

Extraction And GC-MS Analysis of Oil Extract from Cassava Leaf (*Manihot Esculenta*)

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Abstract

Cassava leaves (*Manihot esculenta*) were gotten from farms in Amassoma, Bayelsa State. The leaves were washed, air-dried for 4 days, and later oven-dried at 50°C; they were then ground to powder. 250 g of the powdered *Manihot esculenta* was weighed and soaked in 1000 mL of n-hexane for 72 hours, filtered, and left to evaporate in a water bath to obtain the oil. The GC-MS was used to identify the components in the oil after extraction. Cassava leaves (*Manihot esculenta*) were gotten from farms in Amassoma, Bayelsa State. The leaves were washed, air-dried for 4 days, and later oven-dried at 50°C; they were then ground to powder. 250 g of the powdered *Manihot esculenta* was weighed and soaked in 1000 mL of n-hexane for 72 hours, filtered, and left to evaporate in a water bath to obtain the oil. The GC-MS was used to identify the components in the oil after extraction. The results revealed some various fatty acids present in the oil, which include n-hexadecanoic acid (12.491%), 9-octadecanoic acid (7.400%), 9,12-octadecadienoic acid (2.265%), 9,12,15-octadecatrienoic acid (6.564%), and other components, which include phytol (2.140%), squalene (12.867%), heptadecane (2.191%), tetracosane (0.995%), vitamin E (2.362%), oxirane, hexadecyl (0.791%), caparratriene (0.737%), lanosterol (11.272%), β -amyrone (9.169%), β -amyrin (6.719%), lup-20(29)-en-3-one (20.628%), camphorene (0.669%), and 6-octen-1-ol, 3,7-dimethyl-acetate (0.743%). The components found have been shown to be quite useful in the cosmetics, pharmaceutical, and other sectors. This work points out the value of cassava leaves as a rich source of bioactive compounds with a variety of uses in numerous industrial sectors, while also stressing the significance of the sustainable exploitation of agricultural waste.

Key words: *manihot esculenta*, GCMS, oil, Extraction, Leaves

INTRODUCTION

One of the main sources of substances with nutritional and health benefits is the plant kingdom. Natural bioactive materials are known to help treat a variety of human illnesses. Their extensive range of biological activity and structural diversity make them very interesting to researchers (Atanasov, Zotchev, Dirsch, Suparan, 2021). *Manihot esculenta*, sometimes known as cassava, is an important staple crop in tropical and subtropical regions. It belongs to the Euphorbiaceae family and is mostly produced for its starchy tuberous roots (Montagnac, 2009). Because of its versatility and extensive use, cassava is highly prized as a rich source of essential elements like iron, zinc, calcium, carbohydrates, manganese, magnesium, and vitamins, its leaves offer both nutritional and medicinal benefits (Adewusi and Bradbury 1993, Wobeto, Correa, DeAbreu, Dos Santos, and De Abreu, 2006).

The cold extraction technique is the most appropriate extraction method to apply for this investigation, even though a different extraction method was intended. Alkaloids, flavonoids, phenolic acids, glycosides, and saponins are among the several bioactive substances found in cassava leaves. Because of its high starch content, cassava is the main source of carbohydrates for almost 500 million people in the tropics (Cock, 1985; Moorthy, 2002; Davis, Supatcharee, Khandelwal, Chibbar, 2003; Tonukari, 2004). Cassava has several characteristics that make it an excellent choice for economic growth because of its high nutritional value for both humans and animals.

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Additionally, it may flourish in soil that is quite acidic as well as soil that is heavy in manganese and aluminum. Cassava may be used to produce bioenergy; however, it is not advised to eat it if the soil is highly polluted with inorganic pollutants like nickel, zinc, and cadmium. The portions of the cassava plant that people and animals eat the most frequently are the roots and leaves. Cassava is essential to preserving food security because of its ability to flourish in areas with marginal soil and limited rainfall. Because cassava requires little time, labour, or money, it is preferred by both huge plantations and small farmers. Cassava leaves are used as food in many nations, however because they contain cyanogenic glucosides and antinutrients, there are some worries about eating

them. One effective analytical method for determining and measuring the constituents of a mixture is GC-MS analysis.

A more sustainable and circular economy may benefit from the creation of new goods and uses brought about by the extraction and analysis of bioactive components from cassava leaves. In a 2016 study, Li used gas chromatography–mass spectrometry (GCMS) analysis to determine that the main constituents of the essential oil extracted from fresh cassava leaves using the hexane fraction were nonanal, caryophyllene oxide, and tetradecanal.

Shown below is the picture of cassava leaves in figure 1.



Figure 1. Overview of cassava leaf

Cassava (*Manihot esculenta*) leaves are rich in bioactive compounds with potential medicinal and industrial applications. However, the phytochemical composition of cassava leaf oil remains underexplored. Cassava leaf, a common agricultural by-product, has untapped potential for oil production, but there is a lack of research into effective and sustainable oil extraction techniques. Various solvents have been utilized to extract secondary metabolites from cassava leaves, yet herbicidal compounds have not been identified. This knowledge gap hinders the development of efficient and sustainable extraction processes, limiting the oil's utilization. This study investigates the effect of solvent extraction, cold extraction steam distillation, and ultrasonic assisted extraction on cassava leaf oil yield and chemical composition using GC-MS analysis. The aimed of the study is to; extract oil from cassava leaf using the cold extraction method and the specific objective; are using the cold extraction method to extract oil from cassava leaf, and characterized the oil using GC-MS.

The bioactive and phenolic chemicals found in cassava leaves, including cyanidine, tannins, delphinidine, and

anthocyanidins, which are known to have pharmacological qualities, have been identified and measured through research (Erson, 2013).

According to studies, cassava leaves are also a rich source of phytochemical components, including flavonoids, alkaloids, tannins, and saponins (Ebuehi, Babalola, Ahmed, 2005). Cassava's edible green leaves are frequently used to supplement the rural diet since they are an excellent source of protein, vitamins, and minerals (Bradbury, Howard, Holloway, 1988).

Cassava (*Manihot esculenta*) is a root and tuber crop that has been identified as important food, especially in Africa. In areas where cassava is a main staple, people have developed ways for its processing into storable products such as tapioca, starch, dough and garri. It plays a major role in efforts to alleviate the African food crisis because of its efficient production of food, year-round availability and tolerance to be extreme stress conditions (Hahn, 1987).

Aside from its bioactive components, cassava leaves have a rich nutritional profile; several researches have

determined which macronutrients are found in them. According to Pereira (2016), they are a more abundant source of protein, minerals, and vitamins than several other green vegetables and meals. Although cassava leaves have nutritious potential, their nutritional value may be limited by natural chemicals. Tannins in cassava leaves, for example, can interact with proteins and affect their digestion, potentially reducing the overall protein utilization (Bate-Smith, 1973). Additionally, cassava leaves have higher levels of cyanogenic glucosides than the roots, which can inhibit certain enzyme reactions. Nonetheless, conventional processing techniques like grinding and pounding can aid in lowering the leaves' cyanide concentration. Certain tissues and a number of variables, including the plant's age, variety, location, and environmental circumstances, affect the nutritional makeup of cassava. Since the roots are the primary portion of the plant that is consumed in developing nations, their nutritional value is significant.

MATERIALS AND METHODS

Materials

For the extraction process, we have prepared the following materials and reagents: a manual grinding machine, an electric weighing balance, a sample container, a brown

bottle for maceration, a measuring cylinder, a funnel, a beaker, a retort stand, cotton wool, masking tape for labeling, a sample bottle, and a GC-MS Agilent Technologies 5975CMSD system.

Reagent

N-hexane was employed as the extraction solvent. It is frequently employed as a solvent in many industrial processes and makes up a sizable portion of gasoline. It is composed of a straight chain of six carbon atoms and twelve hydrogen (C_6H_{12}) carbon atoms.

Sample Used: Cassava (*manihot esculenta*) leaves.

Methods

Sample Collection

Cassava leaf were gotten from a cassava farm in Amassoma, Bayelsa State. After that it was taken to the laboratory where it was washed to remove impurities and left to dry.

Sample Preparation

The cassava leaf was gotten from the farm and air-dried for 3-5 days; thereafter, it was oven-dried for a day. Then it was ground to powder using the hand grinding machine (figure 2). After grinding, it was stored in an air tight container prior to the sample extraction.



Figure 2. Image of the manual grinding machine

Extraction Process

The sample was measured (250 g) after it was ground and put into a brown bottle to avoid light rays. 1000 mL of n-hexane was added and left for 72 hours. The content

obtained was filtered, as shown in figure 3 below, and collected. It was then placed in a water bath for 4 days to allow for vaporization of n-hexane from the oil sample. The sample was then taken for GC-MS analysis.



Figure 3. Filtration process.

GC-MS Analysis



Figure 4. Gas Chromatogram-Mass Spectrometer of Agilent Technologies-5975CMSD

RESULTS AND CONCLUSION

Result

The oil extracted from *manihot esculenta* is viscous. The oil is thick and dark green in colour and GC-MS analysis

identified 17 components, including specific fatty acids, with different retention time and percentage abundance. The maximum peak was shown by lup-20(29) en-3-one with a retention time of 16.205 with a peak time of 20.628% as shown in the chromatogram below 5

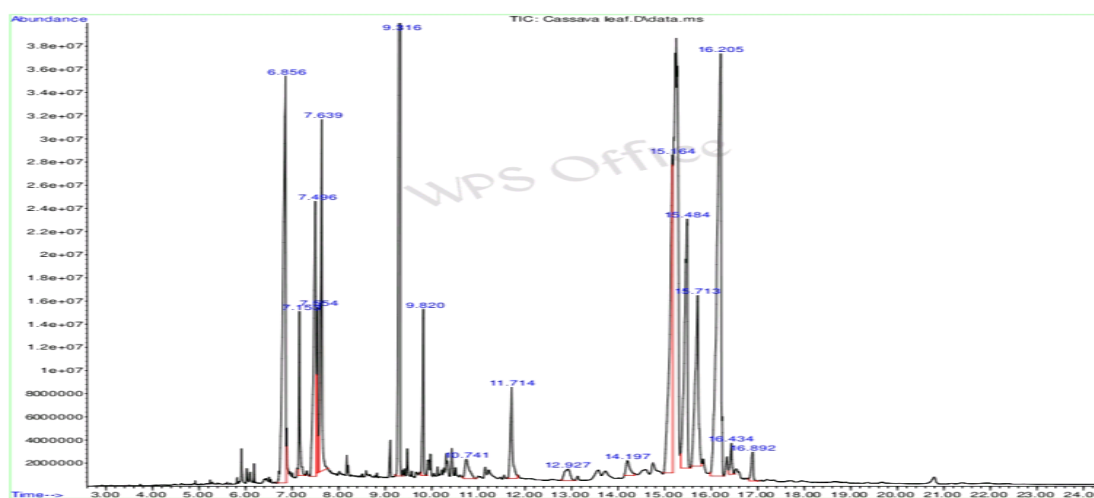


Figure 4. showing the chromatogram of oil extract from cassava leaf *manihot esculenta*

Table 1. showing the components identified in the oil extract from cassava leaf, their retention time and percentage abundance

Peak	Retention time	Components	% of total
1.	6.856	n-hexadecanoic acid	12.491%
2	7.153	Phytol	2.140%
3.	7.496	9-octadecanoic acid	7.400%
4.	7.554	9,12-octadecadienoic acid	2.265%
5.	7.639	9,12,15-octadecatrienoic acid	6.564%
6.	9.316	Squalene	12.867%
7	9.820	Heptadecane	2.191%
8.	10.741	Tetracosane	0.995%
9	11.714	Vitamin E	2.362%
10	12.927	Oxirane, hexadecyl	0.791%
11	14.197	Caparratriene	0.737%

12	15.164	Lanosterol	11.272%
13	15.484	β -amyrone	9.169%
14	15.713	β -amyrin	6.719%
15	16.205	lup-20(29) en-3-one	20.628%
16	16.434	Camphorene	0.669%
17	16.892	6-octen-1-ol, 3-7-dimethyl-acetate	0.743%

The GC-MS analysis of *Manihot esculenta* identified 17 components, four of which were fatty acids: n-hexadecanoic acid with a retention time of 6.859 and a peak of 12.491%; 9-octadecanoic acid with a retention time of 7.496 and a peak of 7.400%; 9,12-octadecadienoic acid with a retention time of 7.554 and a peak of 2.265%; and 9,12,15-octadecatrienoic acid with a retention time of 7.639 and a peak of 6.564%. The remaining components also have specified retention times and peak values. Phytol with a retention time of 7.153 with a peak of 2.140%, Squalene with a retention time of 9.316 with a peak of 12.867%, Heptadecane with a retention time of 9.820 with a peak of 2.191%, Tetracosane with a retention time of 10.741 with a peak of 0.995%, Vitamin E with a retention time of 11.714 with a peak of 2.362%, Oxirane, hexadecyl with a retention time of 12.927 with a peak time of 0.791%, Caparratriene with a retention time of 14.197 with a peak time of 0.737%, Lanosterol with a retention time of 15.164 with a peak time of 11.272%, B-amyrone with a retention

time of 15.484 with a peak time of 9.169%, B-amyrin with a retention time of 15.713 with a retention time of 6.719%, lup-20(29) en-3-one with a retention time of 16.205 with a peak time of 20.628%, Camphorene with a retention time of 16.434 with a peak time of 0.669%, 6-octen-1-ol, 3-7-dimethyl-acetate with a retention time of 16.892 with a peak time of 0.743%.

Discussion

n-Hexadecanoic Acid

N-hexadecanoic acid, commonly referred to as palmitic acid, has the chemical formula $C_{16}H_{32}O_2$. It is a saturated fatty acid with a 16-carbon chain and serves as a significant component of fats and waxes found in animals, plants, and microorganisms. Palmitic acid is prominently present in olive oil, palm oil, and body lipids. Its applications extend to the soap, cosmetics, and food industries, and it plays various biological roles as well.

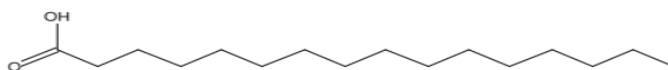


Figure 5. Structure of n-hexadecanoic acid

Phytol

Phytol is a diterpene alcohol with the chemical formula $C_{20}H_{40}O$, serving as a component of chlorophyll and naturally produced by plants. It plays a crucial role in the synthesis of vitamins E and K1 and can be found in various foods and animal products. Moreover, phytol can be converted into phytanic acid, a metabolite that contributes to lipid metabolism and activates peroxisome proliferator-

activated receptors (PPARs). As a long-chain, branched, unsaturated alcohol, phytol is hydrophobic, which means it has low solubility in water but dissolves readily in organic solvents. Its applications extend to fragrances, cosmetics, shampoos, and other products, highlighting its presence in numerous plant-based items, where it also contributes to the process of photosynthesis.

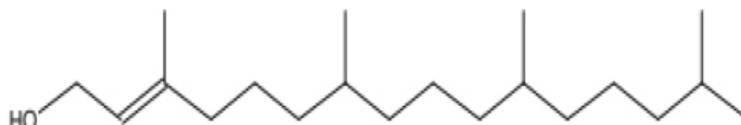


Figure 6. Structure of Phytol

9-OCTADECANOIC ACID (E)-

9-Octadecenoic acid, commonly referred to as oleic acid, is a monounsaturated fatty acid with the molecular formula $C_{18}H_{34}O_2$. It features an 18-carbon chain with a single

double bond located at the 9th carbon from the carboxyl group. This double bond can exist in either a cis (Z) or trans (E) configuration; the cis isomer is known as oleic acid, while the trans variant is called elaidic acid. Oleic

acid is widely distributed and abundant in nature, found in various organisms. It is utilized in the formulation of oleates and lotions, as well as in pharmaceutical applications as a solvent. Additionally, oleic acid is a prominent component

of many vegetable oils, including olive oil and sunflower oil. Research indicates that oleic acid may offer health benefits for the heart, potentially lowering cholesterol levels and reducing inflammation.

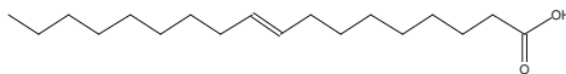


Figure 7. structure of 9-octadecanoic acid (e)-

9,12-Octadecadienoic Acid (Z,Z)

9,12-Octadecadienoic acid, also referred to as linoleic acid, is a polyunsaturated fatty acid that is essential to human nutrition. Its chemical formula is $C_{18}H_{32}O_2$. Being an essential fatty acid, it must be obtained through diet because the body is unable to synthesize it on its own. The 18-carbon chain of linoleic acid has two cis (Z) double

bonds at the 9th and 12th carbon atoms. It is essential for preserving the integrity of cell membranes, encouraging hair growth, bolstering skin health, controlling inflammation, and enabling the synthesis of more fatty acids. The main source of this essential fatty acid is a range of plant-based oils, including soybean, corn, and sunflower oil.

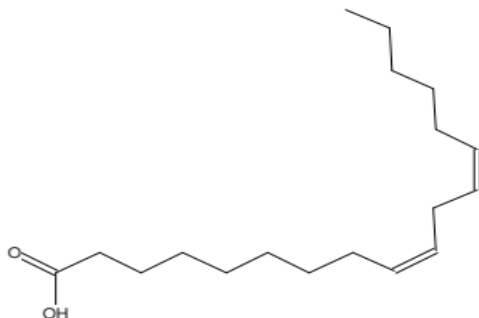


Figure 8. structure of 9,12-octadecadienoic acid (z,z)

9, 12, 15-Octadecatrienoic Acid (Z,Z)

The chemical formula for 9,12,15-octadecatrienoic acid, also known as alpha-linolenic acid (ALA), is $C_{18}H_{30}O_2$. Three double bonds are found at the ninth, twelfth, and fifteenth positions of this 18-carbon polyunsaturated omega-3 fatty acid. Since ALA is an essential fatty acid,

the body is unable to produce it on its own and must get it from food. It is essential to human health since it helps modulate inflammation, brain function, and heart rate. Flaxseed, walnuts, and chia seeds are among the many seeds and oils that contain ALA.

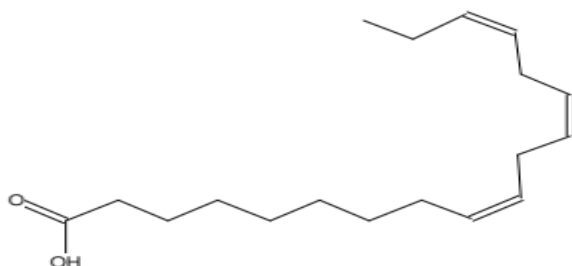


Figure 9. Structure of 9,12,15-octadecatrienoic acid (z,z)

Squalene

With the chemical formula $C_{30}H_{50}$, squalene is a naturally occurring, colorless, oil-like substance that is categorized as a triterpene. It can be found in a variety of animal and plant sources, such as olive oil, and shark liver oil. In both plants and animals, squalene is a precursor to

the manufacture of cholesterol and other sterols. It has been studied for its potential involvement in preventing cancer and is known for its potential uses in skin care as an antioxidant and emollient. This 30-carbon isoprenoid molecule is classified as a polyunsaturated hydrocarbon due to its six double bonds. Impure squalene samples might have a yellow tint, but pure squalene is colourless.

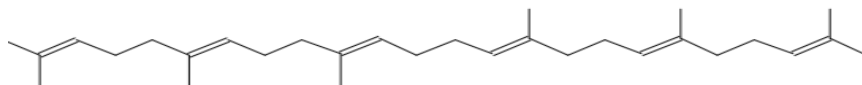


Figure 10. structure of Squalene

Heptadecane

Heptadecane, which has the chemical formula $C_{17}H_{36}$, is a straight-chain alkane. It is a naturally occurring component of many plants and is important for volatile oils. In gas chromatography, hexadecane is frequently utilized as a reference material. At ambient temperature, it appears

as a colorless, odorless liquid made up of 17 carbon atoms connected to hydrogen atoms in a straight chain. It has a high boiling point of 302°C and is comparatively non-reactive. Numerous plant species, such as *Spirulina platensis*, *Vanilla madagascariensis*, and *Camellia sinensis*, contain this chemical.

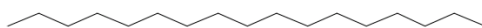


Figure 11. structure of Heptadecane

Tetracosane

Tetracosane ($C_{24}H_{50}$) is a straight-chain alkane hydrocarbon composed of 24 carbon atoms. It appears as a crystalline, waxy solid and is insoluble in water while being soluble in benzene, toluene, and ether. Tetracosane

is found in its mineral form, known as evenkite, and also occurs as a metabolite in plants and as a component of volatile oils. Additionally, it is utilized in various organic synthesis processes.



Figure 12. Structure of Tetracosane

Vitamin E

One important fat-soluble antioxidant that protects cells from harm from free radicals is vitamin E ($C_{29}H_{50}O_2$). It helps the body use other vitamins, like vitamin K, efficiently and is crucial for immune system support. Although it is usually safe in normal dosages, taking too much of it

might cause headaches, nausea, and diarrhea, as well as interfere with some drugs. Vitamin E also helps prevent blood clots and is essential for the production of red blood cells. Additionally, it plays a role in cell signaling and vital cellular functions. Leafy green vegetables, nuts, seeds, and vegetable oils are all excellent sources of vitamin E.

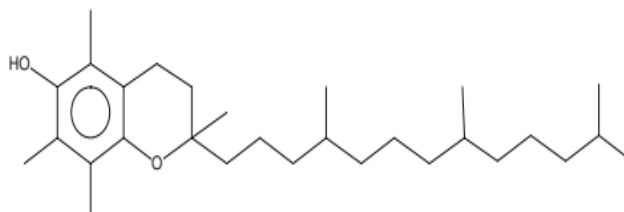


Figure 13. Structure of Vitamin E

Oxirane, Hexadecyl

Oxirane, also known as hexadecyl, is a particular kind of epoxide that has the chemical formula $C_{18}H_{36}O$ and is defined as a long-chain alkyl epoxide with 16 carbon atoms. The term "hexadecyl" refers to the 16-carbon chain that is joined to the compound's three-membered ring structure,

which contains an oxygen atom. Because of its reactivity, oxirane, a cyclic ether with this three-membered structure, is frequently used in chemical synthesis and as a building block in materials research. There are several ways to create oxiranes from alkenes, such as using peroxy acids, dimethyl sulphide, or dimethyl sulphate.

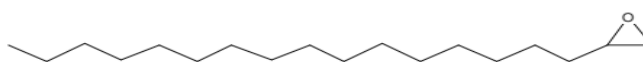


Figure 13. Structure of Oxirane, Hexadecyl

Caparratriene

One sesquiterpene hydrocarbon with anti-leukemia qualities is caparratriene. With the structure (E, E)-3,7,11-trimethyl-2,4,10-dodecatriene ($C_{15}H_{26}$), it was isolated from *Ocotea caparrapi* oil. Studies have shown that it significantly inhibits the proliferation of CEM leukemia

cells. The 2E isomer of caparratriene is the one that occurs naturally. This compound has a critical temperature of 742.01 K and a boiling point of 554.40 K, among other noteworthy physical characteristics. Both Suzuki coupling and Wittig reactions have been used to synthesize ($\pm$)-caparratriene.

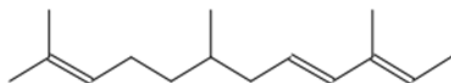


Figure 13. Structure of Caparratriene

Lanosterol

Lanosterol ($C_{30}H_{50}O$) is a tetracyclic triterpenoid and is the chemical from which all animal and fungal steroids are formed. On the other hand, cycloartenol is used to make plant steroids. Lanosterol is a naturally occurring

component of vertebrate eyes that helps to keep the lens healthy. In a single step, lanosterol synthase converts (S)-oxidosqualene into lanosterol, a biosynthetic precursor of cholesterol and other significant steroids.

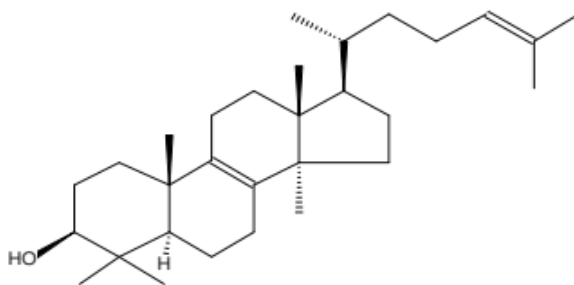


Figure 14. Structure of Lanosterol

β -Amyrone

The triterpene molecule β -amyrone ($C_{30}H_{48}O$) has a wide spectrum of biological actions, such as antiviral, antifungal, and anti-inflammatory effects. It exhibits inhibitory effects on a number of enzymes, including acetylcholinesterase and α -glucosidase. Furthermore, studies suggest that

β -amyrone may have potential as an anti-Parkinsonian drug and as an antioxidant. It is categorized as a derivative of oleanane, a common triterpene present in many different plants, and is a pentacyclic triterpenoid with a structure with five rings. β -Amyrone can be found in a number of medicinal plants.

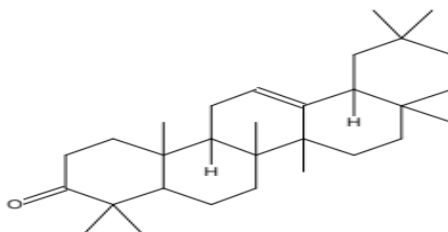


Figure 15. Structure of β -amyrone

β -Amyrin

Composed of six isoprene units, β -amyrin ($C_{30}H_{50}O$) is a common pentacyclic triterpenoid. It is frequently found in a variety of medicinal plants as a derivative of oleanane. With a hydroxy group at the 3 β -position and a double bond between the 12th and 13th positions, this molecule is categorized as a secondary alcohol.

β -amyrin has a variety of pharmacological properties, including as neuroprotective, anti-inflammatory, and antinociceptive actions. It is found in a number of plants, including *Sambucus chinensis*, *Protium heptaphyllum*, and *Camellia sinensis* (tea). Squalene is the starting point for the triterpenoid pathway, which produces β -amyrin. According to research, β -amyrin is usually safe at normal dosages, but high dosages can be dangerous.

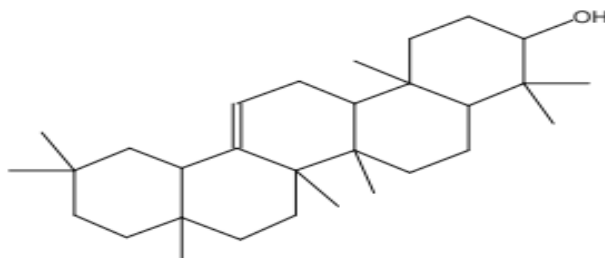


Figure 16. Structure of β -amyrin

LUP-20(29)-EN-3-ONE

Lup-20(29)-en-3-one, also known as lupeol-20(29)-en-3-one, is a naturally occurring triterpenoid ketone that is present in a number of plant species, such as *Pongamia pinnata* var. *pinnata* and *Daphne aurantiaca*. It has the molecular formula $C_{30}H_{48}O$ and is a natural product. Lup-20(29)-en-3-one is a ketone with a carbonyl group ($C=O$)

joined to two additional carbon atoms. Due to its various uses in natural products research and possible biological activity, this molecule has garnered attention. Some research indicates that it may have anti-inflammatory or anti-diabetic qualities, even if the precise mode of action is yet unclear.

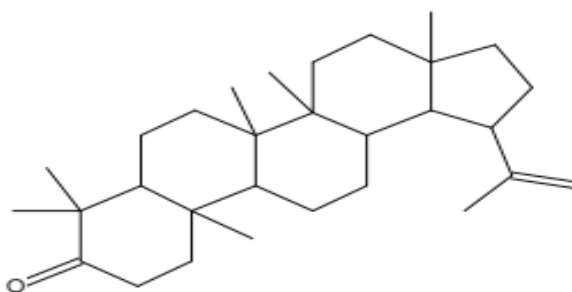


Figure 17. Structure of lup-20(29)-en-3-one

P-CAMPHORENE

P-camphorene, also known as α -camphorate or myrcene, is a diterpene with the chemical formula $C_{20}H_{32}$. This clear, pale yellow liquid serves as a solvent and is utilized in various perfuming agents; however, its use in fragrances

or flavors is not recommended. P-camphorene features a distinctive chemical structure that includes a cyclohexene ring alongside two substituted side chains. Its primary applications are in experimental or research settings.

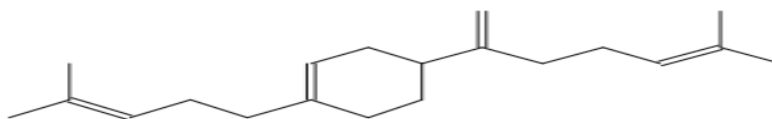


Figure 18. structure of p-camphorene

6-OCTEN-1-OL, 3,7-DIMETHYL-ACETATE

6-Octen-1-ol, 3,7-dimethyl-, acetate, commonly referred to as citronellyl acetate, is an organic compound characterized by the chemical formula $C_{12}H_{22}O_2$. This compound is a derivative of citronellol, an alcohol present

in various essential oils, to which an ester group (acetate) is attached. This esterification process alters the odor profile, resulting in a more floral and fruity fragrance. While it may be utilized as a flavoring agent in food and beverages, it is not widely employed for this purpose.

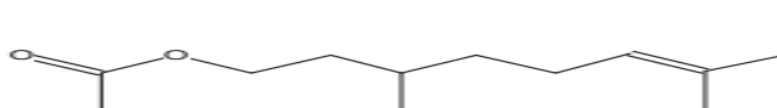


Figure 19. structure of 6-octen-1-ol, 3,7-dimethyl-acetate

CONCLUSION AND RECOMMENDATION

Conclusion

In conclusion, this study has provided a comprehensive background of study, highlighting the statement of research problem with a literature review to support the proposed project title and experimental work. Oil extraction and GC-MS analysis were conducted, revealing various components and fatty acids. Notably, lup-20(29)-en-3-one was identified as the component with the highest percentage of abundance. The presence of these components underscores the potential applications of cassava leaves in the pharmaceutical and cosmetics industries, among others. This research has demonstrated the medicinal value of cassava leaves, which warrants further exploration.

Recommendation

Cassava leaves offer significant advantages due to their rich nutritional profile and therapeutic properties, including anti-inflammatory and antioxidant effects. It is hereby recommended that a comprehensive research and further studies on the oil extract to explore the biological activities of the key compounds, which may have valuable applications in nutrition and medicine. Moreover, advancing oil extraction techniques from cassava leaves could optimize yield and enhance quality. This research could lead to innovative applications in health and industry, utilizing the beneficial properties of cassava leaves.

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