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Extraction, Chemical Composition Analysis and Antioxidant Capacity of Essential Oil from Fresh Leaves of *Lantana camara*

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Abstract:
Conventional hydrodistillation yielded 13.38% of the oil extract. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed 76 components in the oil extract from the leaves of Lantana camara. The identified compounds represent 99.4% of the total oil extracted. The predominant components of the analyzed oil are 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl) (2.7%), 3Z-Hexenol (5.4%), Caryophyllene oxide (5%), (-)-Aristolene (2.3%), 12-Oxabicyclododeca-3,7-diene, 1,5,5,8-tetramethyl (3.7%), 1,6-Cyclodecadiene, Linalool (2.6%), and 2-ethoxy-2-methylpropane (2.8%), with α-Humulene (13.5%) and (E)-Caryophyllene (20.6%) being the two major components.
The total phenolic content of the oil extract is 2.029 ± 0.030 mg GAE/g, and the total antioxidant capacity is 1.255 ± 0.123 mg AAE/g. The percentage 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity was estimated to be 24.41%, with an IC_{50} value of 153.01µg/ml.
These results indicate that the extracted essential oil showed appreciable antioxidant capacity and (DPPH) radical scavenging activity, which could be attributed to the chemical composition of the oil.

Keywords: *ASA (Ascorbic Acid), RSA (Radical Scavenging Activity), GAE (Gallic Acid Equivalent), AAE (Ascorbic Acid Equivalent)*

1. Introduction

1.1. Background of the Study

Plants serve as a source of several biologically active compounds that perform important functions in the treatment of diverse ailments (Pagare *et al.,* 2015). They are abundant with secondary metabolites with impressive biological activities that are suited to different applications and treatments (Ayalew, 2020). These secondary metabolites have different structures and physicochemical properties. An example of such a plant (shrub) is *Lantana camara.* The leaves, flowers, roots, and fruits are all of medicinal use.

There are about 650 species of *Lantana camara* spread across over 60 countries on this planet (earth) (Sonibare & Effiong, 2008). Some species are scathel shrubs owing to the verbanaceae family, growing in tropical and warm regions worldwide. They are mostly cultivated for ornamental purposes as a result of the diversity in the colours of flowers (Sonibare & Effiong, 2008). This enables them to spread faster than other herbal (medicinal) plants. *Lantana camara* is considered a tropical-origin plant in Central, Northern, and South America and the Caribbean (Kokati *et al*., 2012). *Lantana camara* is reported to be in many African countries like Ghana, Kenya, Nigeria, Uganda, Tanzania, and South Africa.

In the last 20 years, the seeds and leaves of *Lantana camara* have been used in herbal medicine; its fruits are employed as dietary supplements (Jagtap *et al*., 2018). Recent studies on the efficacies of *Lantana camara* have shown that the shrub possesses varying medicinal properties, including anticancer activity, anti-inflammatory activity, antidiabetic activity, anthelminthic activity, antibacterial activity, antifungal activity, etc. (Kalita, 2012). *Lantana camara* leaves are aliphatic, and their essential oil extract is reported to have insecticidal properties, occupying bees, flies, and mosquito repellent (Adriana *et al*., 2010). The leaves can also be used to heal wounds per their fast-acting antiseptic and antimicrobial properties (Prasanth *et al*., 2009).

Lantana camara has been used in many parts of the world to treat a wide range of disorders (Ross, 1999). It is found to be used in native remedies for cancers and tumors. Tea is prepared from the leaves and flowers to combat fever, influenza, and stomach ache. In Central and South America, the leaves are made into a cataplasm to treat sores, chicken pox, and measles (Swarbrick, Wilson & Hannan, 1998). In Ghana, perfusion of the whole plant is used in treating bronchitis, and the powdered root in milk is given to children for stomach aches (Irvine, 1961). In Asian countries, the leaves are used to treat rheumatism and ulcers and as a vermifuge (Irvine, 1961). It is believed that a steroid and lancamarone from the leaves exhibit cardiotonic qualities (Sharma & Kaul, 1999).

For the past few years, chromatographic and spectroscopic analyses have revealed the major role of pharmaceutical and biomedical findings in extracts from the leaves of *Lantana camara* (Siddiqui, Alothman & Rahman, 2017). Researchers have used these two methods for multiple studies of plants to ascertain their physicochemical composition, especially gas chromatography-mass spectroscopy (GC-MS) and Fourier transform infrared spectroscopy (FTIR), which are extremely sensitive (Ayalew, 2020). The GC-MS analyses reveal major and minor components of the oil extract. The major components are Caryophyllene, Bicyclogermacrene, Germacrene D, Terpinolene, Sabiene, etc., whereas the minor components are Camphene, α-terpinene, t-sabinene hydrate, α-pinene, Terpin-4-ol, etc. (Jagtap *et al*., 2018).

Antioxidants are compounds that lengthen the storage life of foods, keeping them safe from deterioration as a result of oxidation, for instance, fat and oil rancidity and color changes (Karovicova & Simko, 2000). The ideology of antioxidants plays a key role in using fats and oils as raw materials in food processing. It is, therefore, prudent to understand that the chemistry of fats, mechanisms of oxidation, and functional role of antioxidants in reversing different types of deterioration is dependent on the proper and operative use of antioxidants. These antioxidants, therefore, act by containing free radicals formed from fats and oils by donating a free hydrogen atom (Struckey, 1972). They form stable and low-energy free radicals that depropagate the oxidation of fats and oils. They come in as natural or synthetic antioxidants used as food additives (Karovicova & Simko, 2000). Some synthetic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquione (TBHQ), etc., whereas some natural antioxidants are flavonoids, phenolic acids and their esters, tocopherol, carotenoid, and terpenoids, etc.

A concentrated hydrophobic liquid containing volatile fragrance components from the plant is referred to as an essential oil. Aromatic oils, fragrant oils, steam volatile oils, ethereal oils, or simply "oil of" the plant material from which they were extracted, such as oil of clove, are some of the terms used to describe them. The taste concentrations of essential oils and their resemblance to their respective sources are two of their benefits. As with citrus fruits, the majority of them are relatively stable and contain natural antioxidants and antimicrobial agents (Somesh *et al*., 2015). When used fresh, essential oils are usually colorless. However, as essential oils age, they may oxidize, causing a colostomy. Essential oils are typically colorless, especially when they are fresh. However, it may oxidize when the essential oil ages, causing the hue to darken. As a result, essential oils should be kept in a cool, dry location, properly sealed, and preferably in amber glass containers.

Aromatherapy, cosmetics, incense, medication, household cleaning goods, and food and beverage flavoring all employ essential oils. In the fragrance and food sectors, they are precious commodities. There are around 250 different types of essential oils. Essential oils are manufactured in a variety of nations. India is the world's second-largest exporter of essential oils (Rao *et al*., 2005). Essential oils are extracted from various plant parts. A physical process that does not result in a major change in the chemical makeup of an essential oil is commonly used to separate it from the aqueous phase. The essential oils could then be exposed to a suitable follow-up treatment. Oily aromatic liquids derived from aromatic plant components are known as essential oils. They could be biosynthesized as secondary metabolites in various plant tissues (El-Asbhanni *et al*., 2015).

Oxidative stress is caused by the generation of highly reactive oxygen species (ROS) with a single unpaired electron and is involved in the pathogenesis of a variety of physiological conditions, including cellular injury, aging, cancer, and hepatic, neurodegenerative, cardiovascular, and renal disorders (Losada & Bravo, 2017). Pollutants in the environment, radiation, chemicals, poisons, deep-fried and spicy foods, as well as physical stress, produce reactive oxygen radicals, which cause aberrant protein production and a depletion of antioxidants in the immune system (Agrawal, Kulkarni & Sharma, 2011).

Endogenous antioxidant enzymes, including glutathione peroxidase, catalase, and superoxide dismutase, can deactivate free radicals and maintain normal cellular processes (Kurutas, 2016). However, endogenous antioxidants may not be enough to sustain optimal cellular activities in the face of increased oxidative stress, necessitating the addition of dietary antioxidants (Rahman, 2007).

Natural diets rich in phenolic and flavonoid compounds with antioxidant activity have sparked attention in nutrition and food science in recent decades (Lee *et al*., 2015). Plant secondary metabolites with an aromatic ring and at least one hydroxyl group are known as natural phenolic and flavonoid chemicals (Tungmunnithum *et al*., 2018). Because their hydroxyl groups can directly contribute to antioxidant action, phenolic substances are strong electron donors (Bendary *et al*., 2013). Furthermore, several of them encourage the cell to produce endogenous antioxidant molecules (Cote *et al*., 2010). In biological systems, phenolic compounds display free radical inhibition, peroxide breakdown, metal inactivation, or oxygen scavenging, according to various reports in the literature (Oberoi & Sandhu, 2015). They also reduce the burden of oxidative illness.

Natural antioxidants in leafy vegetables prevent free radical damage (Yen & Chuang, 2000). Many epidemiological studies have linked the consumption of leafy plant vegetables rich in phenolic and flavonoid chemicals with strong antioxidant activity to a reduced risk of cardiovascular disease, cancer, diabetes, and neurological illnesses (Adebooye, Singh & Vijayalajshmi, 2008). Native Nepalese communities use wild edible leafy plants as medicine, salad, juice, and pickles (Shrestha & Dhillion, 2006). Although there has been some research on wild edible plants in Nepal, it is still confined to a survey of traditional use among local people (Uprety *et al*., 2012).

1.2. Statement of the Problem

The rate of developing free radicals and High-Density Lipoprotein (LDL) from internal stresses as well as from daily consumption of saturated fats in the human body is absurd. Also, due to the persistent use of antibiotics in the treatment of various infections, most bacteria have developed high resistance to these antibiotics. To curb the use of synthesized chemicals, which in the long run have damaging effects on the body, there is the need to resort to using plant-

based remedies with minimal effects for such problems. Hence, oil extracts from the leaves of Lantana camara should be used, as they are rich in antioxidants and have high antimicrobial properties.

1.3. Aim

- To ascertain the chemical composition, antioxidant capacity, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of essential oil extract of fresh leaves of Lantana camara.

1.4. Specific Objectives

- To extract essential oil from fresh leaves of *Lantana camara* by conventional hydrodistillation
- To evaluate the chemical compositions of the essential oil extract using GC-MS
- To determine the antioxidant capacity of essential oil extract with prepared standards using Spectrophotometric methods: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay, total antioxidant capacity (TAC) assay, and total phenolic contents (TPC).

1.5. Hypothesis

The essential oil extract would show evidence of antioxidant activity.

1.6. Significance of the Study

The fast rise of degenerative diseases and pathological bacterial infections in Sub-Saharan African countries calls for immediate and prompt alternative remedies to prevent, reduce, and/or cure the diseases. Hence, this study intends to determine the potency and efficacy of an essential oil extract from *L. camara* to curb these problems. The oil extract can, therefore, be used in natural (herbal) medicine or pharmaceutical products to treat or prevent free radical oxidation, causing degenerative diseases. The oil extract can also be incorporated into antibiotics or used wholly to treat microbial infections.

1.7. Limitation

Due to the inability to access the instrument room on time, the oil extract was stored for some time. This may have led to the loss of some vital components and reduced antioxidant capacity since the oil is highly volatile.

1.8. Delimitation

The study was confined to only the leaves of *L. camara* compared to the whole shrub, sampled from the backyard of Christian Home at Ayensu in the University of Cape Coast, Ghana.

2. Literature Review

2.1. Introduction

Lantana camara was introduced in India, America, the Caribbean, Africa, and other parts of the world as an ornamental plant that was entirely naturalized and found throughout India. However, it is enlisted as one of the most important medicinal shrubs ever to have evolved in the world (Ross *et al*., 1999). Thamotharan (2010) states that the shrub, *Lantana camara,* usually known as wild or sage, is the most pervasive species of this genus, and it is a non-herbaceous plant with various flower colors being white, yellow, violet, pink, and red. It is considered an intense odor-semperviren shrub with tingling sensation leaves that cause discomfort on contact with the skin.

A great deal of work has been conducted on the chemical composition of *Lantana camara,* especially the leaf oil extract. A carefully analyzed phytochemical screening of *Lantana camara* leaf oil extract has revealed that it is loaded with phenols, flavonoids, quinine, proteins, carbohydrates, glycosides, saponins, triterpenes, tannins, alkaloids, etc. (Kalita *et al*., 2012). Extracts from the leaves exhibit anti-proliferative, antimicrobial, fungicidal, insecticidal, and nematocidal activities (Sharma, 1999). Pharmacological analysis showed that extracts of the shoots and leaves of *Lantana camara* exhibited strong antioxidant activities (Basu, 2006).

Figure 1: Arial View of the Leaves with Flowers of Lantana camara
Photo Credit: Martey Harrison, 2021

2.2. Biochemical Composition

The phytochemical investigations on the leaves, stem, and root from the yellow, red, and pink flowering taxa of *Lantana camara* showed 13 compounds. These were separated from ethyl acetate, and their identity was established through gas chromatography-mass spectroscopy analysis (Sharma *et al*., 2000). Nougueiras *et al*. (2010) identified more than 30 new compounds with curative medicinal properties; triterpenes, lantadene A and B, betalonic acid, flavonoid, pectolinarigenin, hispidulin, etc. were some of the identified compounds. Those with extreme toxicity showed the least morbidity (Sharma et al., 2011).

2.2.1. Monoterpenes and Sesquiterpenes

According to Ole and Gildermeister (1961), early studies focused on the essential oils produced from *Lantana* species, although they did not produce really high yields. A sample of the oil extract was examined, and it was found that it had a terpene-like, leathery, fatty, and sweaty odor. The components were identified to be mainly (65%) bisabolene derivatives with only minute remnants of monoterpenes. The sesquiterpenes present were B-curcumene (1.5%), (E)-nuciferol and (Z)-nuciferol (3.9%), (-)-ar curcume-15-al (5.6%), g-curcumene (8%), ar-curcumene (9.7%), (-)-epi-b-bisabolol (10%), and (-)-g-curcume-15-al (14.9%). (Ole and Gildermeister, 1961).

Surprisingly, remarkable differences in compositions were noticed with samples collected from different locations in Brazil (Pathak *et al*., 1997). Pathak et al. (1997) identified the oil extract to contain mainly humulene (22%), caryophyllene (15%), and davanone (15%).

2.2.2. Iridoid Glycoside

According to Ford and Bendall (1980), several glycosides have been isolated from *Lantan acamara*, being the pink, red, and white flowering taxa, producing an indefinite amount of these sides present as the sodium in salt. As part of the classification of *Lantana* species, greenhouse-grown plants were observed to contain sodium salt on the sides in both leaves (800 ppm) and roots (900 ppm). However, the virodosiside, the corresponding methyl ester (320ppm), was only present in the roots (Saurbier & Rimpler, 1986).

2.2.3. Flavonoids

The acetone wash of the leaves of *Lantana camara* contained 3-methoxy-3, 7-dimethoxy, and 3, 7, 4-trimethoxyquercetin (Siems *et al*., 1997), whereas hispidulin was isolated from the stems (Huang *et al*., 1998). The flavone, glycoside camaraside, has also been isolated from *Lantanacamara* together with pectolinarigenin 7-O-b-D glycoside (Sharma, 1994).

2.3. Medicinal Properties

Different parts of *Lantana camara* have been used to treat various human ailments. It is one of the most prevalent shrubs with very high and potent medicinal properties. Due to its high therapeutic properties, present surveys focused on the bioactive molecule lantadene and its use.

2.3.1. Anti-cancer Activity

Compounds like flavonoids, alkaloids, triterpenoids, glycosides, and naphthoquinones separated from the oil extract of *Lantana camara* are known to influence different biological activities, not excluding anti-cancer properties. According to Ragha *et al*. (2004), research was conducted on the anti-cancer viability of methanol extracts from different parts of *Lantana camara*, where four (4) cancerous cell lines and normal cell lines were sampled for the study to investigate the in vitro cytotoxic properties of the oil extract. The methanol extract exhibited great cytotoxic activity against the 4 cancerous cell lines, i.e., curtailed their growth to minimal levels, whereas serving as a protective cover for the normal cell lines. Sharma et al. (2006), therefore, concluded that the anti-cancer activity may have been due to the presence of flavonoids and flavonolignans, offering a great ambit for manufacturers in the future.

2.3.2. Insecticidal Activity

A major problem facing farmers in sub-Saharan Africa is the issue of pest infestation during planting and post-harvest. As such, these farmers resort to using synthetic insecticides to stop insect activity in grains and other stored farm produce. These synthetic insecticides, therefore, penetrate stored grain and may be toxic (Lalah *et al*., 1996). According to Ogando *et al*. (2004), when *Lantana camara* leaf extracts were sprayed on stored grains during a 150-day study period, the leaf extract had a toxic and repellent effect against insects of the stored grains. There was a significant percent damage (> 40%) to the grains untreated with *Lantana camara* leaf extracts, whereas the treated ones with leaf extract saw an insignificant percentage of damage (<2%).

During another experimental research, as reported by Binti et al. (2010), spraying 10% *Lantana camara* leaf water extract per week in the field could not predominate *Oulema pectoralis* pest infestation but stimulated the number of leaves, flowers, and stalk dud.

2.3.3. Anti-Inflammatory, Analgesic, Hemorrhoidal Activity

Investigations into the anti-analgesic, anti-inflammatory, and anti-hemorrhoidal activities of *Lantana camara* leaf extract exhibited a mild decrease in volume of 300 mg/kg of the oil extract but showed a significant decrease when treated with 500 mg/kg as white mice were the study organisms (Gidwani *et al*., 2009). Oleanolic acid and urologic acid exhibit

inhibitory properties in inflammation and at various stages of tumor development, whereas thrombin inhibitory activity was investigated to have come from the euphone lactone triterpene, which prohibited blood clotting.

2.3.4. Anti-helminthic Activity

One lesser-known infection that man battles with is the helminthic infection, feigning a greater percentage of the population worldwide. Patel *et al*. (2011) reported a study conducted on the effectiveness of *Lantana camara* essential oil. In the research, 50 healthy Indian earthworms, *Pheratima potuma,* were selected for the study due to their anatomical and pharmacological resemblance with intestinal roundworm parasites. When the worms were fed on ethanolic extracts of the oil for 6 weeks, the worm population was reduced to 23 (46%) motility. This proved how effective the essential oil extract could be when switched from most synthetic drugs.

2.3.5. Cytotoxicity

Research into the aqueous extract of *Lantana camara* (50g dry mass) showed that it had a percentage (0.23%) cytotoxic effect on the HeLa cells with an IC_{50} value of 1500µg/mL in 36 hours. On the other hand, a cytotoxicity test on the Vero cell line indicated that leaf extract concentration up to 500µg/mL rather prohibited the growth of cell cytotoxicity, beginning to dwindle at rarified concentration. As such, it was insinuated that acute oral toxicity of the extract should be very beneficial for clinical study (Pour *et al*., 2011).

2.3.6. Anti-micro-bacterial Activity

Of the many different types of pseudomonas, the one that most often causes infections in humans is *Pseudomonas aeruginosa*, which customarily affects hospitalized or immune-suppressed persons. The infection usually occurs in the respiratory tract and is also frequent in patients with cystic fibrosis as well as those with bronchiectasis (Miskie *et al*., 1997). As these infections are prevalent, *Pseudomonas aeruginosa* develops high antimicrobial resistance and has difficult management.

According to Miskie *et al*. (1997), the results of antibacterial tests by gaseous contact proved that the antibiotic activity of amikacin against *Staphylococcus aureus* was meliorated in the company of oil extract from *Lantana camara* leaves by gaseous contact.

The efficacy of the antibacterial activity of amikacin and gentamicin in abating *Pseudomonas aeruginosa* by the essential oil extract was also authenticated. The boosting of antimicrobial activity against the gram-negative bacteria indicated an instant result, as gram-positive bacteria are more liable to natural products. The essential oil extract has not only shown antibacterial properties but also the capacity to meddle with antibiotic resistance (Sousa *et al*., 2011). Souse *et al*. (2011) also asserted in the study the popular use of *Lantana camara* oil extract to treat respiratory infections.

In another study, the essential oil of *Lantana camara, Ocimum sanctum,* and *Tagetes patula* were effective against all the bacteria strains when cultured in nutrient agar. A higher concentration of *Lantana camara* oil extract was able to inhibit *Escherichia coli* growth (Dharmagadda *et al*., 2005).

Antibacterial activity of bacterial strains, including gram-positive *Staphylococcus aureus* and *Staphylococcus saprophilticus*, gram-negative *Escherichia coli,* and *Pseudomonas aeruginosa,* was determined by the disc method. After incubation time, the inhibition area around the discs was measured. Results indicated that petroleum ether root extract showed less antibacterial activity on *Pseudomonas aeruginosa* and *Staphylococcus saprophilticus*. The inhibition zone size of gram-positive bacteria indicated less sensitivity to the root extract of *Lantana camara*. The maximum inhibitory zone was recounted against *Escherichia coli* in methanol stem extract as juxtaposed to the other extract. Saponins, flavonoids, alkaloids, and tannins were reported to have antibacterial properties (Kensa, 2011). Diverse chloroform extracts of the leaf, stem, and root have an optimum inhibitory effect against *Escherichia coli* (Viji *et al*., 2011).

The literature has proved that gram-positive bacteria are more sensitive to antibiotics, while gram-negative bacteria showed some uniqueness that interdicts antibiotic penetration, but it depends on several influences and is associated with the presence of chemicals.

The antibacterial activities of *Lantana camara* leaves were analyzed, and the flower extracts possessed more powerful antibacterial properties than the paralleling leaf extracts. Analysis showed that the loftiest bacterial inhibitory effects against *Bacillus subtilis* by *Lantana camara* oil extract of the yellow flower ethyl acetate extracts were found to be the most effective against all bacteria except *Staphylococcus aureus* (Ganjewala *et al.,* 2009).

3. Methodology

3.1. Study Area

Fresh leaves of the *Lantana camara* were sampled from the backyard of Christian Home, located in Ayensu, a suburb community of the University of Cape Coast, Ghana.

3.2. Sample Collection

The leaves were sampled using the purposive sampling technique, and only fresh leaves were collected for this study. The sample was collected early in the morning.

3.3. Sample Treatment

The leaves were then sent to the Organic Chemistry Research Laboratory for essential oil extraction and further spectrometry analysis in the Instrument Room**.** The sampled leaves were weighed, washed, and cut into smaller pieces to enhance their surface area for oil extraction, and then packed into a still compartment of the hydrodistillation setup.

3.4. Sample Analysis

3.4.1. Extraction of Essential Oil from the Leaves of *Lantana camara* Using Conventional Hydrodistillation

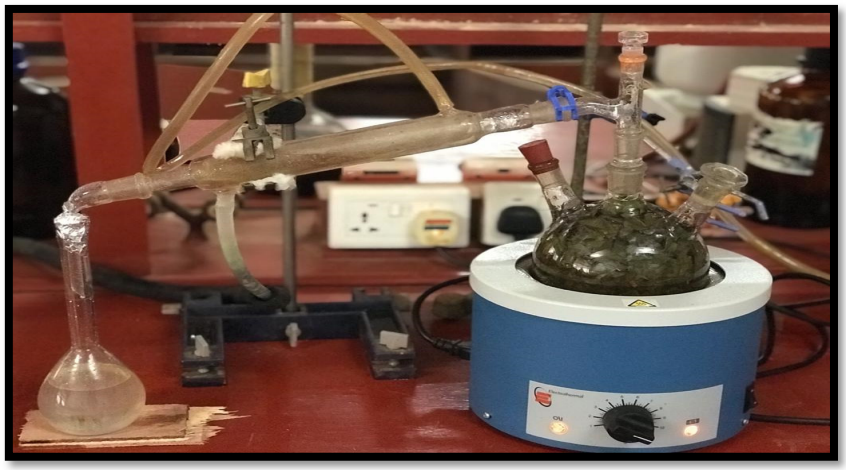

Figure 2: Ahydrodistillation Setup
Credit: Martey Harrison, 2021

3.4.1.1. Working Principle

Hydrodistillation is a traditional method for the removal of essential oils. One of the simplest and oldest methods is water distillation (Meyer-Warnod *et al*., 1984). Essential oils are extracted using this method. Hydrodistillation is a method of extracting essential oils from aromatic and medicinal plants. Hydrodistillation (HD) is a traditional method for extracting essential oils, which are evaporated by heating a mixture of water or another solvent and plant materials, and then the vapors are liquefied in a condenser. A condenser and decanter are also used in the setup to collect condensate and extract essential oils from water, respectively. The extraction principle is based on isotropic distillation. Water or other solvents and oil molecules are present under atmospheric pressure and during the extraction process (heating). The French invented hydro-distillation (HD), which is a type of steam distillation. Essential oil extraction from dried plants and quality control of essential oils in the lab are covered by this pharmacopeia. Water immersion, direct vapor injection, and water immersion and vapor injection are the three types of hydrodistillation. It is a multilateral procedure that can be applied to both large and small businesses. The amount of time required for distillation is determined by the type of plant material being processed. Longer distillation yields a modest amount of essential oil but introduces undesirable high boiling point chemicals and oxidation products.

3.4.1.2. Experimental Procedure

The experimental procedure was set up using the heating mantle, water bath with ice cubes, condenser, round-bottom flask, and flat-bottom flask. 314 g of the treated leaves were packed into the still compartment (round-bottom flask) seated in the heating mantle. 1.5L of distilled water was then added to bring to a boil. As the temperature of the heating mantle was regulated, steam and hot air caused evaporation of the essential oil in the leaves. This caused the generated steam to have a mixture of both water and essential oil. This procedure was allowed to run for about 4 hours. The condenser then condensed the steam and the distillate, collected in the flat-bottom flask, and allowed to cool to room temperature. A 100ml distillate was obtained after the hydrodistillation procedure.

3.4.1.3. Separating Funnel

3.4.1.3.1. Working Principle

Solvent extraction also referred to as liquid-liquid extraction or partitioning, maybe a method to separate a compound supported by the solubility of its parts. This is often done using two liquids that do not mix, for example, water and an organic solvent. Within the solvent extraction method of essential oil recovery, an extracting unit is loaded with perforated trays of volatile oil material and repeatedly washed with the solvent. Perfumes, vegetable oils, and biodiesel are all processed using solvent extraction. On sensitive plants, solvent extraction is employed to provide more essential oils at a reduced cost (Chrissie *et al*., 1996). In material analysis, this can be the most commonly used sample preparation process. Since the procedure is proscribed by the compound solubility within the specific solvent utilized, the sort of additional heat given determines the standard and amount of the extracted mixture. Although the method is simple and effective, it has a variety of drawbacks, including a protracted extraction time, significant solvent consumption, and inconsistent repeatability (Dawidowicz *et al*., 2008).

3.4.1.3.2. Experimental Procedure

The essential oil in the distillate was extracted using a separating funnel. The organic solvent, diethyl ether, was used as the solvent of extraction. For each 250 ml of the distillate poured into the separating funnel, 40 ml of the diethyl ether was added and swirled for about 5 minutes. This was then mounted on a retort for about 10 minutes to separate the organic phase from the inorganic phase. The organic phase (the essential oil) was filtered into a conical flask. This was repeated three times to ensure maximum extraction. The filtered sample was dried using anhydrous sodium carbonate (Na2CO3) as the drying agent (covered with aluminum foil) for about 15 minutes. After this, the filter paper was used to filter the essential oil from the drying agent into a beaker, which was covered with aluminium foil with minute perforated holes in the opening. This was allowed to stand for 48 hours. The essential oil extracted was later transferred into clean, airtight vials and kept in a desiccator for further analysis. A total of 42.0 g of the essential oil was obtained for analysis.

3.4.2. Gas Chromatography – Mass Spectrometry Analysis

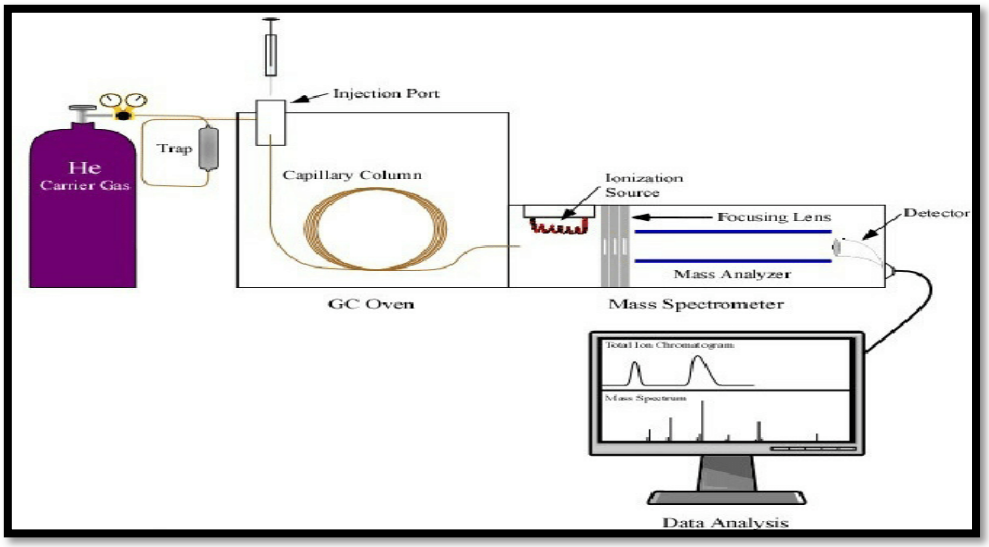

Figure 3: Schematic Diagram of GC-MS
www.researchgate.com

3.4.2.1. Working Principle

The GC/MS equipment separates chemical mixtures (GC component) and identifies the components at a molecular level (MS component). It is one of the most precise equipment for assessing environmental samples on the market. When a combination is heated, it separates into different compounds, which is how the GC works. The new gases are sent via an inert gas-filled column (such as helium). The separated substances pour into the MS as they emerge from the column aperture. The mass of the analyte molecule is utilized in mass spectrometry to identify chemicals. A library of known mass spectra covering several thousand compounds is stored on a computer. Mass spectrometry is taken into consideration as the only definitive analytical detector. The GC generates a chromatogram, a graph during which each separated substance is represented by a peak. The number of peaks within the sample indicates the number of distinct chemicals. The retention time for each compound is indicated by the position of each peak.

3.4.2.2. Sample Preparation

100 mg of the sample was weighed and dissolved in dichloromethane (1 mL), vortexed for 10 s, sonicated for 1hr., centrifuged at 14,000 rpm for 5 min and then dried by passing through anhydrous sodium sulphate before analysis (100 ng/µl, 1µL) by an Agilent Gas Chromatograph 7890A / 5975 C Mass Spectrometer in full scan mode. The extraction and analysis were carried out in triplicates. The following machine conditions were used:

GC Column:	HP-5 MS low bleed capillary column (30 m × 0.25mm i.d., 0.25 µm) (J&W, Folsom, CA, USA)
Flow rate, (He):	1.25 ml/min, constant flow mode
Injection Mode:	split mode
Oven temperature:	35°C (5 min.) to 280°C @10°C/min (10.5min) then to 285°C @50°C/min (29.9 min); run time 70min
Injection volume:	1µl

Table 1

Compound identities were determined using NIST'11, 08, 05, Adams and chemecol mass spectral databases.

3.4.2.3. Experimental Procedure

An Agilent Gas Chromatograph 7890A/5975 C Mass Spectrometer equipped with a Rtx-5mS fused HP-5 MS low-bleed capillary column (30 m × 0.25 mm i.d., 0.25 μm) (J&W, Folsom, CA, USA) and an FID detector were used for the quantitative determination of oil composition. Oven temperature was programmed as follows: 35°C (5 min.) to 280°C @10°C/min (10.5min) then to 285°C @50°C/min (29.9min); run time 70min. It was then ramped at 5°C/min, rising to 280 °C. Injector temperature: 250°C. Carrier gas: helium with a flow rate of 1.25 ml/min, constant flow mode. Detector temperature: 250°C, split ratio: 30:1. Diluted samples (1.0 μL, 1/100, v/v, in dichloromethane, DCM) were injected manually in the split mode. Identification of the oil components was based on their retention indices and mass spectra obtained from GC/MS analysis on an Agilent Gas Chromatograph 7890A/5975C Mass Spectrometer in full scan mode. The GC analysis parameters are listed above, and the MS ones were obtained (full scan mode: scan time: 0.3s, the mass range was m/z 35-450) in the electron ionization mode at 72.4 KPa. All the data were the average of triplicate analyses. Detection and identification of the constituents of the oils were based on comparisons of the individual retention times and mass spectra with those obtained from using NIST'11, 08, 05, Adams, and chemecol mass spectral databases and literature.

3.4.3. Antioxidant Analysis

3.4.3.1. Total Phenolic Content (TPC)

3.4.3.1.1. Working Principle

The Folin-Ciocalteu reagent, which is made up of tungstates and molybdates, is used to study the oxidation-reduction reaction process (Wolf *et al*., 2003). The approach is based on the phenolic compound reducing the mixture of heteropolyphosphotungsates–molybdate, resulting in the synthesis of blue-colored chromogen. Only under basic conditions, such as those provided by a sodium carbonate solution, do phenolic substances react with the Folin-Ciocalteu reagent. Under basic circumstances, the phenolic compound dissociates to generate a phenolate anion, which reduces the Folin-Ciocalteu reagent, which is a combination of tungstates and molybdates, resulting in a blue-colored solution. The absorbance values from a spectrophotometer can be used to determine the color intensity of the generated blue chromogen.

3.4.3.1.2. Experimental Procedure

The total phenolic capacity of the essential oil extract was determined by UV-Vis spectrophotometer using the Folin-Ciocalteu's reagent, as described by Wolf *et al*. (2003). Gallic acid, as a standard, was prepared by dissolving 0.01 g of the gallic acid in a 100mL ethanol; hence, 0.01% (w/v) of gallic acid was obtained. Also, 7.5 g of sodium carbonate (Na_2CO_3) was diluted to 100 mL dilute water to obtain 7.5% (w/v) Na_2CO_3. 10 mL of Folin-Ciocalteu's phenol was prepared to 100 mL by adding distilled water to obtain a concentration of 10% (%V/V). 500 μL of the essential oil was pipetted to 500 μL of ethanol into a test tube. 2 mL of the 7.5% anhydrous sodium carbonate (Na_2CO_3) and 2 mL of the 10% Folin-Ciocalteu's reagent were added to the content of the test tube. This was done for three different test tubes and labeled C1, C2, and C3. The final mixture was then incubated for about 15 minutes at a temperature of 50oC. A blue-black coloration (chromophore) occurred, indicating the presence of phenolic compounds in the essential oil. A blank solution was also prepared—without the essential oil extract. Varying concentrations of different test tubes, labeled, A (100μL GA + 900mL H_2O + 2mL Na_2CO_3), B (200μL GA + 800mL H_2O + 2mL Na_2CO_3), C (300μL GA + 700mL H_2O + 2mL Na_2CO_3), D (400μL GA + 600mL H_2O + 2mL Na_2CO_3), D (400μL GA + 6000mL H_2O + 2mL Na_2CO_3), E (50000μL GA + 500mL H_2O + 2mL Na_2CO_3), F (600μL GA + 400mL H_2O + 2mL Na_2CO_3), G (700μL GA + 300mL H_2O + 2mL Na_2CO_3) and H (800μL GA + 200mL H_2O + 2mL Na_2CO_3). These were also incubated for 15 minutes at 50oC. Using the T70 UV-Vis spectrophotometer, the absorbance was obtained for the samples (C1, C2, and C3) and the standards (A to H), respectively, at a wavelength of 765 nm. A standard calibration curve of absorbance against concentration was constructed from the absorbance values of the gallic acid.

3.4.3.2. Total Antioxidant Capacity (TAC)

3.4.3.2.1. Working Principle

In the total antioxidant capacity assay protocol, the Cu^{2+} ion is converted to Cu^{+} by both small-molecule and protein antioxidants. The Protein Mask prevents Cu^{2+}reduction by proteins, enabling the analysis of only the small-molecule antioxidants. The reduced Cu^{+} ion is chelated with a colorimetric probe, giving a broad absorbance peak of around 570nm, proportional to the total antioxidant capacity.

3.4.3.2.2. Experimental Procedure

The total antioxidant capacity (TAC) of the essential oil extract of *Lantana camara* was examined using a UV-Vis spectrophotometer according to the phosphomolybdenum assay outlined by Wolfe *et al.* (2003). A 1.20 g Na_2SO_4 was dissolved in a 250 mL volumetric flask of H_2SO_4 at an initial concentration of 12.48M. Also, a 1.25g of ammonium molybdate was dissolved into the 250 mL H_2SO_4 volumetric flask. The mixture was swirled to ensure homogeneity. Using ascorbic acid as the standard, a mass of 0.01g of $C_6H_8O_6$ was dissolved with distilled water in 100mL to obtain a concentration of 0.01% (% w/v). 500μL of the essential was pipetted into three different test tubes: C1, C2, and C3. 3mL of the H_2SO_4, together with sodium phosphate and ammonium molybdate (phosphomolybdenum), was added to each of the

three test tubes. The test tubes were incubated for 60 minutes in a water bath at 95 OC and allowed to cool to ambient temperature. Serial dilutions of the standard ascorbic acid were also prepared in ten different test tubes, labeled S1 to S10, in varying concentrations of 10µg/mL to 100µg/mL, respectively, with the addition of 3mL phosphomolybdenum solution. These test tubes were also incubated for 60 minutes at 95OC. A blank solution was also prepared. Using the T70 UV-Vis spectrophotometer, the various abundances were obtained for the standard, essential oil, and the blank. A standard calibration curve of absorbance against concentration was constructed from the abundance values of the ascorbic acid.

3.4.3.3. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Assay

3.4.3.3.1. Working principle

The 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) free radical method produces a violet solution in ethanol and is an antioxidant assay based on electron transfer. In the presence of an antioxidant molecule, this free radical, which is stable at room temperature, is diminished, resulting in a colorless ethanol solution (Prior *et al.,* 2005). The DPPH test is a simple and quick technique to evaluate antioxidants using spectrophotometry, and it may be used to evaluate multiple items at once.

3.4.3.3.2. Experimental procedure

The radical scavenging activity of the essential oil extract was determined as described by Prior *et al.* (2005) using the DPPH assay. 0.001g of the powdery DPPH was weighed and dissolved in a 100ml-volumetric flask using methanol. The flask was covered with aluminium foil as the DPPH is volatile. Ascorbic acid was used as the standard test. 0.01g of C6H8O6 was dissolved in 100mL distilled water in a volumetric flask. 1mL of the 0.001% DPPH was added to C1 (200µL *L. camara*+ 800µL methanol + 1mL C6H8O6), C2 (400µL *L. camara* + 600µL methanol + 1ml C6H8O6), C3 (600µL *L. camara* + 400 µL methanol + 1 mL C6H8O6) and C4 (800µL *L. camara* + 200µL methanol + 1 mL C6H8O6). These were allowed to stand for 30 minutes. A color change from purple to pale yellow indicates the reduction of the oil and their abundance values were determined using the T70 UV-Vis spectrophotometer at a wavelength of 517nm. The same procedure was used for the standard ascorbic acid at varying concentrations of 200 µg/mL, 400µg/mL, 600µg/mL and 800µg/mL. A standard calibration curve of absorbance against concentration was then constructed from the absorbance values obtained. The equation from the curve was used to calculate the concentration of the essential oil per its scavenging activity.

3.4.4. UV-Vis Spectrophotometric Analysis

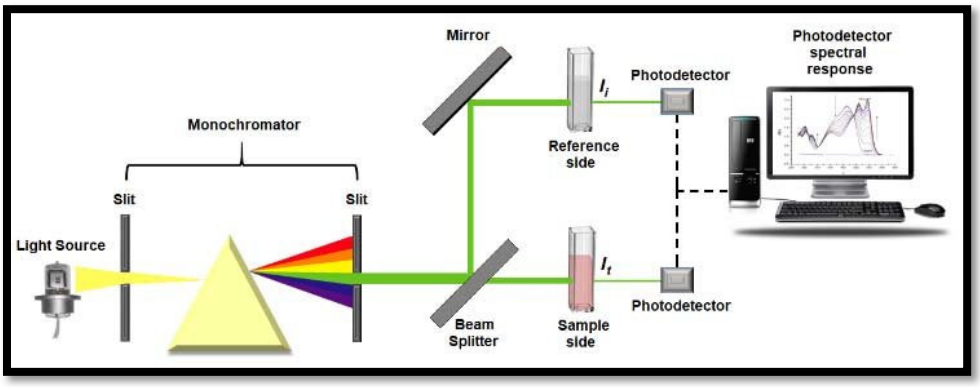

Figure 4: Schematic Diagram of UV-Vis Spectrophotometer
www.researchgate.net

3.4.4.1 Working Principle

The UV-visible Principle of spectrophotometry is based on a chemical compound's absorption of ultraviolet or visible light, which results in the formation of different spectra. The interaction of light and matter is the basis of spectroscopy. Excitation and de-excitation occur as matter absorbs light, resulting in the formation of a spectrum.
When matter absorbs ultraviolet light, the electrons inside it become excited. This leads them to leap from a ground state to an excited state (an energy state having a little amount of energy connected with it) (an energy state with a relatively large amount of energy associated with it). It is worth noting that the difference between the energies of the electron's ground and excited states is always equal to the quantity of ultraviolet or visible energy it absorbs.

3.4.4.2. Experimental Procedure

The inside of the T70 UV-Vis spectrophotometer chamber was checked to ensure the appropriate sample holder was in place for the liquid sample (the essential oil extract). The T70 UV-Vis spectrophotometer was turned on by pressing the power button in the front of the unit. The Carry WinU was opened to click on scan to start the program. The setup button was clicked to set the experimental parameters, the Carry Tab to change the wavelength range setting, and the Baseline Tab to select zero/baseline corrections. The OK button was clicked after the parameters were set. The blank solution was transferred into the cuvette and placed in its holder with the transparent face facing the light source. The

start button was pressed, and the absorbance value was displayed on the monitor. This procedure was repeated for each sample analyzed. Standard calibration curves were then constructed from the standards, and the concentrations of the essential oil extract were determined from the equations obtained.

3.5. Data Analysis

The data were structured in tables, and the results were displayed in graphs. The data were analyzed using MS Excel version 2016 as the statistical package. The data were analyzed utilizing Excel's descriptive statistics tool and Graphpad Prism version 5.0. The oil extract's percent radical scavenging activity was calculated and compared to that of standard ascorbic acid for the DPPH. The TPC and TAC of the oil extract were calculated using the standard calibration curves for gallic acid and ascorbic acid, respectively. The calibration curves were created by graphing absorbance against concentration in MS Excel and Graphpad. The oil extract's total phenolic content and total antioxidant capability were compared to each other. The chemical composition was obtained by GC-MS analysis.

4. Results and Discussion

4.1. Extraction and Percentage Yield of the Oil Extract

Hydrodistillation is a traditional method for extracting essential oils from plant tissues in which a suitable amount of water is poured into the compartment containing the materials, which is then brought to a boil (Azmir *et al*., 2013). This method was used to extract the essential oil from 314.0g of fresh lantana camara leaves, with diethyl ether as the extracting solvent of choice due to its low water solubility (Amerigo & Chai, 2003). The oil extract produced had a mass of 42.0g and a volume of 20.00mL. This resulted in a percentage yield of 13.38%, which is significantly greater than the 12.7% reported by Emmanuel *et al*. (2003).

4.2. GC-Ms Compositional Analysis

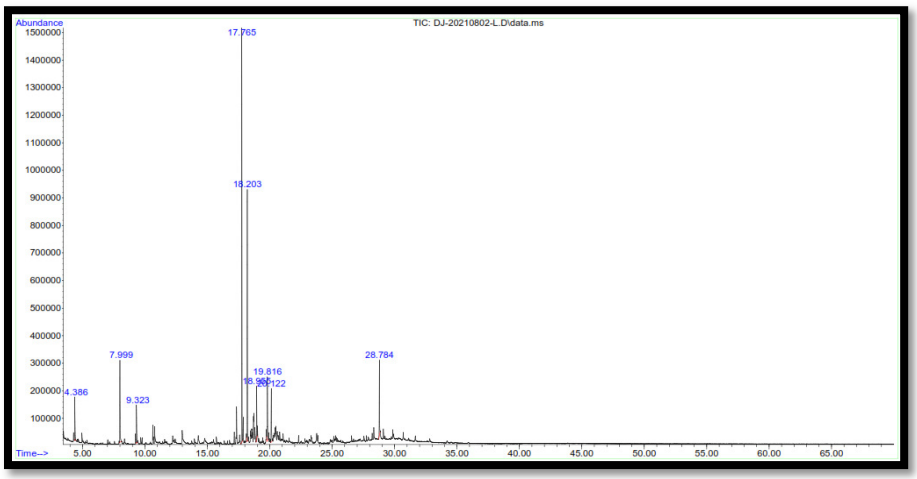

Figure 5: Non Expanded Total Ion Chromatogram with Retention Time Annotation

Results from the GC-MS analysis in figure 5 represent the chemical composition of *Lantana camara* oil extract with respective retention indices and relative peak area, along with their abundance (%). The percentage peak areas were taken to represent the proportion of each compound relative to the total. The analysis resulted in the identification of 76 unique constituents in the oil extract. The oil was shown to be a complex mixture of numerous compounds, many of which were present in low quantities. The identified compounds comprise 99.4% of the total extracted oil. The results could be compared to those reported by Limberger et al. (2001), obtained through the hydrodistillation method of oil extraction and the GC-MS technique of *L. camara.*

The 76 chemical components analyzed by GC-MS can be found in appendix 1. The major chemical components analyzed are 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methyl ethyl) (2.7%), 3Z-Hexenol (5.4%), Caryophyllene oxide (5 %), (-)-Aristolene (2.3%), 12-Oxabicyclododeca-3,7-diene, 1,5,5,8-tetramethyl (3.7%), α-Humulene (13.5%), (*E*)-Caryophyllene (20.6%), 1,6-Cyclodecadiene, cubedol (3.7%), Linalool (2.6%), and 2-ethoxy-2-methylpropane (2.8%). Among these major constituents, caryophyllene and α-humulene are most predominant, with % abundances of 20.6 and 13.5, respectively.

The chemical composition of the essential oils extracted from the *L. camara* leaves agreed quite well with what was previously reported in Passos, Barbosa and Demuner (2012), with some differences within the relative quantities of the volatile compounds. The observed differences in the standard and composition of the extracted oil between studies could be due to factors like genetics, climate, topography, season, etc. Most of the isolated compounds, namely 12-Oxabicyclododeca-3,7-diene, 1,5,5,8-tetramethyl, 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl), and α-Muurolene, are known for pharmaceutical applications (Abirami & Rajendran, 2011); for example, α-Muurolene has been reported as having pain-relieving and anti-inflammatory properties, and it exhibits antifungal activity against dermatophytes. Oxabicyclododeca oil, which is rich in dodecene (> 73%), has been reported to entail antioxidant,

antibacterial, and insecticidal activities (Hemalatha *et al*., 2014). Besides, the medicinal value of *L.camara* oil and the sustained demand for synthetic flavorings and fragrances to be used within the pharmaceutical, food, and cosmetic industries make this essential oil valuable for exploitation in these industries.

4.3. Total Phenolic Content (TPC)

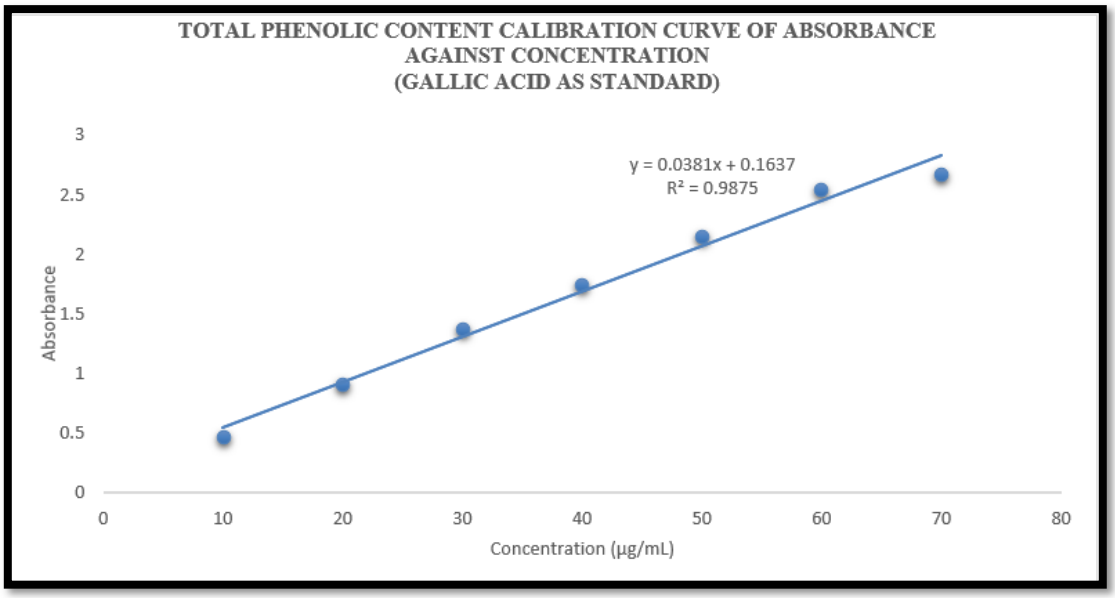

Figure 6: Standard Calibration Curve of Gallic Acid

TPC activity is the process of figuring out the amount of phenolic content in samples. Phenolic compounds that are contained in plants have redox properties, which allow them to act as antioxidants (Shoid & Shashid, 2015).

Phytochemical screening of the diethyl ether extract of the *Lantana camara* and the standard gallic acid was conducted, and both showed the presence of phenolic compounds. The total phenolic contents were determined using the Folin-Ciocalteu method in terms of gallic acid equivalent (GAE) in mg/g of the extract. Phenolic compounds are important plant components with redox properties responsible for antioxidant activity (Soobrattee *et al*., 2005). The hydroxyl groups in the oil extract are responsible for enhancing free radical scavenging.

The total phenolic content was calculated using the graph in figure 6 and the standard curve equation is y = 0.0381x + 0.1637, with R2 = 0.9875, implying a 99 % correlation between the data sets. The concentrations of essential oil extract were calculated using this equation, and the average is 2.029±0.030µg/mL. In the oil extract (20 mL), the total phenolic content (gallic acid equivalent, mg/g) was determined to be 2.029 ± 0.030 mg GAE/g. The TPC and TAC of the oil extract were compared in figure 7 below, with the oil extract having a greater TPC (approximately two-folds) than the TAC. The R2= 0.9033 (90%) indicates a stronger correlation between the two data sets. The P-value = 0.0036, therefore P<0.05, meaning there is a statistically significant difference in their mean value. This means there are more phenolic contents present in the oil extract, with a lower antioxidant capacity.

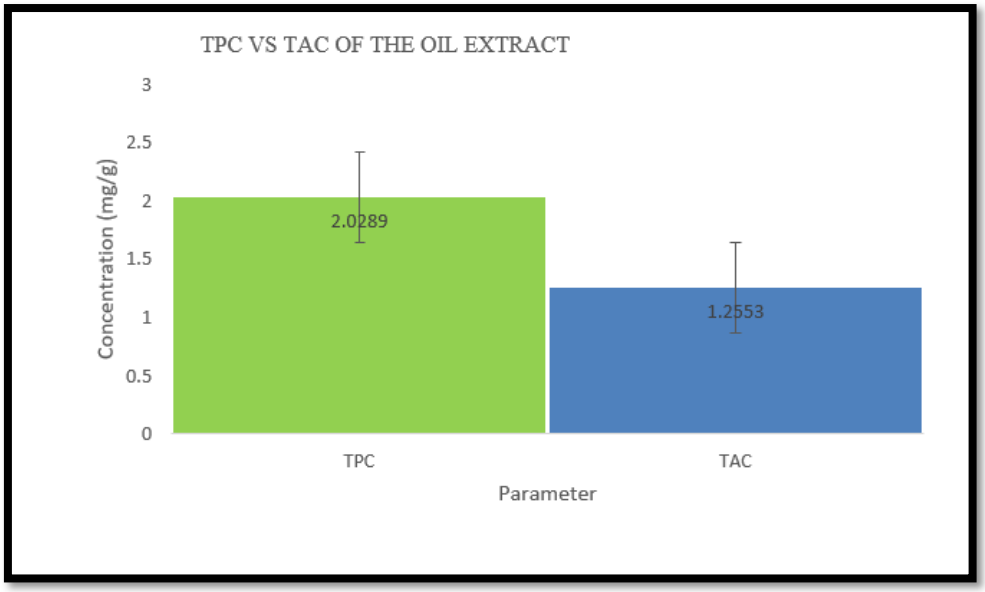

Figure 7: Comparison of TAC and TPC Analysis of the Oil Extract

A phenol loses an H+ ion to generate a phenolate ion, which decreases the Folic-Ciocalteu reagent in the basic reaction conditions (Fernandes et al., 2012). The change is measured using spectrophotometry. Because phenolics (including many flavonoids) include polar phenolic hydroxyl group(s), gallic acid's greater TPC is understandable. Similarly, the diethyl ether extract's lower TAC could be explained in the same way.

4.4. Total Antioxidant Capacity (TAC)

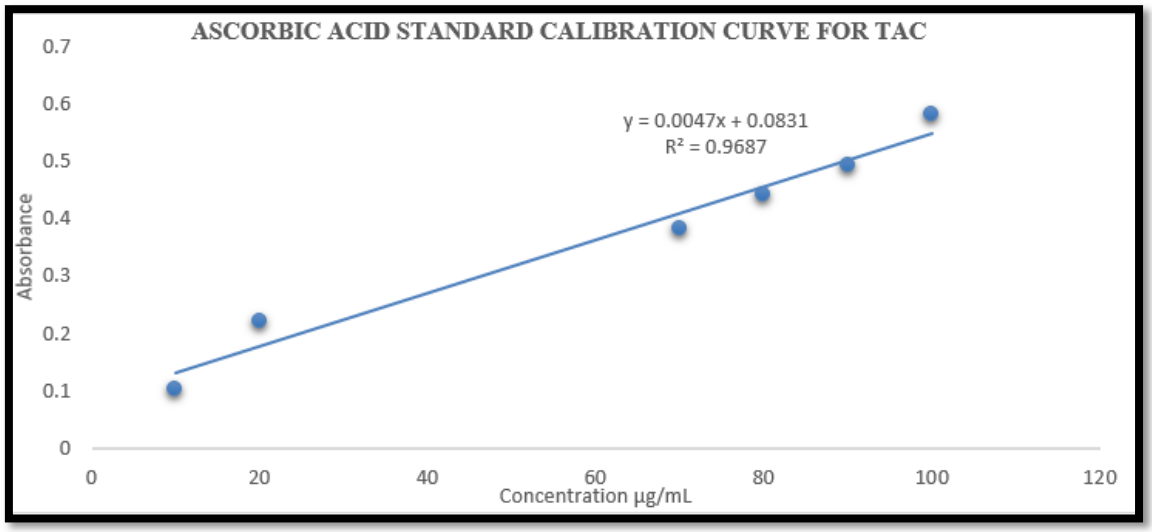

Figure 8: Standard Calibration Curve of Ascorbic Acid

Total antioxidant capacity (TAC) is a commonly used analyte for determining the antioxidant status of biological samples and can assess the antioxidant response to free radicals produced in a given disease (Kohen & Nyska, 2002). Trolox equivalent antioxidant capacity (TEAC), ferric reducing ability of plasma (FRAP), and cupric reducing antioxidant capacity (CUPRAC) are three different assays used to calculate a sample's TAC. This study reports the TAC values in mg AAE/g, where AAE is the ascorbic acid equivalent. The assay is based on the fact that molybdenum (VI) is reduced to molybdenum (V) in the presence of a reducing agent (antioxidant), forming a green phosphomolybdate (V) complex, which can be evaluated spectrophotometrically at 765 nm (Prieto, Pineda & Aguilar, 1999). The assay involves an electron transfer (ET) mechanism. Many natural products, including phenols and flavonoids, can cause this reduction.

Figure 8 above shows a plot of concentration against absorbance of the standard ascorbic acid, which gave a linear regression graph of $y = 0.0047x + 0.0831$, with $R^2 = 0.9687$, showing approximately a 97% regression of how well the data set fit the model. This equation was then used to calculate the concentration of the *lantana camara* oil extract to be 1.255±0.123µg/ml. The total antioxidant capacity (ascorbic acid equivalents, mg/g) in the oil extract was reported as 1.255 ± 0.123 mg AAE/g, indicating the TAC of the essential oil extract in the 20mL. This TAC value of the extract could have been quite higher if it were extracted with hexane or methanol or even a different extraction method like Soxhlet extraction, Clevenger-Assisted hydrodistillation, etc. Nevertheless, the presence of antioxidant properties in the essential oil provides the basis for its (*Lantana camara*) wide use in ayurvedic medicine.

Enzymes such as superoxide dismutase, catalase, and glutathione peroxidase are part of these antioxidant systems, as are macromolecules such as albumin, ceruloplasmin, and ferritin, and a variety of small molecules such as ascorbic acid, -tocopherol, -carotene, reduced glutathione, uric acid, and bilirubin (Koracevic, D. *et al.,* 2001). Cooperation among antioxidants provides greater protection against reactive oxygen or nitrogen species than any single compound alone. Thus, the overall antioxidant capacity may provide more relevant biological information than individual component measurements because it takes into account the cumulative effect of all antioxidants present in the oil extract (Koracevic, D. *et al*., 2001).

4.5. DPPH Radical Scavenging Activity

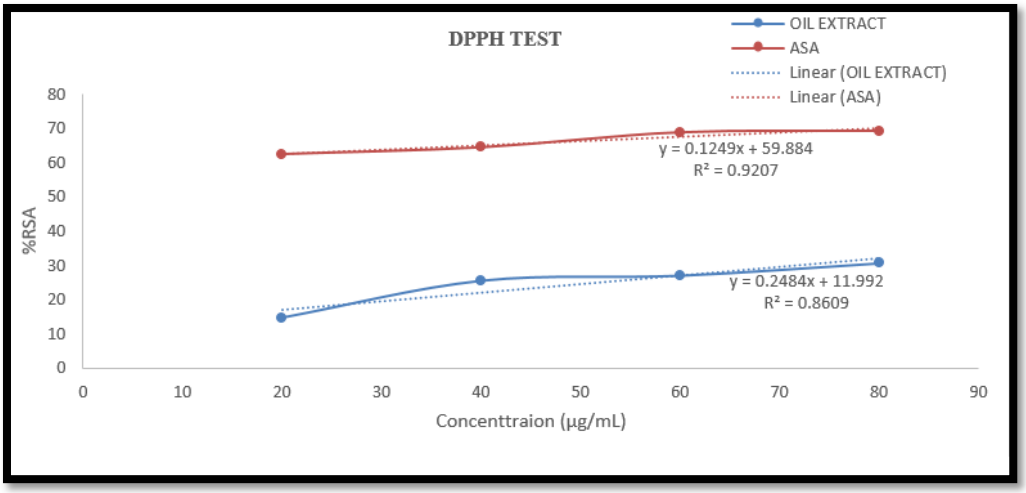

Figure 9: %RSA of Both Lantana camara Oil Extract and Standard Ascorbic Acid

The DPPH assay relies on the aptitude of an antioxidant to donate hydrogen radicals or an electron to the DPPH radical, which is a stable radical with a deep violet colour. When an odd electron becomes paired within the presence of an atom scavenger of an antioxidant agent, DPPH radicals get reduced to corresponding hydrazine, DPPH-H form (Piaxao *et al.,*2007), and therefore the solution gets decolorized from its initial deep violet to light yellow color. The degree of fall within the absorbance measured is proportional to the concentration of the antioxidant. The measured absorbance at various concentrations was used to calculate the percentage radical scavenging activities of both the oil extract and standard ascorbic acid, obtaining a regression line of y = 0.2484x + 11.991 and a coefficient of correlation, R2 = 0.8609, which showed a comparatively good correlation (86%) between the %RSAs with a mean of 24.41 ± 6.91%. This could be observed in figure 9**.** Also, the %RSA of the standard ascorbic acid from figure 8 above gave a linear equation of y = 0.1221x + 60.171 and coefficient of correlation, R2= 0.9296, which showed a much higher correlation (93%) than that of the oil extract, averaging 66.27 ± 3.27%. These %RSA averages of the samples indicated that the radical scavenging activity of the standard ascorbic acid had a higher capability against the free DPPH compared to the *Lantana camara* oil extract.

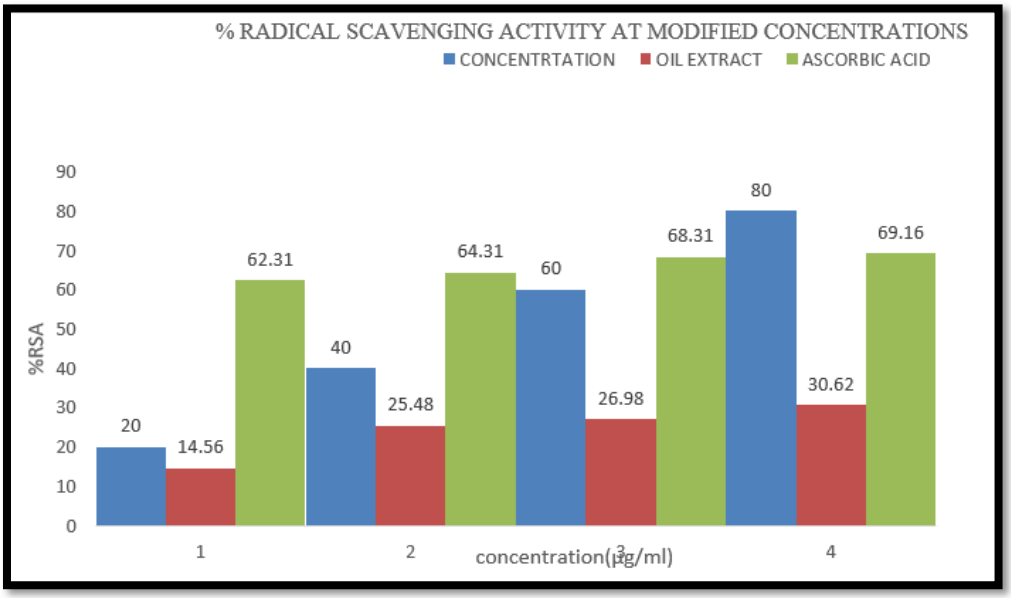

Figure 10: DPPH Radical Scavenging Activity of Oil Extract and Standard Ascorbic Acid at Different Concentrations

Figure 10 above compares the %RSA of the essential oil extract and the ascorbic acid at different concentrations. It could be observed that as the concentration increases, the corresponding %RSA also increases appreciably. This explains that to enhance the radical scavenging activity of any antioxidant-containing oil or sample, the concentration should be proportionally increased as well.

At 95% confidence level, Graphpad analysis of the %RSA of the oil extract and ascorbic acid revealed a P-value of P = 0.003, which is lower than the P-value of 0.05. A P-value is a measure of the probability that an observed difference could have occurred between the two means. The lower P-value tells that there is a statistically significant difference

between the %RSA of the oil extract and the ascorbic acid. An R2= 0.9119 (92%) depicts a strong positive correlation between the two means.

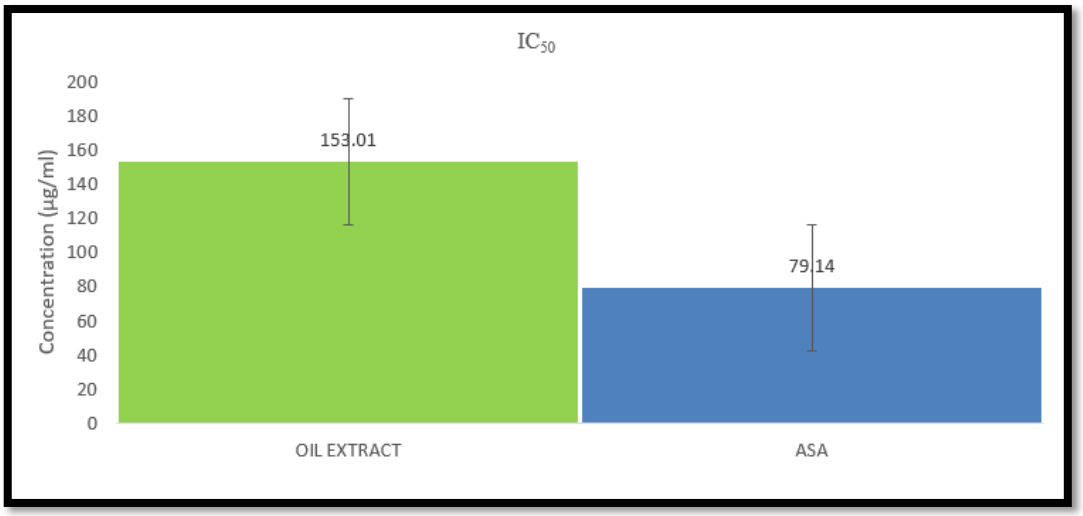

Figure 11: Comparison of the IC_{50} of Lantana camara Oil Extract and Standard Ascorbic Acid

IC_{50} (half-maximal inhibitory concentration) is the concentration of a substance or drug that can scavenge 50% of the DPPH free radical scavenging method (Prior, Wu & Schaich, 2005). The IC_{50} value is inversely proportional to the free radical scavenging activity or antioxidant property of the substance or drug. From figure 11 above**,** the IC_{50} of the *Lantana camara* essential oil extract was estimated to be 153.01 (± 6.91) µg/ml; from the linear regression, y = 0.2484x + 11.992; and that of the ascorbic acid was calculated to be 79.14(±3.27) µg/ml from y = 0.1249x + 59.884. This clearly shows that to inhibit free radical DPPH, a higher concentration (153.01µg/ml) of the essential oil extract is required, whereas one would need a much lower concentration (79.14µg/ml) of the standard ascorbic acid. The scavenging activity of free radicals in the sample is due to the presence of molecules known as antioxidants.

The results of the investigation indicated that *Lantana camara* leaf oil extract is often used as a home herbal remedy and is a rich source of appreciable polyphenol compounds (Halliwell & Gutteridge, 1990). They are also one of the most commonly used components of Ayurvedic formulations. The consumption of the oil may have beneficial implications in human health, such as the treatment and prevention of cancer, cardiovascular diseases, and other pathologies, by delaying or inhibiting the oxidation of lipids or other macromolecules and inhibiting the initiation and propagation of oxidative chain reactions (Piaxao et al., 2007). In addition, the easy availability of plant oil makes it a promising source of natural antioxidants and other bioactive compounds in the food and pharmaceutical industries.

5. Conclusion and Recommendation

5.1. Conclusion

This study involved the extraction of essential oil from the fresh leaves of *Lantana camara,* ascertaining the total phenolic content (TPC), total antioxidant capacity (TAC), and radical scavenging activity (RSA), as well as determining its chemical composition.

The extraction method, conventional hydrodistillation, was able to extract essential oil from the fresh leaves of the *Lantana camara*, using diethyl ether as the solvent of interest. The percentage yield from this was quite higher as compared to one reported by Emmanuel et al. (2003). The color of the oil was observed to be slightly yellowish, possessing a very strong aromatic scent. This could be due to the aromatic compounds present.

Results from the DPPH assay showed that the %RSA of the oil extract was half that of the standard ascorbic acid. This suggests that the oil extract had the capacity to scavenge half of the DPPH compared to the standard ascorbic acid, which scavenged twice that of the oil extract.

The GC-MS analysis showed the presence of numerous chemicals in the essential oil extract. Most of these compounds were known to be of minor components, while a few were identified as major components.

This study has, therefore, revealed that the antioxidants present in the oil extracts were able to scavenge DPPH. Inculcating this oil extract in manufactured food products or pharmaceuticals would provide a much better health benefit than synthetically manufactured antioxidants.

Based on the radical scavenging activity of the oil extract, it can be concluded that it contained phenols and antioxidants.

5.2. Recommendation

Different extraction methods and solvents should be used for extracting the essential oil. Also, different parts (flowers, roots, seed, bark, etc.) of the *L. camara* should be used for the study.

Based on the IC_{50}, a higher concentration of the oil should be used to enhance its radical scavenging activity.

The food and pharmaceutical industries are recommended to inculcate antioxidants from organic sources, like *L. camara,* in producing finished products, as these antioxidants offer minimal health risks.

6. References

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Appendices

Appendix 1: Components of Essential Oil Extract of Lantana camara with Abundance from the GC-MS Analysis

RT	Library/ID	Peak Area_1	Peak Area_2	Peak Area_3	Mean Area	Abundance (%)
3.48	2-Pentanone	368884	405772	331995	368884	0.3
4.29	2,3-dimethyl-2-butanol	638409	702249	574568	638409	0.5
4.38	2-ethoxy-2-methylpropane	3532653	3885918	3179387	3532653	2.8
4.6	4-methyl-1-hexene	287393	316132	258653	287393	0.2
4.67	Methyl Isobutyl Ketone	354603	390063	319142	354603	0.3
4.95	3-methyl-3-pentanol	981055	1079160	882949	981055	0.8
5.23	4-methyl-2-pentanol	184856	203341	166370	184856	0.1
5.37	4-methylheptane	325097	357606	292587	325097	0.3
5.6	2-methyl-3-pentanol	125631	138194	113067	125631	0.1
7.02	2,4-dimethylheptane	384267	422693	345840	384267	0.3
7.57	2,4-Dimethyl-1-heptene	243982	268380	219583	243982	0.2
7.94	2E-Hexenal	306006	336606	275405	306006	0.2
8	3Z-Hexenol	6698962	7368858	6029065	6698962	5.4
8.15	4-methyl-octane	388874	427761	349986	388874	0.3
8.32	2E-Hexen-1-ol	131700	144870	118530	131700	0.1
8.36	1-Hexanol	728995	801894	656095	728995	0.6
9.78	α-pinene	499798	549777	449818	499798	0.4
10.63	β-pinene	1310637	1441700	1179573	1310637	1
10.69	limonene	650071	715078	585063	650071	0.5
10.78	1-Octen-3-ol	2466746	2713420	2220071	2466746	2
11	Cyclohexane, 1-methylene-4-(1-methylethenyl)-	289537	318490	260583	289537	0.2
11.1	3-Octanol	224100	246510	201690	224100	0.2
11.53	2,2,3,3-tetramethylhexane	139476	153423	125528	139476	0.1
11.59	4-methyldecane	423667	466033	381300	423667	0.3
11.64	Benzene, 1-ethyl-2,4-dimethyl-	244307	268737	219876	244307	0.2
11.71	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)-	252120	277332	226908	252120	0.2
11.76	Eucalyptol	279391	307330	251451	279391	0.2
12.32	Nonane, 5-methyl-5-propyl-	428925	471817	386032	428925	0.3
12.43	Sabinene hydrate<trans->(IPP vs OH)	662909	729199	596618	662909	0.5
12.99	Linalool	3212801	3534081	2891520	3212801	2.6
13.43	1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-	279159	307074	251243	279159	0.2
13.75	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	464306	510736	417875	464306	0.4
14.09	endo-Borneol	175186	192704	157667	175186	0.1
14.28	Terpinen-4-ol	1551363	1706499	1396226	1551363	1.2

14.79	Verbenone	1218253	1340078	1096427	1218253	1
14.88	4,8-dimethyltridecane	786100	864710	707490	786100	0.6
15.28	5-Ethyl-5-methylnonadecane	175269	192795	157742	175269	0.1
15.37	4,6-dimethyldodecane	522073	574280	469865	522073	0.4
16.61	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	321992	354191	289792	321992	0.3
17.16	α-Copaene	867247	953971	780522	867247	0.7
17.3	β-Bourbonene	493989	543387	444590	493989	0.4
17.34	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-	3390201	3729221	3051180	3390201	2.7
17.54	Tetradecane, 4-ethyl-	453627	498989	408264	453627	0.4
17.58	α-Selinene	665609	732169	599048	665609	0.5
17.63	Nonadecane	346868	381554	312181	346868	0.3
17.76	(*E*)-Caryophyllene	25704219	28274640	23133797	25704219	20.6
17.87	β-Copaene	2193445	2412789	1974100	2193445	1.8
17.96	α-Guaiene	305053	335558	274547	305053	0.2
18.13	(*Z*)-β-Farnesene	1298872	1428759	1168984	1298872	1
18.2	α-Humulene	16887538	18576291	15198784	16887538	13.5
18.3	α-Muurolene	876525	964177	788872	876525	0.7
18.45	γ-Muurolene	1125100	1237610	1012590	1125100	0.9
18.49	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	669011	735912	602109	669011	0.5
18.62	β-Selinene	1057188	1162906	951469	1057188	0.8
18.7	4-epi-cubedol	1862459	2048704	1676213	1862459	1.5
18.73	(-)-Aristolene	2856078	3141685	2570470	2856078	2.3
18.81	Phenol, 2,4-bis(1,1-dimethylethyl)-	2241693	2465862	2017523	2241693	1.8
18.96	cubedol	4578873	5036760	4120985	4578873	3.7
19.02	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]-	1802365	1982601	1622128	1802365	1.4
19.34	Guaia-1(10),11-diene	522893	575182	470603	522893	0.4
19.44	1,Z-5,E-7-Dodecatriene	549712	604683	494740	549712	0.4
19.5	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-	379053	416958	341147	379053	0.3
19.62	4aH-cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-	148571	163428	133713	148571	0.1
19.74	Spathulenol	1486019	1634620	1337417	1486019	1.2
19.82	Caryophyllene oxide	6194854	6814339	5575368	6194854	5
19.92	Globulol	1175725	1293297	1058152	1175725	0.9
19.99	1,2-Dihydropyridine, 1-(1-oxobutyl)-	516360	567996	464724	516360	0.4
20.12	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-	4651637	5116800	4186473	4651637	3.7

20.3	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	1030000	1133000	927000	1030000	0.8
20.43	Epizonarene	2052237	2257460	1847013	2052237	1.6
20.5	α-Cubebene	1943027	2137329	1748724	1943027	1.6
21.35	Aromadendrene	713340	784674	642006	713340	0.6
22.17	Cycloisolongifolene, 8,9-dehydro-	224530	246983	202077	224530	0.2
24.58	Eicosane	122456	134701	110210	122456	0.1
25.13	Phytol	1055072	1160579	949564	1055072	0.8
38.91	Stigmastanol	168780	185658	151902	168780	0.1
					1.25E+08	

Appendix 2: Absorbance and Concentration Values of TPC

Sample	Absorbance	Concentration (µg/mL)
C1	0.241	2.029
C2	0.243	2.081
C3	0.239	1.976
AVERAGE		**2.029**

Sample	Absorbance	Concentration (µg/mL)
C1	0.090	1.0426
C2	0.086	1.4681
C3	0.089	1.2553
AVERAGE		**1.2553**

Appendix 3: Absorbance and Concentration Values for TAC

$$\%\text{RSA} = \frac{Absorbance\ of\ Control - Absorbance\ of Sample}{Absorbance\ of\ Control} \times 100$$

Concentration (µg/mL)	% RSA Oil Extract	% RSA Ascorbic Acid
20.00	14.61 ± 6.91	62.31 ± 3.27
40.00	25.48 ± 6.91	64.31 ± 3.27
60.00	26.98 ± 6.91	68.74 ± 3.27
80.00	30.62 ± 6.91	69.17 ± 3.27
AVERAGE	**24.41 ± 6.91**	**66.27 ± 3.27**

Appendix 4: DPPH Radical Scavenging Activity of Oil Extract and Standard Ascorbic Acid at Modified Concentrations

	Oil Extract	Standard
TPC	2.029 ± 0.03 mg GAE/g	N/A
TAC	1.255 ± 0.123 mg AAE/g	N/A
RSA	24.41 ± 6.91 %	66.27% ± 3.27%
IC_{50}	153.01 µg	79.14%

Appendix 5: Summary of TPC, TAC, RSA and IC_{50} Values of Oil Extract and Standards