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DETERMINATION OF CHEMICAL COMPOSITION OF VOLATILE OIL OBTAINED FROM *Calotropis procera* LEAF THROUGH GC-MS ANALYSIS

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Abstract: **In this study, volatile oil was extracted from matured leaves of *Calotropis procera* plant using standard procedures. GC-MS analysis revealed Pregn-4enetrione, 2,2 - Bischorophenlypropane, Cercosporin, Lycoxanthin, O-Acetyl Anodendroside and 4 –Isopropylidene bisdibromophenoxy with %Area values of 26.05%, 22.33%, 13.70%, 12.33%, 13.70% and 12.33% respectively as the chemical components of the volatile oil. The order of their composition in the volatile oil were Pregn-4enetrione >2,2- Bischorophenyl propane > Cercosporin=O-Acetyl Anodendroside > Lycoxanthin = 4-Isopropylidenebisdibromophenoxy with Pregn-4enetrione and 2,2-Bischorophenyl propane detected in higher amounts than other chemical components in volatile oil of *Calotropis procera* leaf. The present study showed that volatile oil from *Calotropis procera* leaf contained some chemical components of biochemical, physiological and pathological importance. Isolation and characterization of each chemical component detected in the present study in animal model are recommended for further study.**

Keywords: **GC-MS, volatile oil, chemical, *Calotropis procera*, importance.**

1. INTRODUCTION

Gas chromatography is an analytical methodology using a gas chromatograph is called gas chromatograph, gas chromatography systems are widely commercialized and used in various industries such as pharmaceutical, food and beverages, environmental, petrochemicals, chemicals and energy and gas. It is applicable for many types of analysis in the markets such as residual solvent analysis, food and beverage component analysis, simulated distillation, air, water and soil studies. Gas purity analysis and artificial photosynthesis research respectively (Shimadzu Corporation, 2020).

According to de Hoffmann and Stroobant (2007), mass spectrometry's characteristics have raised it to an outstanding position among analytical methods unequalled sensitivity, detection limits, speed and diversity of its applications. In analytical chemistry, the most recent applications are mostly oriented towards biochemical problems, such as proteome, metabolome, high throughput in drug discovery and metabolism, and so on. Other analytical applications are routinely applied in pollution control, food control, forensic science, natural products or process monitoring. Other applications include atomic physics, reaction physics, reaction kinetics, geochronology, inorganic chemical analysis, ion–molecule reactions, determination of thermodynamic parameters and many others. The advancement in the employment of Mass spectrometry principle in research analysis has spanned within ten years of its discovery in recent time. Shimadzu Corporation (2020) reported that GC-MS is superior in qualitative and quantitative capability and, with greatly improved performance and operability; it is rapidly spreading in the market.

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The use of plants in tradition folklore in the treatment of several ailments and diseases is as old as the existence of man, among such plants with herbal healing properties includes *Calotropis procera* plant. Medicinal plants have been identified and used throughout human history. Flowering plants were the original source of most plant medicines. Some herbs and spices originate from flowering plants. Modern medicine makes use of many plant-derived compounds as the basis for evidence-tested pharmaceutical drugs (Ajiboso *et al.,* 2015; Meena *et al.,* 2011).

Calotropis procera plant with the common name – Soddom apple in English; Bomubomu in Yoruba; Tumfafiya in Hausa and Epuko in Nupe languages contains high amount of oil which predominates in its leaf and thus gives the leaf the waxy texture. This oil is volatile in nature and thus requires appropriate organic solvent such as hexane for maximum and total extraction.

There are acclaimed uses of *Calotropis procera* plant in its ethno-botanical survey in the treatment of ailments, diseases and infections, it is used for the treatment of disorders such as diabetes mellitus, bronchial asthma, rheumatoid arthritis and nervous disorders among others (Ajiboso *et al.*, 2015; Meena *et al.*, 2011). In Nigeria, different parts of *Calotropis procera* plant have been reported to exhibit anti-inflammatory, analgesic and antioxidant properties (Ajagbonna *et al.*, 1999).

Therefore, this study aimed at determining the chemical composition of volatile oil obtained from the leaves of *Calotropis procera* plant through GC-MS analysis.

2. MATERIALS AND METHODS

Collection of Plant material

2.0 kg leaves from matured *Calotropis procera* plant were collected from botanical garden of Nasarawa State University Keffi – Nigeria, West – Africa in August 2022. The plant was identified by a Plant Biologist from Department of Plant Science Nasarawa State University Keffi – Nigeria, West – Africa before harvesting the leaves. The leaves were cleaned by removing dirts and other foreign materials, after which were chopped into pieces and dried under laboratory condition (12 hrs day / 12 hrs night) at 33.1±0.1^{o}C for 5 days.

Volatile oil extraction

Modification of procedures described by Farnaz *et al.*, (2019) and Negahban *et al.*, (2007) were used to extract volatile oil from leaves of *Calotropis procera* plant. Dried chopped leaves were milled into fine powder which was allowed to pass through 75µg mesh size, the chopped leaves were milled into powder form using a milling machine (Corona manual hand mill, PC 5740364). To 92g of the powdered sample, 1000 mL distilled water was added, were subjected to hydrodistillation using a modified Clevenger-type apparatus (Model. AK-500 China) for 3 hours. Volatile oil dried over anhydrous sodium sulfate was transferred into amber-colored vials at 4 °C for further tests. After hydrodistillation, a quantity of 5 µL oil was mixed with 100 µL hexane and 1ml sodium sulfate. Then 1 µL from the new mixture was injected into the chromatograph.

Gas chromatography/ Mass spectrometry analysis of volatile oil

Principle:

(a). Gas chromatography

The injected sample enters a gas stream which transports the sample into a separation tube known as the "column". Helium is used as the carrier gas; the components are separated inside the column. The detector measures the quantity of the components that exists the column.

(b). Mass spectrometry

The mass spectrometer generates multi ions from the sample under investigation, it separates the sample according to their specific mass to charge ratio (m / z), and then records the mass to charge ratio according to relative abundance of each ion type.

Procedure

The gas chromatography /mass spectrometry (GC \ MS) analysis of the volatile oil was performed using Agilent 6890 N Network gas chromatographic (GC) system equipped with a flame ionization detector. The GC oven temperature started at

100^{0}C and was held for 1 minute at 260ºC and then for 10 minutes with 4ºC min^{-1} program rate. While the injector and detector temperatures were set at 250^{0}C and 230^{0}C respectively, the GC/MS instrument was set-up overnight to get warm and was zero before analysis, I ml of volatile oil was put into a vial in the GC / MS instrument, the sample was diffused in a 30 m long and 0.25 mm thick DB-5MS column (Aglient Technologies, J & W Scientific products, USA) with the carrier gas being helium. Separation of the sample into various peaks at different retention times, % height and % area taken at 26 minutes including the active compounds and their structural formulas were obtained from the data processor.

3. RESULTS AND DISCUSSION

The chromatogram pattern of volatile oil extracted with steam/hexane from *Calotropis procera* leaves was shown in Figure 1. Four peaks with retention time (RT) of 11.14 minutes were detected; maximum RT used for the separation was 26 minutes. Apex RTs for 1st, 2nd, 3rd and 4th peaks were 7.02, 8.04, 9.40 and 10.40 minutes respectively. The result of mass spectroscopic analysis was presented in Table 1. As shown in Figures 2-7, six (6) major chemical components that were significant and relevant to some of the medicinal potency and efficacy of *Calotropis procera* plant were detected in the volatile oil content of the leaf. The chemical components detected were Pregn-4enetrione, 2,2 – Bischorophenyl propane, Cercosporin, Lycoxanthin, O-Acetyl Anodendroside and 4 –Isopropylidene bisdibromophenoxy with %Area values of 26.05%, 22.33%, 13.70%, 12.33%, 13.70% and 12.33% respectively (Table 1). The order of the detected chemical components composition in the volatile oil were Pregn-4enetrione >2,2- Bischorophenyl propane > Cercosporin=O-Acetyl Anodendroside > Lycoxanthin = 4-Isopropylidene bisdibromophenoxy with Pregn-4enetrione and 2,2- Bischorophenyl propane detected in higher amounts than other chemical components composition in volatile oil of *Calotropis procera* leaf.

The biochemical, physiological and pathological importance of these chemical components have been documented in one study or the other. In adrenal steriodogenesis, Pregnenolone serves as a principal precursor to all the other steroid hormones synthesized by the ovary, testes, or adrenals. It appears that the rate-limiting step for the synthesis of progesterone is side-chain cleavage of cholesterol by P450scc. Pregnenolone is then converted into progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD) (FSHP, 2019). Preg-4-enetrione is a product of progesterone alkaline oxidation and also an inflammatory and immunosuppressive agent (Zhang *et al.*, 2011).

Clinical research has shown Pregnenolone to be important for nerve and brain health, having also been found to be a potent memory and learning skill enhancer – maybe the most important so far discovered. It is also known to relieve arthritis. Pregnenolone has been used in treating depression, relieving stress, improving mood and sense of well-being. Additionally, it stimulates clear thinking, improves concentration, enhances alertness and promotes an overall better mental function, it all supporting improved creativity, keeping the brain functions at peak capacity and increasing learning desire. Pregnenolone also possibly influences the (psychic) energy levels. In studies Pregnenolone has been found to have a positive effect on Alzheimer's, Multiple Sclerosis, Parkinson's, seizures, Chronic Fatigue Syndrome (CFS), Diabetes as well as on Autoimmune disorders, such as Lupus and Scleroderma, Heart Disease, Muscle Building, the Immune System, Cholesterol levels, vision and hearing, PMS, Benign Prostate Hyperplasia, skin quality and weight loss. Pregnenolone has also been tested with promising results in spinal cord and nerve injuries, due to accidents or disease (FSHP, 2019).

2,2-bischlorophenyl propane is in the same group of dichloro compounds with N,5-bis-4-chlorophenylpropan-2-ylimino-3,5-dihdrophenazi-2-amine which is also known as Clofazimine. Clofazimine is a standard drug used for the treatment of leprosy and also an alternative treatment for diabetes mellitus through increase in level of cellular phospholipase (Arbiser and Moschella, 1995). 2,2-bischlorophenyl propane is also a derivative of Chalcones. Chalcones are well known for their biological activities, these have been reported as potential antifungal, chemotherapeutic, anti-infective & anti-inflammatory agents (Ahmed *et al.*, 2019). Chalcones are prepared by the condensation of acetophenone with appropriately substituted aromatic aldehydes in ethanol medium employing sodium hydroxide as catalyst (Fun *et al.*, 2010).

Lycoxanthin and other xantophll carotenoids are effective for the reduction or prevention of oxidative stress through singlet oxygen quenching, direct radical scavenging and lipid peroxidation chain breaking (Terao, 1989). Cercosporin is a membrane sensitizer and potent producer of singlet oxygen. Cercosporin causes peroxidation of membrane lipids leading to membrane lipid breakdown and death of cell (Daub and Chung, 2007). 2,2-o-isopropylidene-5-fluorodine, a derivative of 4,4-isopropylidene bis-dibromophenoxyl ethanol is used as a PDE4 inhibitor for the treatment of diabetes mellitus (Leibowitz, 1995).

4. CONCLUSION

The present study showed that volatile oil from *Calotropis procera* leaf contained chemical components of biochemical, physiological and pathological importance.

5. RECOMMENDATIONS

The following recommendations were deduced from this study:

1. Volatile oil from *Calotropis procera* leaf is a good source of Pregn-4enetrione, 2,2 - Bischorophenlypropane, Cercosporin, Lycoxanthin, O-Acetyl Anodendroside and 4 –Isopropylidenebisdibromophenoxy.

2. Isolation and characterization of each chemical component detected in volatile oil from *Calotropis procera* leaf in the present study in animal model.

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Table 1: Chemical components of Volatile oil extracted from *Calotropis procera* leaf

Minutes					Extract
ApexRT	StartRT	EndRT	%Area	%Height	Components of Steam/Hexane
7.02	6.91	7.16	26.05	25.66	Pregn-4-enetrione
8.04	7.95	8.16	26.08	27.35	Cercosporin, o-Acetyl anodendroxide E2
9.40	9.36	9.56	23.96	24.66	Lycoxanthin, 4,4-isopropylidene bisdibromophenoxy ethanol
10.40	10.26	10.52	23.91	22.33	2,2-bischlorophenyl propane

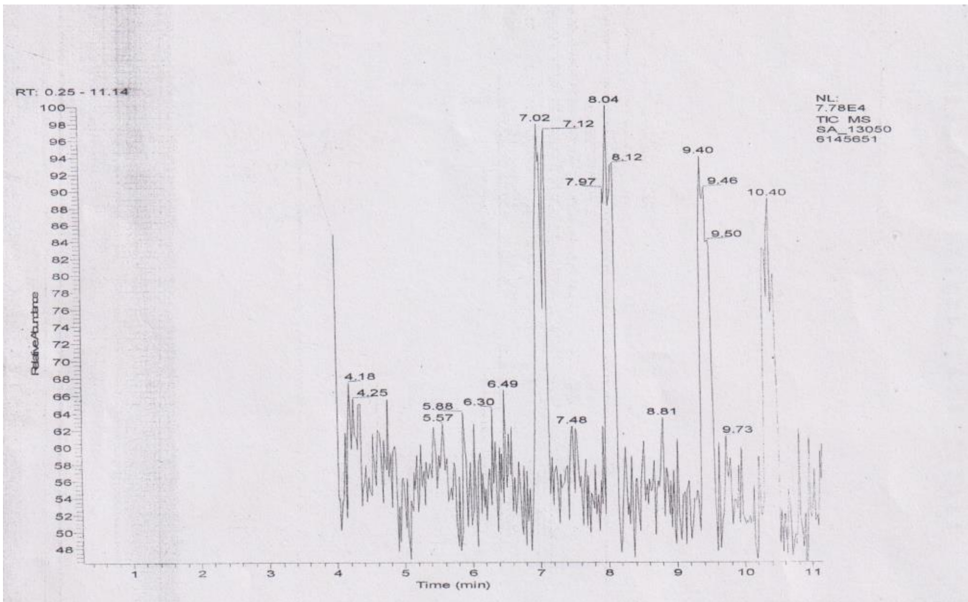

Figure 1: GC Chromatogram of Volatile oil extracted from *Calotropis procera* leaf

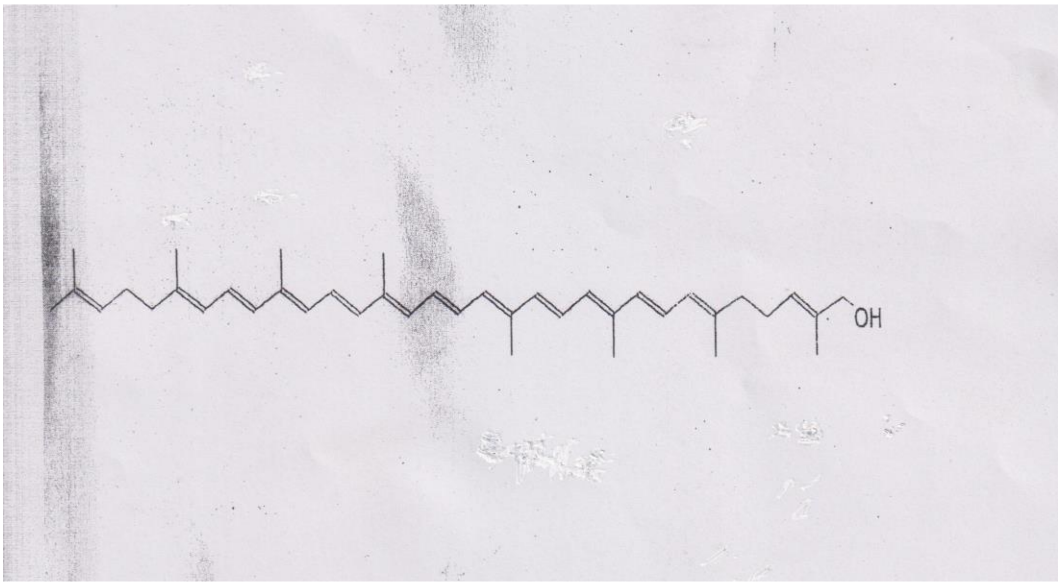

Figure 2: Chemical structure of Lycoxanthin

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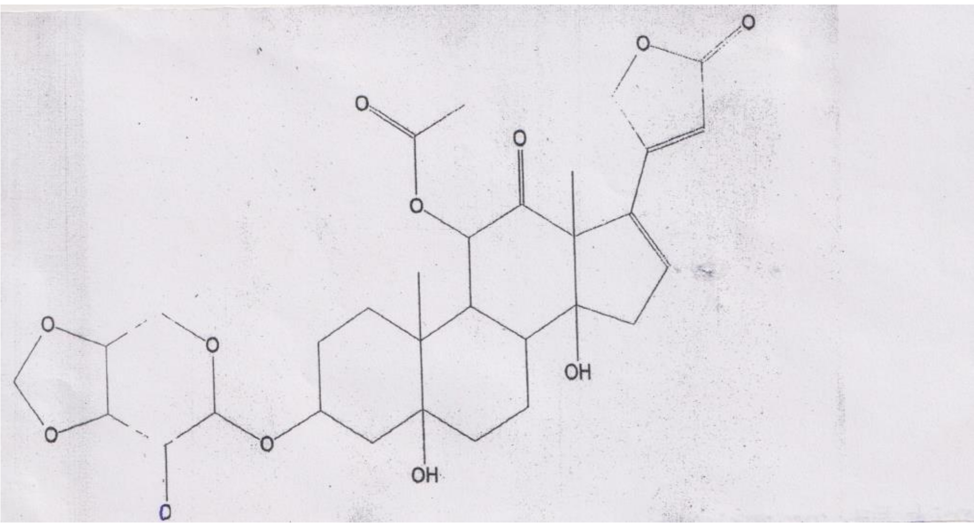

Figure 3: Chemical structure of Anodendroxide E2

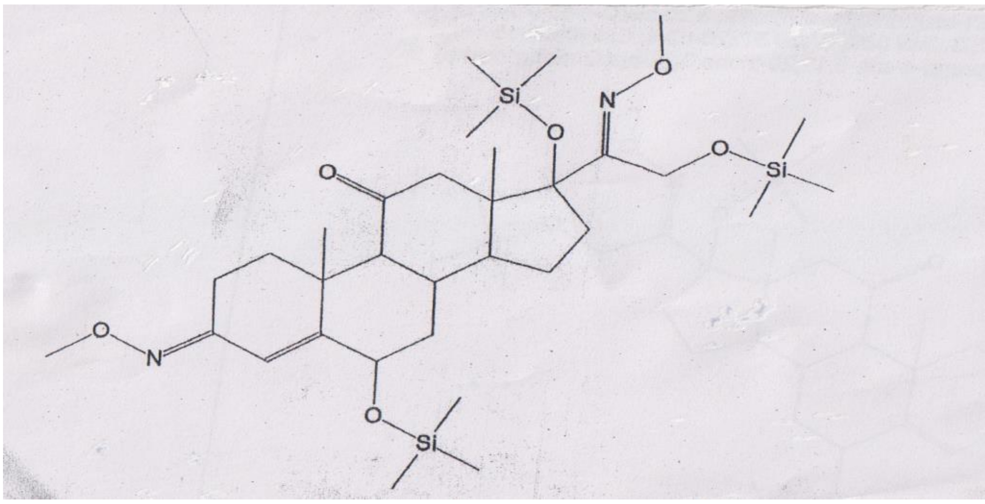

Figure 4: Chemical structure of Pegn-4-enetrione

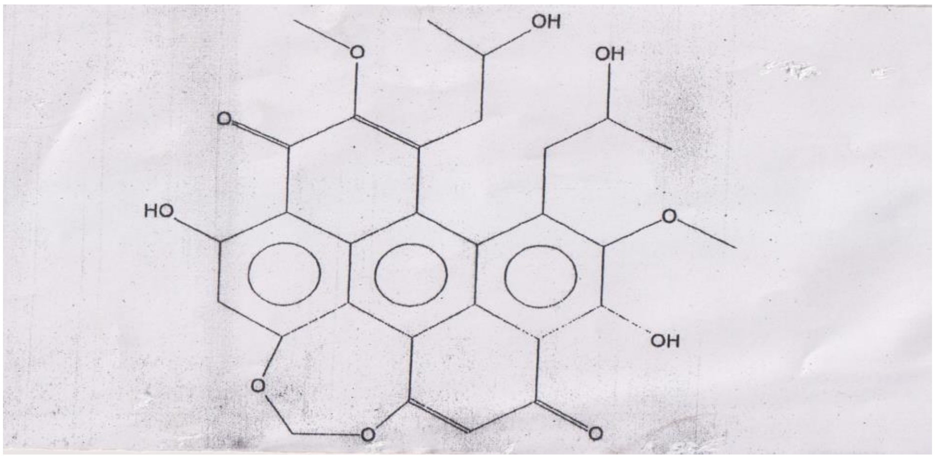

Figure 5: Chemical structure of Cercosporin

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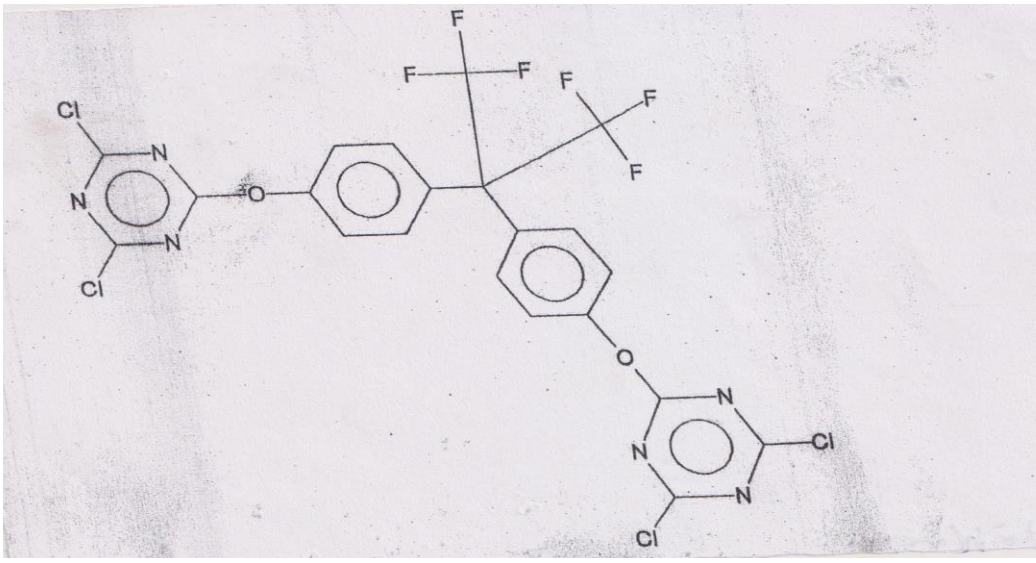

Figure 6: Chemical structure of 2,2-bisdichlorophenyl propane

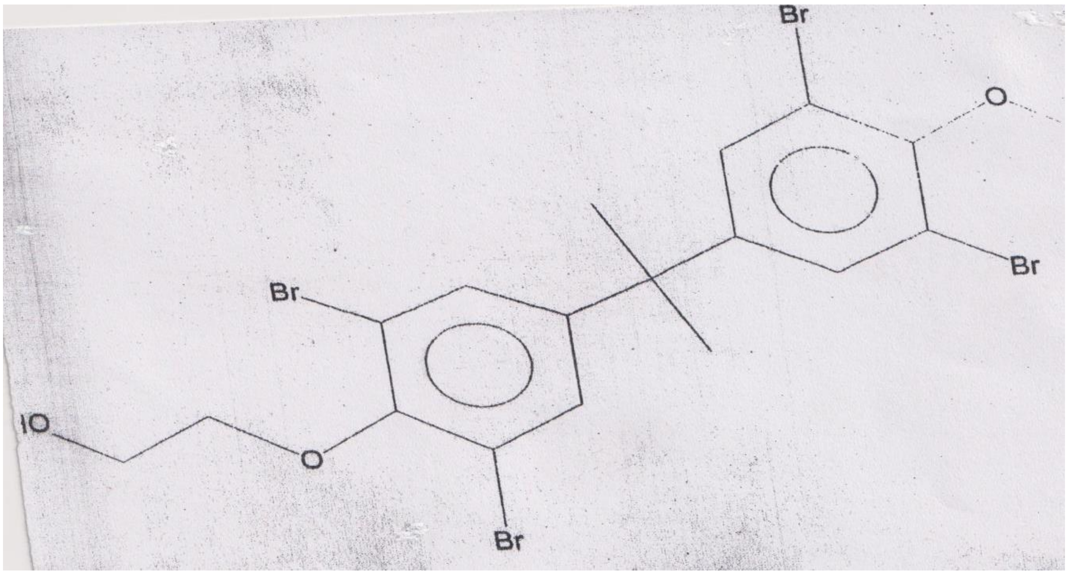

Figure 7: Chemical structure of 4,4-isopropylidene bisdibromophenoxy ethanol