compounds, retention time, peak area, molecular weight, molecular formula and bioactivity have been tabulated (Table 1). The structures of the suggested phytocompounds are presented in Figures 4 – 13.

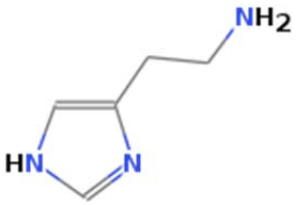


**Figure 4: Histamine**

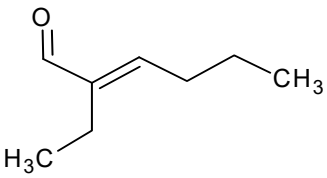


**Figure 5: 2-Ethyl-2-hexen-1-al**

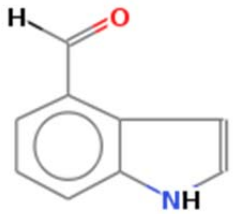


**Figure 6: 1H-Indole-4-carboxaldehyde**

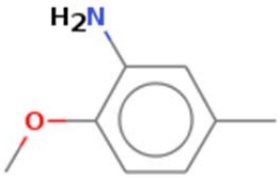


**Figure 7: p-Cresidine**

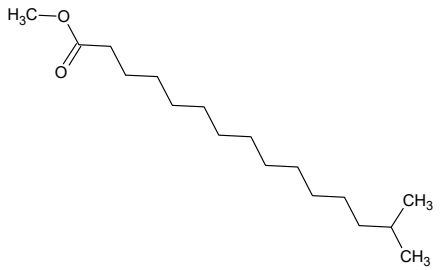


**Figure 8: Hexadecanoic acid also known as Palmitic acid**

**Figure 9: Hexadecanoic acid, ethyl ester**

**Figure 10: 9,12-Octadecadienoic acid, methyl ester**

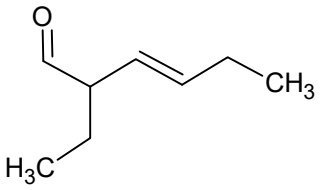


**Figure 11: 2-ethylhex-3-enal**

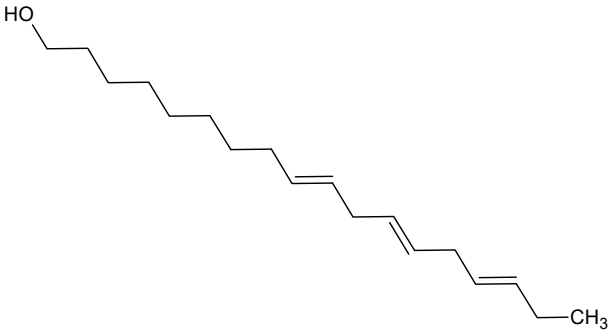


**Figure 12: 9,12,15-Octadecatrien-1-ol**

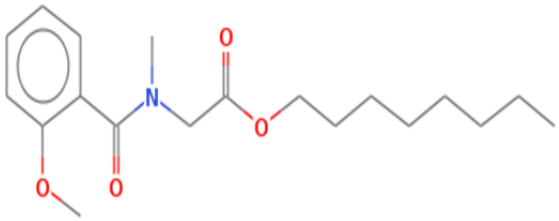


**Figure 13: Sarcosine, N-(2-methoxybenzoyl)-, octyl ester**

Antihistamines are compounds that inhibit histamine receptors in the body. They are used in the treatment of allergic nose reactions, insomnia, motion sickness, peptic ulcers and acid reflux. Chromolaena odorata leaf ethanol extract may act as histamine inhibitor because it contains histamine.

Hexadecanoic acid and hexadecanoic acid, ethyl ester have been reported to be acidifier, acidulant and arachidonic acid inhibitor [15]. Acidifiers are chemicals that reduce the pH of the body. They help in food digestion in patients suffering from achlorhydria . These patients are not able to secret HCl for food digestion. These compounds may be beneficial since they increase gastric acid when ingested. Arachidonic acid is present in the brain, muscles, and liver [16]. Arachidonic acid is a fatty acid that is polyunsaturated in nature and responsible for the repair and growth of skeletal body tissue [17]. Arachidonic acid does not cause cancer but studies have proven that it might be a major cause of inflammation [18 -21]. Additives that reduce the pH of food in order to add a tart taste and characteristic tang are called acidulants. They also have preservative and antioxidative properties [22].

Pancreatic cancer is the fourth most common cause of death in the world. Statistics have shown that 266,000 and 331,000 patients died of pancreatic cancer in 2008 and 2012 respectively [23, 24]. Survival rate of pancreatic cancer is low because diagnosis is always at an inoperatable stage [25, 26].

Catechol-o-methyl-transferase is involved in the degradation of neurotransmitters. 9,12-Octadecadienoic acid, methyl ester , a catechol-o-methyl-transferase-inhibitors opposes the degradation of neurotransmitters. Parkinson's disease is treatable with catechol-o-methyl-transferase-inhibitors [27].

Anaphylaxis is a serious infection that might lead to death if not checked because its duration is within minutes to hours [28]. This allergic reaction is triggered by insect bites, medication, food or any foreign substance [29, 30]. It is an allergic reaction that is accompanied with low blood pressure, itching, swelling of the tongue, shortness of breath, vomiting, and lightheadedness [31, 32]. Sarcosine, N-(2-methoxybenzoyl)-, octyl ester with retention time 24.41 minutes and peak area 7.32% is an antidote for anaphylaxis infection.

9, 12, 15-Octadecatrien-1-ol, one of the isolates of Chromolaena odorata with molecular weight 264.44 and peak area 19.52% , is an oligosaccharide provider [15]. It helps in cell division and cell binding. It is also improves gastrointestinal health, energy levels and performance. Oligosaccharide provider simply means little or few sugar [33].

**TABLE I**
**SUGGESTED COMPOUNDS, RETENTION TIME, PEAK AREA, MOLECULAR WEIGHT, MOLECULAR FORMULA AND BIOACTIVITY OF CHROMOLAENA ODORATA**

| S/No | Name of Compound | Retention time | Peak area (%) | Molecular weight | Molecular formula | Bioactivity |
|---|---|---|---|---|---|---|
| 1 | Histamine | 3.824 | 1.37 | 111.14 | $C_5H_9N_3$ | Histamine-Inhibitor |
| 2 | 2-Ethyl-2-hexen-1-al | 3.927 | 3.908 | 126.19 | $C_8H_{14}O$ | Not found |
| 3 | 1H-Indole-4-carboxaldehyde | 12.012 | 11.38 | 145.15 | $C_9H_7NO$ | Hepatocarcinogenic |
| 4 | p-Cresidine | 14.953 | 4.71 | 137.17 | $C_8H_{11}NO$ | Anticancer (Pancreas) |
| 5 | Hexadecanoic acid | 16.551 | 17.37 | 256.42 | $C_{16}H_{32}O_2$ | Acidifier,acidulant, arachidonic-Acid-Inhibitor. |
| 6 | Hexadecanoic acid, ethyl ester | 16.723 | 14.17 | 284.47 | $C_{18}H_{36}O_2$ | Acidifier,acidulant, Arachidonic-Acid-Inhibitor, |
| 7 | 9,12-Octadecadienoic acid, methyl ester | 18.257 | 13.53 | 294.47 | $C_{19}H_{34}O_2$ | Catechol-O-methyl-transferase-inhibitor |
| 8 | 2-ethylhex-3-enal | 18.651 | 9.35 | 126.19 | $C_8H_{14}O$ | Not found |
| 9 | 9,12,15-Octadecatrien-1-ol | 19.451 | 19.52 | 264.44 | $C_{18}H_{32}O$ | Oligosaccharide provider, |
| 10 | Sarcosine,N-(2-methoxybenzoyl)-, octyl ester | 24.414 | 7.32 | 335.43 | $C_{19}H_{29}NO_4$ | Anaphylactic (antidote) |

## V. CONCLUSIONS

Ten compounds have been identified in the ethanol extract of Chromolaena odorata. The bioactivities of these compounds have been highlighted. This identified phytocompounds will enhance the development of drugs from this plant. The traditional usage of Chromolaena odorata in treatment of ailment will be enhanced due to the identified bioactive compounds. These phytocompounds needs further pharmacological investigation in order to develop new drugs for the treatment of specific diseases. Thus, GC-MS analysis is the pioneer step in understanding the nature of active components in Chromolaena odorata.

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