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PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF HEXANOLIC EXTRACT OF *LANTANA CAMARA* LINN.

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ABSTRACT: *Lantana camara* Linn. is an important ethnomedicinal plant with several medicinal properties. It is widely used in the traditional medicinal system as carminative, anti-inflammatory, antipyretic and respiratory system infections. The present investigation was carried out to determine the qualitative analysis of phytochemical screening and the presence of bioactive compounds from the hexanolic extract of *Lantana camara* leaves using GC-MS. The GC-MS analysis of hexanolic extract reported the presence of cis-Caryophyllene, caryophyllene oxide, isophytol acetate, 13-docosenamide, (Z) - and Squalene as major compounds. The presence of terpenes in extract confirms its application as anti-cancer, antioxidant and anti-microbial agents along with traditional medicine application.

INTRODUCTION: Medicinal plants are a treasure house of many potential drugs. There has been an increase in awareness and interest in the importance of medicinal plants in recent years. Drugs obtained from the medicinal plants are easily available, cheaper, safe and having minimum side effects. According to WHO, 80% of the world's population from developed countries depend on traditional medicines for primary health care [1]. Medicinal plants contain bioactive compounds that participate in physiological action on the human body [2]. *Lantana camara* Linn. is listed as one of the most important medicinal plants of the world [3]. It is a significant weed with 650 varieties growing in 60 countries.

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It is commonly known as wild or red sage and regarded both as a notorious weed and ornamental garden plant and it belongs to family Verbenaceae [4]. In India, it was introduced at the National Botanical Gardens, Calcutta in 1807 as an ornamental plant [5]. *Lantana camara* Linn. is an evergreen strong aromatic, hairy shrub. It grows to a height 1 to 3 meters and it can spread to 2.5 meters in width. The stems and branches are sometimes armed with prickles or spines. The leaves are arranged in opposite pairs, elliptic about 3 inches long and 1.5 inches wide with pointed tip and base rounded and toothed in the margins.

Flowers are small held in clusters (called umbels) with various colors pink, red, orange, yellow, white or violet. The fruits are fleshy berries in clusters, shiny and globose in shape, green-colored, which changes to black on ripening [7, 8, 9]. *Lantana camara* has been historically useful as a medicinal and ornamental plant for a very long time. All parts of this plant have been widely used in traditional medicines for the treatment of malaria, ulcers,

cancer, high blood pressure, tetanus, tumors, eczema, cut, catarrhal infections, chickenpox, measles, rheumatisms, asthma, colds, fevers, swellings, *etc*. [10] *Lantana camara* has been scientifically proven to possess anti-bacterial, anti-fungal, anti-filarial, anti-hypertensive, hemolitic, anti-ulcerogenic, anti-inflammatory, anti-proliferative, anti-fertility and anti-oxidant activities [11]. The plant has also been reported to possess antipyretic, anti-cancer, anti-mutagenic, anti-hyperglycemic, hepatic protective, larvicidal, termiticidal activities [12]. The plant has been reported to contain the important phytochemicals such as essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridoid glycosides, phenylethanoid, oligosaccharides, anthraquinone, saponins, steroids, triterpenes, sesquiterpenes, tannins, *etc*. [13] it is reported that this plant contains the number of terpenoids. Nowadays, the number of anti-cancer drugs belongs to terpenoid class, like glycyrrhizic acid, Oleanolic acid, ursolic acid, Nomilin, *etc*. [14, 15, 16, 17, 18]

Taxonomy:

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Superdivision	:	Spermatophyta
Division	:	Magnoliopsida
Subclass	:	Asteridae
Order	:	Lamiales
Family	:	Verbenaceae
Genus	:	Lantana
Species	:	*Lantana camara* Linn. [6]

FIG. 1: *LANTANA CAMARA* PLANT

MATERIAL AND METHOD:

Collection of Plant Material and Powder Preparation: The fresh leaves of Lantana camara were collected from Bela Village, near Nagpur city. This city is located at the central part of India. The sample of plant was identified by senior plant taxonomist, Dr. S. U. Borkar, Department of Botany, Govt. Institute of Science, Civil Lines, Nagpur (Maharashtra), India. The specimen was submitted to the herbarium of the department with authentication code, IoSc./Bot.Sci/09/1/2018-19/192. The collected plant is shown in **Fig. 1.** The plant leaves were washed thoroughly with clean water to remove dust and dirt and then air-dried under shade at room temperature for about 15 days. The dried leaves were ground to a fine powder using pestle and mortar and a domestic electric grinder. The material was stored in an airtight brown color glass bottle for further use.

Preparation of Extract: The coarsely powdered plant material of *Lantana camara* was extracted with n-hexane (≥99% pure, Merck) solvent by cold (maceration) method. In each batch, 25 grams of plant material were dissolved in 250 ml of n-hexane solvent in airtight stopper brown bottle. After every eight hours, the bottle was shaken for 1 hour using a shaking machine. The process was repeated for 3 days. The solution was then filtered through Whatman filter paper no. 1. The filtrate was evaporated using a rotary evaporator and further dried at room temperature. The obtained dried crude extract was collected in an airtight container and stored in a refrigerator at 4 °C for further analysis.

Preliminary Phytochemical Analysis: The crude extract was subjected to the qualitative chemical tests for the detection of various phytochemicals using standard procedures as described by Harbone, Trease, Evans and Sofowora [19, 20, 21]. The observations for chemical tests are shown in **Table 1**. It is reported that the extract contains flavonoids, saponins, terpenoids, carbohydrates and phyto-sterols in major quantities.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis: The GC-MS analysis was carried out using Thermo scientific TSQ 8000 GC-MS system and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the column TG5MS (30 m × 0.25 mm, 0.25 µm, composed of 100% dimethylpolysiloxane). For GC -MS detection, an electron ionization system with

energy of 70 *ev* was used. Helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1ml/min. The oven temperature was raised from 60 °C to 280 °C (raising at the rate of 10 °C/min). The total GC running time was about 31 min. The mass spectra were carried out coupled with Gas chromatography by maintaining 70 *ev* voltage and keeping the source temp of 230 °C.

The inlet line temperature was maintained at 280 °C and the mass scan rate was tuned to 50-700. The total MS running time was about 34 min.

TABLE 1: PHYTOCHEMICAL SCREENING OF HEXANE EXTRACTS OF *LANTANA CAMARA* LEAVES

S. no.	Phytoconstituents	Test Procedure [19, 20, 21]	Observation in Hexanoic Extract
1	Alkaloids	2 ml of the extract was treated with a few drops of Hager's reagent (picric acid dissolved in benzene). The yellow precipitate was not formed, indicating the absence of Alkaloids	-- (Negative Result)
2	Flavonoids	1 ml of the extract was treated with 10% of 1 ml Pb(OAC)4 . Formation of intense yellow colour indicates the presence of Flavonoids	++ (Positive Result)
3	Phenols	2 ml of the extract was treated with 3 to 4 drops of $FeCl_3$ solution. The extract was not converted to bluish-black colour, indicating the absence of phenols.	-- (Negative Result)
4	Saponins	To 5 ml of extract, a drop of sodium bicarbonate solution was added. The test tube was shaken and allowed to stand for 3 min. Formation of honeycomb-like froth indicates the presence of Saponins.	++ (Positive Result)
5	Terpenoids	2 ml extract was treated with 2 ml $(CH_3 CO)_2O$ and 2 to 3 drops of conc H_2SO_4. The red colour appeared, indicating the presence of Terpenoids	++ (Positive Result)
6	Carbohydrates	1 ml of the extract was mixed with 2 drops of alcoholic (ethanolic) naphthol solution in a test tube and was shaken properly. After that, 2 ml of conc H_2SO_4 was added carefully along the side of the tube. Violet ring formed at the junction indicating the presence of Carbohydrates	++ (Positive Result)
7	Tannin	1 ml of the extract was treated with 2 to 3 drops of $FeCl_3$ solution. The green precipitate was not observed, indicating the absence of Tannin.	-- (Negative Result)
8	Coumarins	2 ml the extract was treated with 3 ml of 10% NaOH solution. The solution didn't turn yellow indicating the absence of Coumarins.	-- (Negative Result)
9	Glycosides	Liebermann's test: 2 ml the extract was mixed with 2 ml of chloroform and 2 ml of acetic acid. There is no change in colors, indicating the absence of Glycosides.	-- (Negative Result)
10	Anthraquinones	Borntrangers test: 3 ml of the extract was treated with 3 ml of benzene and 5 ml of 10% NH_3. No change in color in ammonical layer indicating the absence of Anthraquinones.	-- (Negative Result)
11	Phytosterols (Salkowski's test)	Salkowski test: Extract was treated with 2 ml chloroform and then filtered The filtrate was treated with few drops of H_2SO_4, the solution was shaken gently and allowed to stand. Golden color (reddish-brown) appeared, indicating the absence of Phytosterols	++ (Positive Test)

RESULTS AND DISCUSSION:

Phytochemicals Extraction and Analysis: The preliminary phytochemical screening of the hexanoic extract of *Lantana camara* leaves revealed the presence of flavonoids, carbohydrates, steroids, saponins and terpenoids. Among the variety of secondary metabolites in *Lantana camara*, terpenoids are predominant compounds present in it. The presence of the high amount of terpenoid compounds revealed that *Lantana camara* may have anti-cancer activity. **Table 1** shows the results of the phytochemical screening of hexanolic extract of *Lantana camara* leaves.

Gas Chromatography-Mass Spectrometry (GC-MS): The obtained spectrum of GC-MS analysis of hexane extract of leaves of *Lantana camara* is shown in **Fig. 2.** The spectrum shows more than 19 prominent peaks having higher retention time. Interpretation of the mass spectrum of GC -MS was conducted using the database of the National Institute of Standard and Technology (NIST). The

spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. Retention time, molecular weight, molecular formula and percentage composition were used to identify the components. A list of identified compounds and their retention time along with molecular formula are listed in **Table 2.**

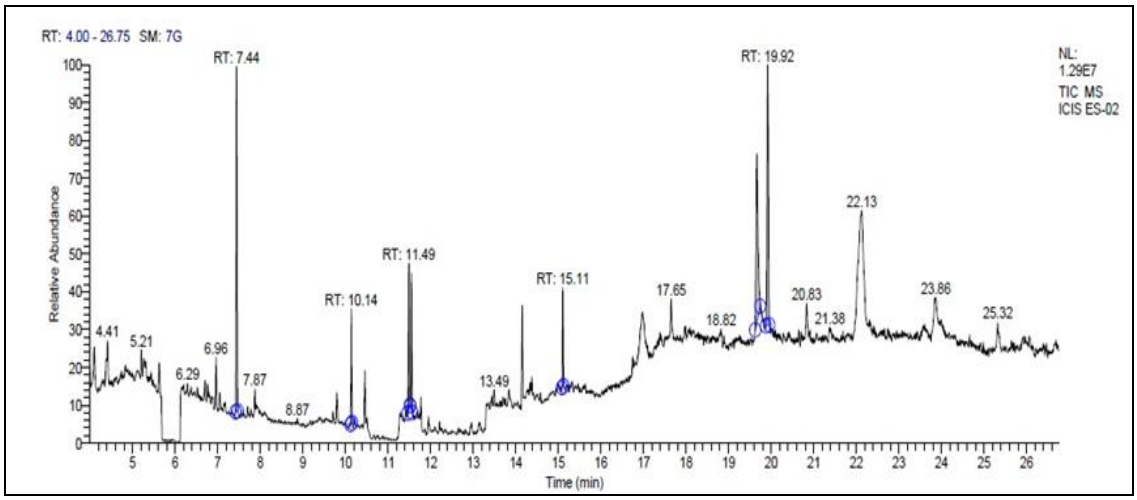

FIG. 2: GC-MS CHROMATOGRAM OF HEXANE EXTRACT OF *LANTANA CAMARA* LEAVES

TABLE 2: COMPOUNDS IDENTIFIED IN THE HEXANOIC EXTRACT OF *LANTANA CAMARA* LEAVES BY GC-MS ANALYSIS

S. no.	Name of compound	Retention time	Molecular formula	Molecular weight	Nature of compound
1	Bicyclo[2.2.1]heptan-2-one,1,7,7,-trimethyl-, (1S)-	7.44	$C_{10}H_{16}O$	152.23	Terpenoid
2	Camphor	7.44	$C_{10}H_{16}O$	152.23	Terpenoid
3	(+)-2-Bornanone	7.44	$C_{10}H_{16}O$	152.23	Terpenoid
4	Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8 methylene- (cis-Caryophyllene)	10.14	$C_{15}H_{24}$	204.35	Terpenoid
5	Caryophyllene	10.14	$C_{15}H_{24}$	204.35	Bicyclic sesquiterpenes
6	Isocaryophillene	10.14	$C_{15}H_{24}$	204.35	Sesquiterpenoid
7	(-)-Spathulneol	11.49	$C_{15}H_{24}O$	220.354	Tricyclic sesquiterpenoid
8	1H-Cycloprop[e] azulen -7-ol,decahydro- 1,1,7-trimethyl-4–methylene-,[1ar-(1aa,4aa,7a,7aa,7ba)]-	11.49	$C_{15}H_{24}O$	220.354	Tricyclic sesquiterpenoid
9	Tricyclo[5.2.2.0(1,6)undecan-3-ol,2- methylene-6,8,8-trimethyl-	11.49	$C_{15}H_{24}O$	220.356	Tricyclic sesquiterpenoid
10	Caryophyllene oxide (beta-Caryophyllene epoxide)	11.55	$C_{15}H_{24}O$	22.0356	Bicyclic sesquiterpenes
11	Isoaromadendrene epoxide	11.55	$C_{15}H_{24}O$	220.356	aromadendrene sesquiterpenoid
12	Alloaromadendrene oxide-(1)	11.55	$C_{15}H_{24}O$	220.356	Oxygenated sesquiterpenoid
13	Phytol	15.11	$C_{20}H_{40}O$	128.1705	Acyclic diterpene
14	3,7,11,15,-Tetramethyl- 2 -hexadecen -1- ol	15.11	$C_{20}H_{40}O$	296.53	Acyclic diterpene
15	Isophytol, acetate	15.11	$C_{20}H_{42}O_2$	338.56	Terpenoid alcohol
16	13-Docosenamide,(z)-	19.67	$C_{22}H_{43}NO$	337.592	Amide
17	trans -13- Docosenamide	19.67	$C_{22}H_{43}NO$	337.592	Amide
18	Bis(cis -13- Docosenamide)methane	19.67	$C_{45}H_{86}N_2O_2$	687.195	Amide
19	Squalene	19.92	$C_{30}H_{50}$	410	Triterpenoid
20	2,6,10,14,18-Pentamethyl- 2,6,10,14,18-eicosapentaene	19.92	$C_{25}H_{42}$	342	Terpene
21	Supraene	19.92	$C_{30}H_{50}$	410	Triterpenoid

The results pertaining to GC-MS analysis led to the identification of a mixture of numerous compounds. The majority among them belong to terpenoids. **Table 3** shows the identified phytochemicals using GC-MS and their known biological activities.

TABLE 3: IDENTIFIED PHYTOCHEMICALS AND THEIR KNOWN BIOLOGICAL ACTIVITIES

S. no.	Compound	Biological Activity [22, 23, 24, 25]
1	Bicyclo[2.2.1]heptan-2-one,1,7,7,-trimethyl-, (1S)- (L Camphor)	Antimicrobial
2	Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene (cis-Caryophyllene)	Antimicrobial
3	(-)-Spathulneol	Anti-fungal, Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities
4	Caryophyllene oxide (β-Caryophyllene epoxide)	Inhibited potassium ion fluxes, Analgesic, Anti-inflammatory activity, and Anti-fungal activity
5	13-Docosenamide,(z)-	Antimicrobial, Antinociceptive and anti-inflammatory activities
6	Squalene	Anticancer, Antioxidant, Drug carrier, Detoxifier, Skin hydrating agent

CONCLUSION: GC-MS analysis revealed the presence of many important terpenoids compounds in the hexanoic extract of leaf of *Lantana camara*. The major components of the extract are cis-caryophyllene, β-Caryophyllene epoxide, L-camphor, 13-docosenamide, (z) - and squalene.

In this investigation, cis-caryophyllene is uniquely identified as it is not reported by any literature. Also, the major compound belongs to terpenoids and hence it justifies the medicinal importance of *Lantana camara* leaves for the treatment of several ailments by traditional practitioners. Further, isolation of active phytoconstituents and subjecting it to biological activity from the plant will definitely give fruitful results.

It could be concluded that *Lantana camara* leaves is a rich source of important secondary metabolites like terpenes. It is a plant with phytopharmaceutical importance.

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CONFLICTS OF INTEREST: Nil

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