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Research Article

# GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *CALLISTEMON PALLIDUS* OF MEKELLE, ETHIOPIA

Muuz Mehari, Wold Amha, Unnithan CR*

Department of Chemistry, Mekelle University, Ethiopia

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*Corresponding Author: **Dr. C.R. Unnithan**
Professor of chemistry Mekelle, Ethiopia Email: crunnithan@yahoo.co.in

## ABSTRACT

The constituents of essential oil isolated by hydro distillation of the aerial parts of *Callistemon pallidus*, Myrtaceae family, from Ethiopia was examined by GC-MS. A total of 13 components were identified accounting for 81.58% of the oil of *C.pallidus*. The oil contained, as the main components, 1, 8-cineloe (56.75%), caryophyllene (3.78%), caryophyllene oxide (2.89%), α -pinene (2.87%), 3-carene (2.54 %), cubenol (2.09%) and fenchol(1.91%). The essential oil of *C.pallidus* exhibited significant anti bacterial activity against gram positive (*Staphylococcus auerus*) than gram negative bacteria (*Escherichia coli*).

**Keywords**: *Callistemon pallidus, Myrtaceae,* essential oil, antibacterial activity

## INTRODUCTION

*Callistemon pallidus* commonly known as lemon bottlebrush is a shrub in the family of Myrtaceae. This species is endemic to eastern Australia. The genus *Callistemon* is known in folk medicine and their volatile oils have been used as antimicrobial and antioxidant agents[1-6] .

## MATERIALS AND METHODS

The aerial parts of *Callistemon pallidus* plant was collected during the month of September from Mekelle, Ethiopia in 2013. The plant was identified by the authors and its herbarium sheet was deposited at the Chemistry department, Mekelle University, Mekelle, Ethiopia.

**Chemical reagents:** All chemicals used in the present study were of analytical grade and obtained from Sigma Co. (St. Louis, MO, USA).

**Essential oil extraction:** The shade dried aerial parts of *Callistemon pallidus* plant collected (1Kg) was subjected to hydro distillation in a Clevenger apparatus for 3h. The essential oil was separated from aqueous layer using a 100 mL capacity separatory funnel. The collected essential oil was dried over anhydrous sodium sulphate and filtered using a Whatman filter paper no. 40. The extracted essential oil was light yellow liquid in appearance which was stored at 4°C in dark brown 5-mL capacity sample bottle until analysis. The yield (fresh weight/w) was < 0.1%.

**GC and GC-MS analysis:** GC analyses were carried out in Agilent Technology 6890N gas Chromatograph data handling system equipped with a split-split less injector and fitted with a FID using N2 as carrier gas. The column was HP-5capillary column (30m x 0.32mm, 0.25µm film thickness) and temperature program was used as follows: initial temperature of 60$^{o}$C( hold : 2 min) programmed at a rate of 3$^{o}$C/min to a final temperature of 220 $^{o}$C (hold: 5 min). Both the temperature of injector and FID were maintained at 210$^{o}$C. The GC-MS was performed by Perkin Elmer Clarus 500 gas chromatograph equipped with a split-split less injector (split ratio 50:1) data handling system. The column was an Rtx®-5 capillary columns (60 min x 0.32 mm, 0.25µm film thickness). Helium was used as carrier gas at a flow rate of 1.0ml/min. The GC was interfaced with Perkin Elmer 500 mass detector operating in EI+ mode. The mass spectra was recorded over 40-500 amu and revealed the total ion current chromatograms. The temperature program remained the same as in GC. The temperatures of injector and transfer line were kept at 210 $^{o}$ C and that of ion source at 200 $^{o}$C. Identification of the oil components was done by comparison of their mass spectra with the NIST/Wiley library as well as by comparing them with those reported in literature. The identification of each compound was also confirmed by comparison of its retention index with those of authentic compounds [8].

**Antibacterial activity:** The study was conducted by using standard disc diffusion method. In each experiment, microorganisms were cultured at 37°C for 24 hours and prepared to turbidity which is equivalent to 0.5 McFarland standards (National Committee of Clinical Laboratory Standards) [9-11]. Mueller-Hinton (MH) agar 38g was dissolved in 1000 ml of distilled water. Then it was boiled on heating mantle to dissolve the media completely and then sterilized by

autoclaving at 15 lbs. and 121°C for 15 min. After it was autoclaved at indicated conditions, it was poured to the sterilized petridishes and allowed to set at room temperature until the agar has solidified. It was then incubated at 37°C for 24 hours to be ready for susceptibility test. The stock solution of the crude *C.pallidus* oil in Chloroform (20mg/ml) and test discs were prepared from Whatman filter paper[12]. A 0.5 McFarland standard was prepared as described in National Committee of Clinical Laboratory Standards (NCCLS) [13, 14]. One percent V/V solution of sulfuric acid and 1.175% W/V solution of barium chloride were prepared and made it turbidity standard. A small volume of this turbid solution was transferred to a screw capped tube and vigorously shaken on a mechanical vortex mixer to have a uniform turbid appearance and stored in the dark at room temperature. Purely cultured Mueller-Hinton agar petridishes were labeled with different names of bacteria. Then 5 ml of sterile Normal Saline Solution (NSS) was pipetted out into a three different sterile screw-cap tubes. These tubes were labelled according to the type and number of bacteria used to test (*E. coli* and *S. aurous)*. To prepare inoculums, 3 well isolated colonies of the same morphological types were selected from an agar plate culture. The top of each colony is touched with a loop, and growth was transferred into a tube containing 5 ml of NSS that corresponds to each bacterium names. These inoculums containing tubes were mixed by using mechanical vortex mixer and their turbidity was compared accurately. The sterile discs which were prepared by office perforator were inserted in to different concentrations of *C.pallidus* oil with stock solution of 20mg/ml. It was impregnated in to negative and positive controls petroleum ether and chloroform, and amoxicillin respectively. After that, discs with different concentrations were placed on the inoculated plates using a pair of sterile forceps. Seven discs were placed on a 90 cm diameter petridish plate and the space between each disc was given as 24 mm gap from centre of the disk to the centre of petridish. The pressed discs were completely stacked the agar surface, plates were inverted and placed in an incubator at 37 C for 24 hour. After overnight incubation, the diameter of each zone (including the diameter of the disc) were measured and recorded.

## RESULTS AND DISCUSSION

The composition of essential oil of *Callistemon pallidus* is shown in the table 1. A total of 81.58% was identified. The major components identified were 1, 8-cineloe (56.75%), caryophyllene (3.78%), caryophyllene oxide (2.89%), α -pinene (2.87%), 3-carene (2.54 %), cubenol (2.09%) and fenchol (1.91%). The class of compounds identified from the oil of *Callistemon pallidus* by the authors contain monoterpenes (68 .21%) and sesquiterpenes (9.68%). The major identified constituents ,especially 1,8-cineole in the present study are same as reported in early studies[15-18]. A few of the identified constituents in the oil of *C. pallidus* in our study are un reported. They include 3-none-5-one (1.32%),4-isopropyl toluene (1.10%), fenchol (1.91%), linalylanthranilate (1.09%) and cubenol (2.09%). These variations in composition at different regions in chemotypes of *Callistemon* species were reported [19-21]. The crude oil of *Callistemon pallidus* showed considerable antibacterial activity (table 2) against gram positive bacteria (*S.aureus* ) than gram negative (*E.coli* ) bacteria.

## CONCLUSION

The major compounds of the oil of *Callistemon pallidus*, from Mekelle are 1, 8-cineloe (56.75%), caryophyllene (3.78%), caryophyllene oxide (2.89%), α -pinene (2.87%), 3-carene (2.54 %), cubenol (2.09%) and fenchol (1.91%) showed significant anti bacterial activity against gram positive (*Staphylococcus auerus*) bacteria.

**Table 1: Chemical compositions of essential oil of *Callistemon pallidus***

| Peak No | Retention Time ($R_T$) | Identified compounds | Percentage composition (%) | Method of identification |
|---|---|---|---|---|
| 1 | 4.876 | 1, 8-cineole | 56.75 | GC, GC-MS |
| 2 | 5.204 | 3-carene | 2.54 | GC, GC-MS |
| 3 | 5.567 | α-pinene | 2.87 | GC, GC-MS |
| 4 | 5.878 | 3-none-5-one | 1.32 | GC, GC-MS |
| 5 | 6.248 | unidentified | 1.02 | GC, GC-MS |
| 6 | 6.896 | α-terpineol | 2.86 | GC, GC-MS |
| 7 | 6.989 | unidentified | 1.01 | GC, GC-MS |
| 8 | 7.967 | 4-isopropyl toluene | 1.10 | GC, GC-MS |
| 9 | 8.972 | fenchol | 1.91 | GC, GC-MS |
| 10 | 9.576 | linalyl anthranilate | 1.09 | GC, GC-MS |
| 11 | 10.245 | cubenol | 2.09 | GC, GC-MS |
| 12 | 11.234 | caryophyllene | 3.78 | GC, GC-MS |
| 13 | 12.342 | caryophyllene oxide | 2.89 | GC, GC-MS |
| 14 | 14.132 | methyl isoborneol | 1.28 | GC, GC-MS |
| 15 | 15.542 | unidentified | 1.06 | GC, GC-MS |
| 16 | 16.986 | unidentified | 0.78 | GC, GC-MS |
| 17 | 17.890 | spathulenol | 1.10 | GC, GC-MS |
| Total Identified Compounds | | 13 Compounds | 81.58 | GC-GC-MS |
| Total unidentified or unknown compounds | | | 3.87 | GC-GC-MS |

**Table 2: *In vitro* antibacterial activity of crude essential oil of *Callistemon pallidus***

| Sl. No. | Test Organisms | | Zone of inhibitions(mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Concentration of *Callistemon oil*(µg/ml) | | | | | Negative control | Positive control |
| | | | 10 | 20 | 40 | 80 | St | Chloroform | AM (30µg/disk) |
| 1. | *Gram Positive* | *S.aureus* | 1.05 ± 0.098 | 1.468 ± 0.086 | 1.00 ± 0.083 | 1.004 ± 0.149 | 1.135 ± 0.170 | - | 13.086 ± 0.281 |
| 2. | *Gram negative* | *E.coli* | 0.831 ± 0.060 | 0.816 ± 0.070 | 0.802 ± 0.079 | 1.180 ± 0.177 | 0.949 ± 0.070 | - | 7.980 ± 0.428 |

**All the values are given as mean ±STD which were analyzed in triplicate, St: - Stock solution, -:- Has no activity, AM: - Amoxicillin, *S.aureus:- Staphylococcus aureus, E.coli:- Escherichia coli.***

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