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GC-MS ANALYSIS OF EXTRACTS AND VOLATILE OIL OF *CALLISTEMON CITRINUS (CURTIS) SKEELS* LEAVES

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ABSTRACT
Callistemon citrinus (Curtis) Skeels is an ornamental plant belonging to Myrtaceae family. The investigation was carried out to determine the possible bioactive components of the leaves of *Callistemon citrinus (Curtis) Skeels* using GC-MS analysis. The chemical compositions of the petroleum ether, chloroform and methanolic extracts and also the volatile oil of the leaves of *Callistemon citrinus (Curtis) Skeels* was investigated using Gas Chromatography – Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the NIST library. The petroleum ether extract showed the presence of aspidinol (17.08%) and palmitic acid (9.20%) as major constituents. The chloroform extract showed the presence of squalene (45%) and aspidinol (12.63%) as major chemical constituents. The methanolic extract showed the presence of m-Mentha-1 (7), 8-diene (10.08%) and L-terpinen-4-ol (4.11%) as major chemical constituents. The volatile oil showed the presence of dihydrocarveol (17.85%) and isopulegol (12.72%) as major constituents.

KEYWORDS: GC-MS analysis, *Callistemon citrinus (Curtis) Skeels*, bioactive constituents, Myrtaceae.

INTRODUCTION
Callistemon is a genus of 34 species of shrubs in the family Myrtaceae, all of which are endemic to Australia. It is sometimes considered a synonym of Melaleuca. Callistemon species are commonly referred to as bottlebrushes because of their cylindrical, brush like flowers resembling a traditional bottle brush. They are found in the more temperate regions of Australia, mostly along the east coast and south-west, and typically favor moist conditions so when planted in gardens thrive on regular watering. The genus Callistemon is known in folk medicine for its anticough, antibronchitis, and insecticidal effects and its volatile oils have been used as antimicrobial and antifungal agents.[1] *Callistemon lanceolatus* D.C. (Myrtaceae) is a slow-growing ornamental shrub that grows to a height of around 10 meters. It is commonly known as crimson bottle brush tree because of its spiky inflorescence that resembles a bottle brush. The plant has been used by tribal communities of India for the treatment of gastrointestinal disorders, pain, and infectious diseases.[2] *Callistemon citrinus* syn. *Callistemon lanceolatus* has shown to possess activities like anticaries, antioxidant, spasmolytic, anti-inflammatory, hypoglycemic, hepatoprotective, cytotoxic action, cardio protective, anti-*Helicobacter pylori* and antibacterial activity.[3-12] It mainly contains neolignans- callislignan A and B; triterpenoid- 30-hydroxyalphitolic acid, alphitolic acid, lupenol, 3-acetoxy-olean-18-en-28-oic acid, betulinic acid, ursolic acid, betulinic acid 3-*O*caffeate, morolic acid 3-*O*-caffeate, ursolic acid 3-*O*-caffeate; 4',5-dihydroxy-6,8-dimethyl-7-methoxyflavanone, eucalyptin, 8- demethyleucalyptin, sideroxylin, syzalterin, quercetin, kaempferol 3-O-beta-Dgalacturonopyranoside, quercetin 3-O-(2''-O-galloyl) - beta-D-glucoronopyranoside. It also contains pelargonidin-3, 5-diglucoside, cyanidin-3, 5-diglucoside, kaempferol, pyrogallol, catechol, betulic acid, α- amyrin, oleanolic acid, β- sitosterol; 3', 4', 7- trihydroxy flavanol; 3', 4', 7- trihydroxy flavones; 3', 4', 7- trihydroxy flavanol-3- glucoside; 3', 4', 7- trihydroxy flavone-7- galactoside; 5, 7- dihydroxy-3, 8, 4'- trimethoxy-6-C- methyl flavones; 2 α-hydroxy ursolic acid; uvaol; myrtucommulone; gallic acid; ellagic acid; 3, 3'-di-O-methyl ellagic acid; 3, 3', 4-tri-O-methyl ellagic acid. It contains volatile oil which mainly consists of 1, 8-cineole, β-pinene, α-pinene, α-terpineol and limonene.[13, 14] Hence it was our intention to investigate the presence of new phytoconstituents from the leaves of *Callistemon citrinus (Curtis) Skeels* which have not yet been reported in it.

MATERIALS AND METHODS
Plant Material
The fresh leaves of *Callistemon citrinus* were collected from Karad, Maharashtra in July2012. A voucher specimen (APS 01) for the plant material was identified

by Dr. G. G. Potdar at Yashwantrao Chavan College of Science (Karad).

Extraction

The leaves were washed and dried in shade overnight and grounded to coarse powder. The volatile oil was extracted from leaves by using Clevenger apparatus. 100gm of powdered leaves of *Callistemon citrinus (Curtis) Skeels* was taken in 1000ml round bottom flask to which 400ml of water and 100ml glycerine was added. Few porcelain pieces were added to avoid bumping during distillation. The flask was heated on heating mantle. The heating was continued till the oil was no more collected in the graduated tube. The heating was done for 3 to 4 hours. The oil was then collected from the graduated tube, filtered through anhydrous sodium sulfate and collected in amber colored bottle and stored in refrigerator until further analysis. These leaves were dried and further pulverized to form coarse powder which was subjected to continuous Soxhlet extraction. 60gram of powder was weighed and subjected to extraction with 350ml of petroleum ether, followed by chloroform and then methanol. After 30 cycles of each solvent extraction, the extracts were filtered, collected and the solvent was removed by means of distillation and then evaporated on heating mantle to obtain syrupy mass which was then air dried to complete dryness. The extract was thus obtained in powder form which was stored in desiccator till further process.

GC-MS Analysis

The Gas Chromatography- Mass spectroscopy analysis of the extracts was performed in the CFC laboratory, Shivaji University, Kolhapur using a GC-MS Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF- 5ms fused silica capillary column of 30m length, 0.25mm internal diameter and 0.25µm film thickness. For GC-MS detection quadrapole system was used. Helium gas (99.99%) was used as carrier gas at a constant flow rate of 1 ml/min. The oven temperature was programmed from 80 to 240^0C at 10^0 C/min, held isothermal for 2min and finally raised to 280^0C at 10^0 C/min. 1µl of respective samples was manually injected in split less mode, with split ratio of 50:50 and with mass scan range up to 650 amu. Total running time of GC-MS is 20 mins.

RESULTS AND DISCUSSIONS

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the different extracts of *Callistemon citrinus (Curtis) Skeels*. These compounds were identified through mass spectrometry attached with GC. The GC-MS chromatogram of petroleum ether, chloroform, methanol extract and volatile oil are shown in Fig. 1, Fig. 2, Fig. 3 and Fig. 4 respectively.

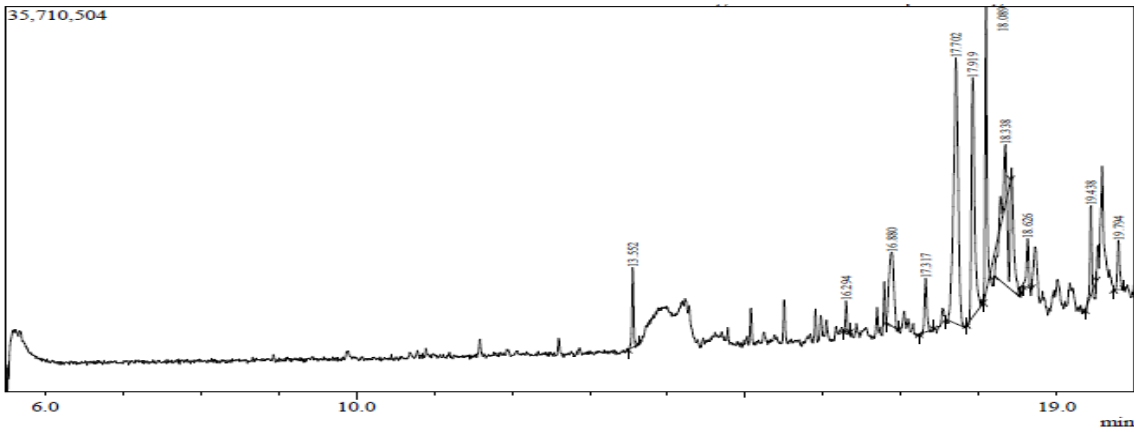

Fig 1: Gas Chromatogram of Petroleum ether extract of *Callistemon citrinus (Curtis) Skeels* leaves.

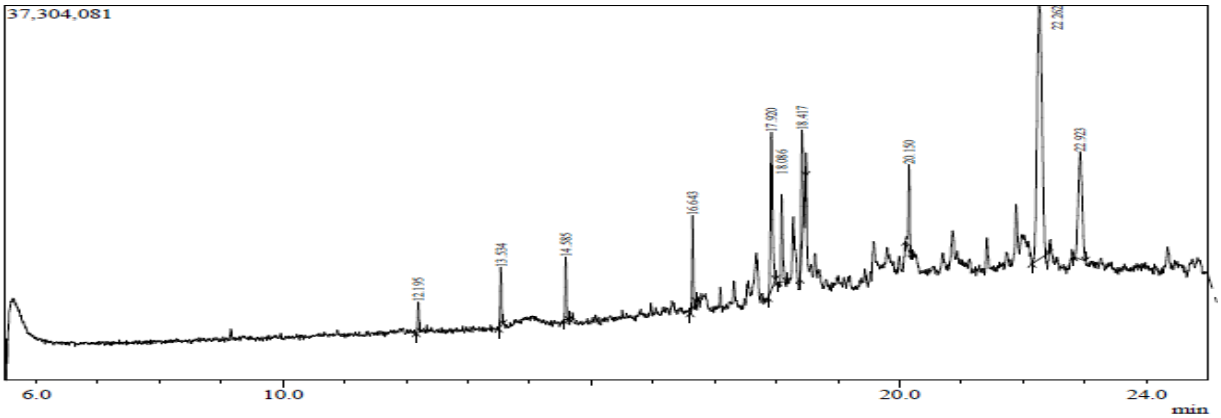

Fig 2: Gas Chromatogram of Chloroform extract of *Callistemon citrinus (Curtis) Skeels* leaves.

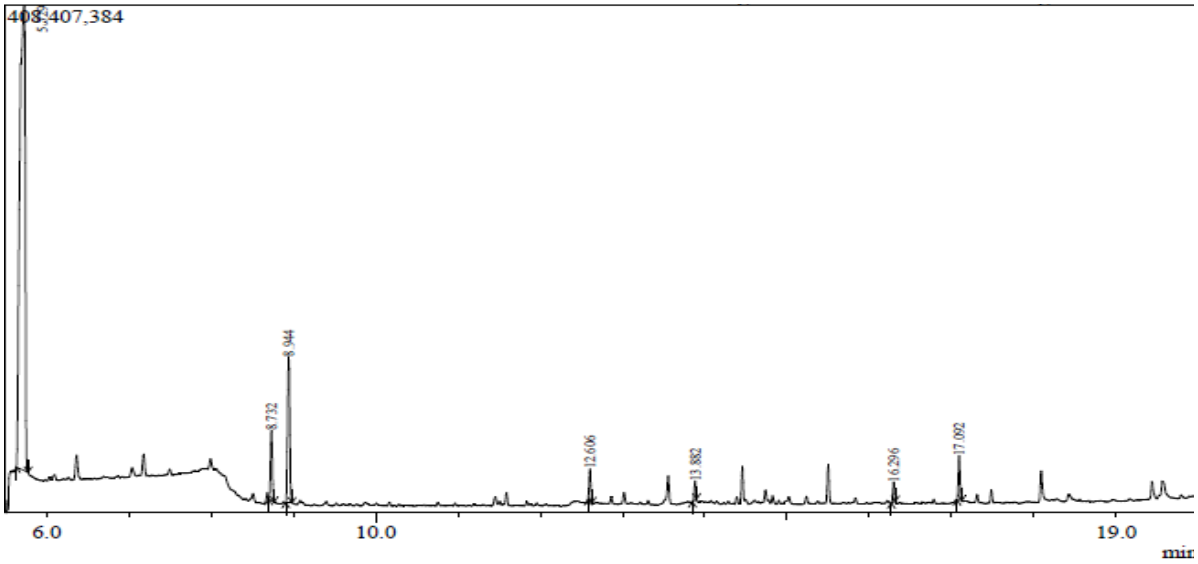

Fig 3: Gas Chromatogram of Methanol extract of *Callistemon citrinus (Curtis) Skeels* leaves.

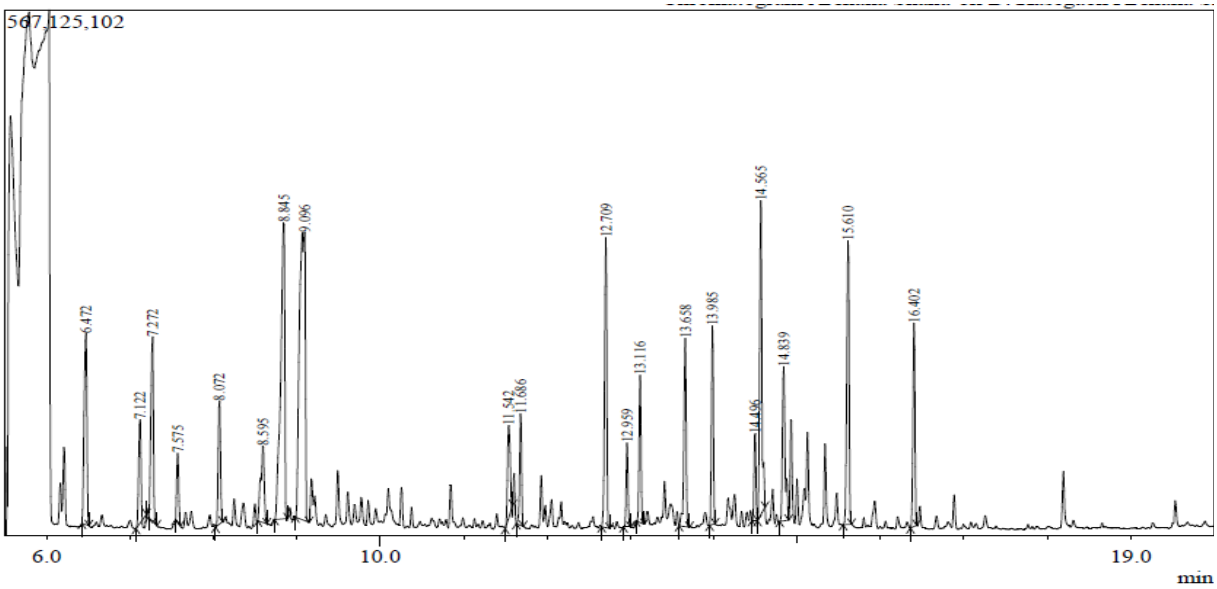

Fig 4: Gas Chromatogram of volatile oil of *Callistemon citrinus (Curtis) Skeels* leaves.

The compounds present in the petroleum ether extract, chloroform extract, methanol extract and the volatile oil of *Callistemon citrinus (Curtis) Skeels* were identified by GC-MS analysis. The mass spectra of the phytoconstituents was compared with the library data of NIST and identified. Seven compounds in the petroleum ether extract were identified out of which aspidinol (17.08%) and palmitic acid (9.20%) was found to be predominant active principles. Nine compounds in the chloroform extract were identified out of which squalene (45%) and aspidinol (12.63%) was found to be predominant active principles. Five compounds in the methanolic extract were identified out of which m-Mentha-1 (7), 8-diene (10.08%) and L-terpinen-4-ol (4.11%) was found to be predominant. Seventeen compounds were identified in the volatile oil out of which dihydrocarveol (17.85%) and isopulegol (12.72%) was fond to be predominant.

The active principles identified in the petroleum ether extract, chloroform extract, methanolic extract and the volatile oil with their retention time, molecular formula, molecular weight and concentration (%) in the extract of *Callistemon citrinus (Curtis) Skeels* are represented in Table 1, Table 2, Table 3 and Table 4 respectively.

Table 1: Phytoconstituents identified in petroleum ether extract of *Callistemon citrinus (Curtis) Skeels* leaves

Sr. No.	Compound	Retention time	Molecular Weight	Molecular Formula	Concentration (%)
1	7- isopropyl-7-methyl-nona-3, 5-diene-2, 8-dione	13.552	208	$C_{13}H_{20}O_2$	2.92
2	4, 4, 8-trimethyl tricycle[6, 3, 1, 0(1, 5)]dodecane-2, 9-diol	17.317	238	$C_{15}H_{26}O_2$	2.59
3	Aspidinol	17.919	224	$C_{12}H_{16}O_4$	17.08
4	n- hexadecanoic acid (palmitic acid)	18.089	256	$C_{16}H_{32}O_2$	9.20
5	9-butylfluoren-9-ol	18.626	238	$C_{17}H_{18}O$	2.22
6	Phytol	19.438	296	$C_{20}H_{40}O$	3.04
7	Octadecanoic acid (stearic acid)	19.794	284	$C_{13}H_{36}O_2$	2.43

Table 2: Phytoconstituents identified in chloroform extract of *Callistemon citrinus (Curtis) Skeels* leaves.

Sr. No.	Compound	Retention time	Molecular Weight	Molecular Formula	Concentration (%)
1	1-Heptadecene	12.192	238	$C_{17}H_{34}$	1.53
2	2, 4-di-tert-butylphenol	13.534	206	$C_{14}H_{22}O$	2.76
3	cyclohexadecane	14.585	224	$C_{16}H_{32}$	2.83
4	1-octadecene	16.643	252	$C_{18}H_{36}$	4.18
5	Aspidinol	17.920	224	$C_{12}H_{16}O_4$	12.63
6	n- hexadecanoic acid (palmitic acid)	18.086	256	$C_{16}H_{32}O_2$	4.78
7	Desaspidinol	18.417	210	$C_{11}H_{14}O_4$	6.07
8	1-docosanol	20.150	326	$C_{22}H_{46}O$	4.55
9	Squalene	22.262	410	$C_{30}H_{50}$	45.62

Table 3: Phytoconstituents identified in methanolic extract of *Callistemon citrinus (Curtis) Skeels* leaves.

Sr. No.	Compound	Retention time	Molecular Weight	Molecular Formula	Concentration (%)
1	L-terpinen-4-ol	8.732	154	$C_{10}H_{18}O$	4.11
2	m-mentha-1 (7), 8-diene	8.944	136	$C_{10}H_{16}$	10.08
3	Caryophyllene	12.606	204	$C_{15}H_{24}$	1.65
4	Coumarin-6-ol, 3, 4-dihydro-4, 4-dimethyl-7-nitro-	16.296	237	$C_{11}H_{11}NO_5$	1.10
5	1, 4-eicosadiene	17.092	278	$C_{20}H_{38}$	2.26

Table 4: Phytoconstituents identified in volatile oil of *Callistemon citrinus (Curtis) Skeels* leaves.

Sr. No.	Compound	Retention time	Molecular Weight	Molecular Formula	Concentration (%)
1	3-octen-5-yne, 2, 7-dimethyl-	6.472	136	$C_{10}H_{16}$	5.64
2	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	7.122	136	$C_{10}H_{16}$	2.26
3	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1. Alpha, 2. Beta, 5. Alpha)-	7.272	154	$C_{10}H_{18}O$	4.91
4	Fenchol	7.575	154	$C_{10}H_{18}O$	1.43
5	Trans-pinocarveol	8.072	152	$C_{10}H_{16}O$	2.97
6	Isoborneol	8.595	253	$C_{13}H_{19}NO_2S$	2.83
7	Isopulegol	8.845	154	$C_{10}H_{18}O$	12.72
8	Dihydrocarveol	9.096	154	$C_{10}H_{18}O$	17.85
9	7-benzofuranol, 2, 3-dihydro-2, 2-dimethyl-	11.542	164	$C_{10}H_{12}O_2$	2.53
10	Bicyclo[2.2.1]heptan-2-ol, 1,5,5-trimethyl-	11.686	154	$C_{10}H_{18}O$	2.28
11	Eudesma-4(14), 11-diene	12.709	204	$C_{15}H_{24}$	6.65
12	2-methyl-2-(4-methyl-3-oxopropyl)cyclohexane	12.959	210	$C_{13}H_{22}O_2$	1.58
13	1, 5-cycloundecadiene, 8,8-dimethyl-9-methylene-	13.116	190	$C_{14}H_{22}$	2.90
14	1-cyclohexene-1-acetaldehyde, 2,6,6-trimethyl-	13.658	166	$C_{11}H_{18}O$	4.44
15	Spathulenol	14.496	220	$C_{15}H_{24}O$	1.74
16	4,8,13-cyclotetradecatriene-1,3-diol	14.565	306	$C_{20}H_{34}O_2$	7.64
17	2-dodecen-1-yl(-) succinic anhydride	14.839	266	$C_{16}H_{26}O_3$	4.42

CONCLUSION

The GC-MS hyphenated technique was used for separation and identification of phytoconstituents present in the leaves of the plant *Callistemon citrinus (Curtis) Skeels* after the extraction of volatile oil. This paper highlights the need to thoroughly investigate the detailed pharmacognostic profile of the plant for its characterization and standardization of the extracts.

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