*Journal of Applied and Natural Science*
12(4): 532 - 540 (2020)
Published online: November 24, 2020
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

*Research Article*

# Antidandruff activity of *Cassia auriculata* and *Cassia alata* through fatty acids mediated inhibition of *Malassezia furfur*

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***Article Info***
https://doi.org/10.31018/jans.v12i4.2390
Received: October 4, 2020
Revised: November 15, 2020
Accepted: November 20, 2020

***How to Cite***
Prabhu V. et al. (2020). Antidandruff activity of *Cassia auriculata* and *Cassia alata* through fatty acids mediated inhibition of *Malassezia furfur*. *Journal of Applied and Natural Science*, 12(4):532 - 540. https://doi.org/10.31018/jans.v12i4.2390

**Abstract**
Susceptibility of *Malassezia furfur* to certain medium chain fatty acids shed light onto novel strategies to control dandruff. This study explored antidandruff activity of the fatty acids and other bioactive compounds from flowers of *Cassia auriculata* and *Cassia alata.* The idea was supplementing the growth medium with fatty acids which are inhibitory to *Malassezia* so that plant-based antidandruff formulations could be developed based on the results. Chloroform and ethanolic flower extracts were tested there *in vitro* efficacy against *M. furfur* and the potential antidandruff compounds were identified by gas chromatography-mass spectrophotometry (GC-MS). Minimum inhibitory concentrations were determined for both the extracts and $IC_{50}$ values of 50 and 88 µM for chloroform extract of *C. auriculata* and *C. alata* were recorded. For ethanol extract, $IC_{50}$ values of 75 and 70 µM were exhibited by *C. auriculata* and *C. alata,* respectively. Inhibition of *M. furfur* through fatty acids from Cassia is the first report, and it is possible to include specific fatty acids in the growth media to inhibit the growth of *Malassezia* which could be later served as lead molecules in antidandruff formulations. Further, the presence of citronellol, pinitol, anthracenedione and chrysine in *Cassia* flower extracts and their antidandruff activity reported in this study needed further research on those compounds to formulate effective treatment of *Malassezia* associated diseases.

**Keywords:** Anti-dandruff, *Cassia alata, Cassia auriculata,* Fatty acids, *Malassezia furfur*

## INTRODUCTION

Scalp has thick terminal hair, large numbers of sweat glands and sebaceous glands. Sebum secreted in stratum corneum provides a nutrient-enriched environment for microbial growth. *Malassezia* species are found on mammalian skin (Findley *et al*., 2013, Gemmer *et al*., 2002; Sampaio *et al*., 2011; Takemoto *et al*., 2015) associated with several common skin disorders (Sugita *et al*., 2010). *Malassezia* is a lipophilic yeast that uses sebum lipids as a nutrient

source and leaves unsaturated free acids to result in dryness of the stratum corneum that ultimately leads to dandruff (Ashbee and Evans, 2002). Dandruff is characterized by redness on the scalp with loosely attached oily flakes on the skin (Gupta *et al*., 2004). Ketoconazole, zinc pyrithione, selenium sulfide and salicylic acid-containing preparations are commonly used in antidandruff shampoo formulations, but high resistance among *Malassezia* sp, recurrent dandruff rates and toxicity limits their usage on the human scalp.

*Cassia* belongs to the family Caesalpiniaceae and many studies have reported the use of *Cassia* due to its pharmacological potential. Various parts of *Cassia auriculata* and *Cassia alata* were studied for their antimicrobial activity (Murugan *et al*., 2013; Timothy *et al*., 2012). *Cassia* species is also reported in treating skin diseases (Singhal and Kansara, 2012; Khare 2007; Duke, 2002; Maity *et al*., 2001; Atarzadeh *et al*., 2017). Antifungal activity against *Malassezia furfur* by *C. auriculata* (Kumar *et al*., 2008) and *C. alata* (Damodaran and Venkataraman, 1994) were reported earlier. However, the active ingredients present in the plants were not explored from the point of antidandruff activity. Further, *Malassezia* spp. are susceptible to certain medium chain fatty acids (Papavassilis *et al*., 1999; Mayser, 2015; Bhattacharyya *et al*., 2017). In light of this information, this study explored antidandruff activity of the fatty acids and their derivatives from flowers of *C. auriculata* and *C. alata.* The idea was supplementing the growth medium with fatty acids which are inhibitory to *Malassezia* so that plant based antidandruff formulations could be developed based on the results.

## MATERIALS AND METHODS

**Plant materials:** Fresh flowers of *C. auriculata* and *C. alata* were collected from Pollachi (10.669823°N, 76.980639°E), Tamil Nadu, India. The flowers were identified and authenticated in the Department of Botany, Bangalore University. The flowers were washed and air-dried and powdered.

**Preparation of flower extract:** Flowers of *C. auriculata* and *C. alata* (50 g) was extracted successively with 500 ml of chloroform and 500 ml of ethanol by using a soxhlet extractor for 4-6 h at a temperature not exceeding the boiling point. The chloroform and ethanol extracts were collected after filtration and evaporated to dryness under reduced pressure using a Rota vapour. The dried extracts were used for further studies.

**Test organism:** *Malassezia furfur* MTCC 1374 was obtained from Microbial Type Culture Collection, Chandigarh for this study.

**Anti-malassezial activity:** The antimicrobial activity of the test organisms to the *C. auriculata* and *C. alata* plant extracts was screened by using the agar well diffusion method. An inoculum suspension ($1.2 \times 10^3$ CFU/ml) was swabbed uniformly to solidified 20 ml Sabouraud's Dextrose Agar (SDA) and was allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar using a sterile cork borer. Aliquot of 10 µl from each plant extract (10, 20, 40, 80, 160 and 320 µM) was added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37°C for 24 h. The resulting inhibition zones were measured in millimetres (mm). Ketoconazole was used as positive control and the studies were performed in triplicates.

**Minimum inhibitory concentration assay:** In vitro antidandruff activity was performed in 96 well microtitre plates using *M. furfur* MTCC 1374 in Sabouraud dextrose broth (Sibi *et al*., 2014). Extracts in the concentrations of 200 µM, 180 µM, 160 µM, 140 µM, 120 µM, 100 µM, 50 µM, 25 µM, 10 µM and 1 µM were tested against *M. furfur*. Cell density at $1.2 \times 10^3$ CFU/ml was measured by following McFarland 0.5 standard solutions used. Optical density absorbance at 630 nm was read out in microtiter plate reader. Ketoconazole was used as a control in the study. The lowest concentration which inhibited the growth of the fungi was considered the minimum inhibitory concentration (MIC) of each extract. The inhibitory concentration ($IC_{50}$) value was calculated using the linear relation between the minimum inhibitory percentage and concentration of the extract.

**GC-MS Profiling of bioactive compounds:**To identify the compounds present in *C. auiculata* and *C. alata*, GC-MS analysis was carried out using an Agilent technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length x 250 µm in diameter x 0.25 µm thickness of film). Spectroscopic detection by GC-MS involved an electron ionization system which utilized high energy electrons (70ev). Pure helium gas (99.995%) was used as the carrier gas with a flow rate of 1ml/min. The initial temperature was set at 45-150$^o$C with the increasing rate of 8$^o$C/min and holding time of about 4 min. Finally, the temperature was increased to 290°C for 4 min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in a splitless mode. The relative quantity of the chemical compounds presents in each of the extracts of *C. auriculata* and *C. alata* was expressed as a percentage based on peak area produced in the chromatogram. Compounds were identified by comparing the mass spectral data with the NIST library (Prabhu *et al*., 2020).

**Statistical analysis:** The experiments were done in triplicate and the data reported as the mean ± SD. Analysis of variance was performed by one way ANOVA using SPSS statistical software, version 20. A probability value at $P < 0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

An antidandruff activity using well diffusion assay revealed the inhibitory activity of both *C. auriculata* and *C. alata* against *M. furfur*. were prepared in chloroform

and ethanol. Quantitative results of the effect of chloroform and ethanol extracts of flowers at the concentrations of 20, 40, 60, 80 and 100 µg/ml on the size of the clear zones are represented in the table-1. The range of inhibition values observed between the extracts varied, and not all preparations exhibited the same anti-malassezzial properties.

Anti-malassezial activity of ethanol extracts was higher than chloroform extract and the inhibitory was increased when the extract concentrations were higher. It was found that inhibitory concentration ($IC_{50}$) values of 50 and 88 µM were recorded for chloroform extract of *C. auriculata* and *C. alata* flowers, respectively. For ethanol extract, $IC_{50}$ values of 75 and 70 µM were exhibited by *C. auriculata* and *C. alata* respectively (Fig. 1).

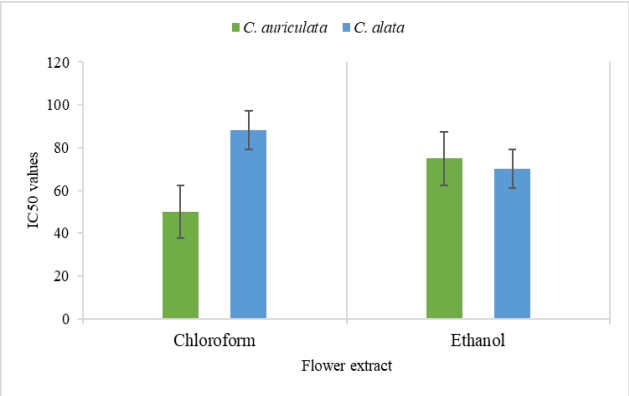


**Fig. 1.** *$IC_{50}$ values of C. auriculata and C. alata flower extracts against M. furfur MTCC 1374.*

The peaks are marked with retention time in the GC-MS chromatogram of the chloroform and ethanol extract of the flowers of *Cassia auriculata and Cassia alata*. Their retention time (RT) and the amount of their presence are indicated in Table 2, 3, 4, 5. A total of 59 and 37 compounds identified in chloroform and ethanol extract of *C. auriculata* and *C. alata* are shown in Fig. 2 and 3.

GC-MS analysis of chloroform extracts indicated the presence of fatty acids (i-Propyl 11,12-methylene-octadecanoate, Octadecanoic acid, Hexadecanoic acid), fatty alcohols (Tetracosanol, Hexadecanol), sesquiterpenes and aromatic organic compounds in *C. auriculata* and *C. alata*. Of the total 59 compounds identified in the chloroform extracts, 30 were present in *C. auriculata*. The various compounds observed from chloroform extracts of *C. auriculata* and *C. alata* did not differ significantly from each other.

Fatty acids are carboxylic present in various plants as either saturated or unsaturated compounds with known antimicrobial activity (Rahuman *et al*., 2000; Cerdeiras *et al*., 2000; Dilika *et al*., 2000; Lee *et al*., 2002; McGraw *et al*., 2002; Kumar *et al*., 2010). Similarly, fatty acids esterified into polyhydric alcohols with antimicrobial effectiveness is reported (Mukherjee *et al*., 2013; Togashi *et al*., 2007; Kubo *et al*., 1993). In our study, anti-malassezial activity of chloroform extracts of *Cassia* was due to the presence of fatty acids and fatty alcohols. This is the novel report that fatty acids from *Cassia* is inhibitory to *M. furfur* thus could help in the control of dandruff.

Anti-malassezial activity of medium-chain fatty acids is reported in earlier studies (Mayser, 2015; Bhattacharyya *et al*., 2017), but the presence of fatty acids and fatty alcohols in *Cassia* species and their inhibitory activity against *Malassezia furfur* is reported for the first time through this study. From the results, it is possible to include specific fatty acids in the growth media to inhibit the growth of *Malassezia,* which could be later served as lead molecules in antidandruff formulations.

Various bioactive compounds were present in ethanol extract of *C. auriculata* and *C. alata* as revealed from GC-MS analysis. Some of the important phytoconstituents identified in this study were terpenoids (Citronellol), polyols (pinitol), glycosides (Anthracenedione, Mannopyranoside) flavonoids (Resorcinol), flavones (Chrysine) along with carbohydrates (Fig. 4 and 5; Table 3 and 4). Antimicrobial activities of the above identified phytochemical compounds from various sources are widely documented in earlier studies. For example, resorcinol is known for its keratolytic and anti-dermatophytic activities (Romagnoli *et al*., 2016; Martindale, 1989). Ethanol extract of *C. auriculata* flowers showed the presence of resorcinol (0.66%) in this study which may be attributed to the antidandruff activity of plant. Inhibitory activity of citronellol against the fungi *Trichophyton*

**Table 1.** Anti-malassezial activity of *C.auriculata* and *C. alata* flowers against *M. furfur*. (Values are of triplicates).

| Concentration (µg/ml) | Chloroform extract | | Ethanol extract | |
|---|---|---|---|---|
| | Zone of Inhibition (mm) | | Zone of Inhibition (mm) | |
| | ***C. auriculata*** | ***C. alata*** | ***C. auriculata*** | ***C. alata*** |
| 20 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.00 | 0.0 ± 0.0 |
| 40 | 0.7 ± 0.47 | 0.3 ± 0.06 | 0.0 ± 0.06 | 0.1 ± 0.01 |
| 80 | 3.3 ± 0.11 | 2.6 ± 0.10 | 3.1 ± 0.20 | 4.3 ± 0.22 |
| 160 | 6.7 ± 0.20 | 4.7 ± 0.01 | 7.3 ± 0.01 | 7.7 ± 0.03 |
| 320 | 6.8 ± 0.25 | 5.2 ± 0.51 | 7.1 ± 0.33 | 7.8 ± 0.05 |
| Ketoconazole (30 µg) | 5.9 ± 0.74 | | | |

**Table 2.** Chemical constituents present in chloroform extract of *C. auriculata* flowers.

| Name of the compound | Molecular formula | Retention time (min) | Total Percentage |
|---|---|---|---|
| Hexadecane | $C_{16}H_{34}$ | 14.927 | 0.583 |
| 1-Octadecanol | $C_{18}H_{38}O$ | 15.619 | 3.2 |
| Heptadecane | $C_{17}H_{36}$ | 16.993 | 0.984 |
| Pentadecane,2,6,10,14-tetramethyl- | $C_{19}H_{40}$ | 17.121 | 1.103 |
| Octadecane | $C_{18}H_{38}$ | 18.968 | 1.910 |
| Nonadecane | $C_{19}H_{40}$ | 20.883 | 1.455 |
| Eicosane | $C_{20}H_{42}$ | 22.707 | 1.363 |
| 1-Eicosene | $C_{20}H_{40}$ | 23.350 | 2.330 |
| Heneicosane | $C_{21}H_{44}$ | 24.468 | 0.959 |
| Docosane | $C_{22}H_{46}$ | 26.146 | 0.644 |
| 1-Docosene | $C_{22}H_{44}$ | 26.778 | 2.054 |
| Phenol,2,4-bis(1,1-dimethylethyl)- | $C_{14}H_{22}O$ | 27.263 | 3.860 |
| Tricosane | $C_{23}H_{48}$ | 27.775 | 0.996 |
| Hentriacontane | $C_{31}H_{64}$ | 29.328 | 0.414 |
| Hexadecanoic acid,butyl ester | $C_{20}H_{40}O_2$ | 29.512 | 0.626 |
| n-Tetracosanol-1 | $C_{24}H_{50}O$ | 29.949 | 1.792 |
| Pentacosane | $C_{25}H_{52}$ | 30.793 | 0.852 |
| Phthalic acid, hept-4-yl isobutyl ester | $C_{19}H_{28}O_4$ | 31.274 | 4.034 |
| Phthalic acid, butyl-2-pentyl ester | $C_{17}H_{24}O_4$ | 31.814 | 5.364 |
| Heptacosane | $C_{27}H_{56}$ | 31.971 | 1.348 |
| Dibutyl phthalate | $C_{16}H_{22}O_4$ | 32.164 | 17.63 |
| 1-Hexacosene | $C_{26}H_{52}$ | 32.399 | 3.072 |
| 12-Oxotricyclo [5.3.1.1(2,6)]dodeca-3,8-diene,11-acetoxy-4,5,9-trichloro | $C_{14}H_{13}Cl_3O_3$ | 33.697 | 0.793 |
| Nonacosane | $C_{29}H_{60}$ | 33.849 | 9.685 |
| 17-Pentatriacontene | $C_{35}H_{70}$ | 34.196 | 1.247 |
| l-(+)-Ascorbic acid 2,6-dihexadecanoate | $C_{38}H_{68}O_8$ | 34.407 | 0.888 |
| i-Propyl 11,12-methylene-octadecanoate | $C_{22}H_{42}O_2$ | 35.145 | 1.768 |
| Hexadecanoic acid | $C_{18}H_{36}O_2$ | 36.103 | 0.632 |
| Bis(2-ethylhexyl)phthalate | $C_{24}H_{38}O_4$ | 37.089 | 26.104 |
| Octadecane,-3-ethyl-5-(2-ethylbutyl)- | $C_{26}H_{54}$ | 37.289 | 2.33 |

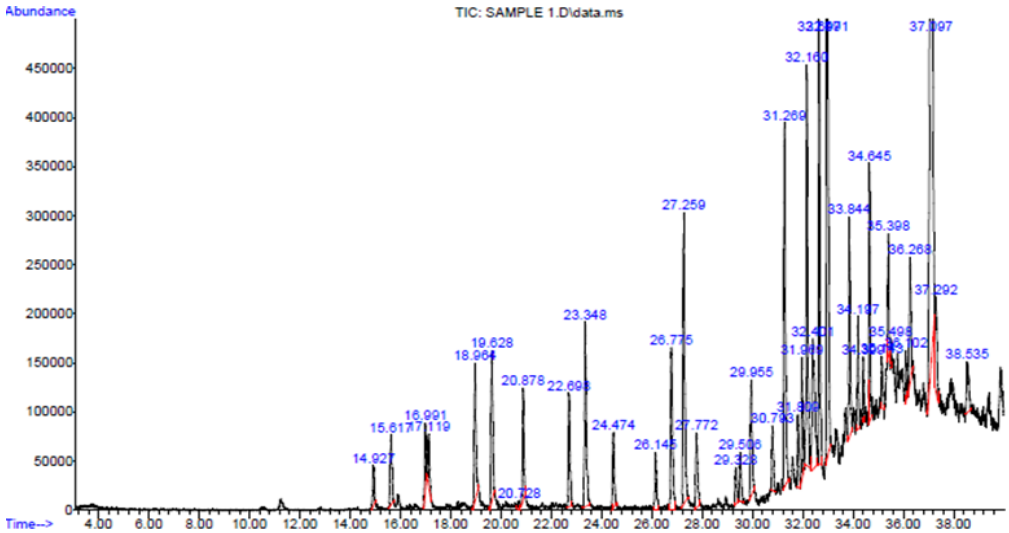


**Fig. 2.** *Chromatogram of chloroform extract of C. auriculata flowers.*

**Table 3.** Chemical constituents present in chloroform extract of *C. alata* flowers.

| Name of the compound | Molecular Formula | Retention time (min) | Total Percentage |
|---|---|---|---|
| Hexadecane | $C_{16}H_{34}$ | 15.012 | 0.556 |
| 1-Hexadecanol | $C_{16}H_{34}O$ | 15.704 | 0.975 |
| Heptadecane | $C_{17}H_{36}$ | 17.031 | 0.875 |
| Hexadecane, 2,6,10-trimethyl- | $C_{19}H_{40}$ | 17.134 | 1.015 |
| Octadecane | $C_{18}H_{38}$ | 18.977 | 1.656 |
| 1-Nonadecene | $C_{19}H_{38}$ | 19.635 | 0.752 |
| Benzyl alcohol | $C_7H_8O$ | 20.024 | 0.923 |
| Nonadecane | $C_{19}H_{40}$ | 20.865 | 2.178 |
| Eicosane | $C_{20}H_{42}$ | 22.673 | 1.757 |
| 1-Eicosene | $C_{20}H_{40}$ | 23.308 | 1.006 |
| Heneicosane | $C_{12}H_{44}$ | 24.413 | 1.856 |
| Docosane | $C_{22}H_{46}$ | 26.112 | 1.683 |
| Benzene,1,2,3-trimethoxy-5-(2-propenyl)- | $C_{12}H_{16}O_3$ | 26.490 | 0.513 |
| n-Tetracosanol-1 | $C_{24}H_{50}O$ | 26.713 | 0.788 |
| 1,3-Benzodioxole, 4-methyl-6-(2-propyl)- | $C_{11}H_{12}O_3$ | 26.879 | 0.265 |
| Phenol, 2,4-bis(1,1-dimethylethyl)- | $C_{14}H_{22}O$ | 27.154 | 3.196 |
| Tetracosane | $C_{24}H_{50}$ | 27.726 | 6.097 |
| Hexadecanoic acid, butyl ester | $C_{20}H_{40}O_2$ | 29.437 | 0.279 |
| Heptafluorobutyric acid, n-octadecyl ester | $C_{22}H_{37}F_7O_2$ | 29.900 | 0.783 |
| Pentacosane | $C_{25}H_{52}$ | 30.787 | 6.624 |
| Phthalic acid, butyl isohexyl ester | $C_{18}H_{26}O_4$ | 31.182 | 4.17 |
| Tetratriacontane | $C_{34}H_{70}$ | 31.937 | 18.98 |
| Phthalic acid, butyl hex-3-yl ester | $C_{18}H_{26}O_4$ | 32.572 | 2.163 |
| 1-(+)-Ascorbic acid 2,6-dihexadecanoate | $C_{38}H_{68}O_8$ | 34.323 | 6.188 |
| Nonacosane | $C_{29}H_{60}$ | 34.638 | 22.356 |
| Octadecanoic acid | $C_{18}H_{36}O_2$ | 36.000 | 1.890 |
| Octadecane, 3-ethyl-5-(2-ethylbutyl)- | $C_{26}H_{54}$ | 37.281 | 5.694 |
| (2S,2'S)-2,2'-Bis[1,4,7,10,13-pentaoxacyclopentadecane] | $C_{20}H_{38}O_{10}$ | 37.922 | 1.702 |
| 2-Hexadecanol | $C_{16}H_{34}O$ | 41.361 | 5.080 |

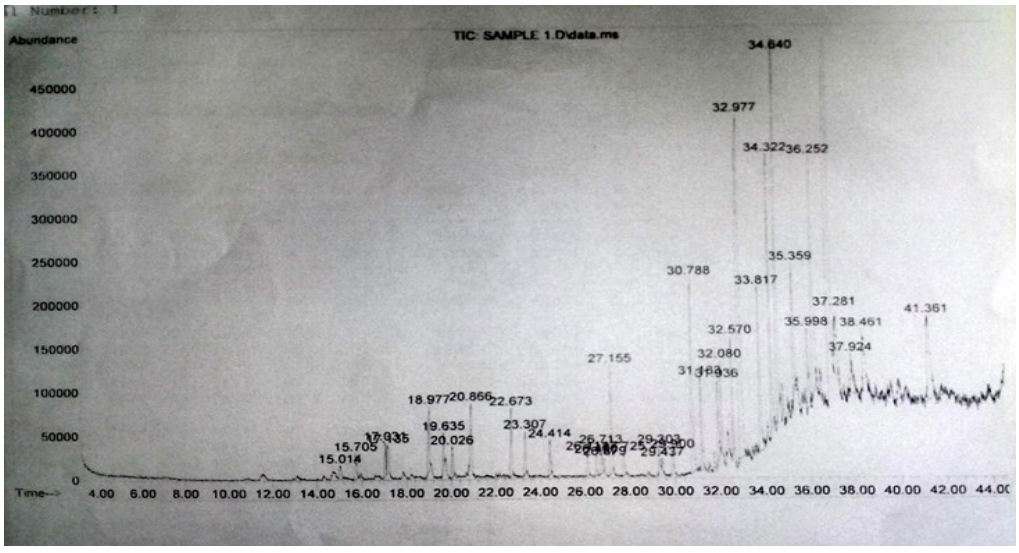


**Fig. 3.** *Chromatogram of chloroform extract of C. alata flowers.*

**Table 4.** Chemical constituents present in ethanol extract of *C. auriculata* flowers.

| Name of the Compound | Molecular formula | Retention time | Total Percentage |
|---|---|---|---|
| Resorcinol | $C_6H_6O_2$ | 14.4722 | 0.665 |
| 2-Oxiranemethanol, α-(1-methylethyl)-3-[1-(trimethylsilyloxy)pentyl]- | $C_{14}H_{30}O_3Si$ | 20.993 | 1.063 |
| D-Pinitol, pentakis(trimethylsilyl) ether | $C_{22}H_{54}O_6Si_5$ | 21.713 | 3.600 |
| 1,5-Anhydro-D-sorbitol, tetrakis(trimethylsilyl) ether | $C_{18}H_{44}O_5Si_4$ | 22.003 | 5.520 |
| Lactulose, octakis(trimethylsilyl) ether (isomer 1) | $C_{36}H_{86}O_{11}Si_8$ | 22.324 | 5.002 |
| Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)- | $C_{24}H_{60}O_6Si_6$ | 22.744 | 22.725 |
| D-(-)- Erythrofuranose, tris(trimethylsilyl) ether (isomer 2) | $C_{13}H_{32}O_4Si_3$ | 22.95 | 12.160 |
| D-(+)-Glucuronic acid γ-lactone, tris(trimethylsilyl) ether, methyloxime | $C_{16}H_{35}NO_6Si_3$ | 23.463 | 9.551 |
| Sedoheptulose, o-methyloxime, hexakis-O-(trimethylsilyl)- | $C_{26}H_{65}NO_7Si_6$ | 23.61 | 5.028 |
| d-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1Z)- | $C_{22}H_{55}NO_6Si_5$ | 23.845 | 0.894 |
| Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, scyllo- | $C_{24}H_{60}O_6Si_6$ | 24.331 | 5.093 |
| D-(-)-Tagatose, pentakis(trimethylsilyl) ether | $C_{21}H_{52}O_6Si_5$ | 25.983 | 6.350 |
| Chrysin, bis(trimethylsilyl) ether | $C_{21}H_{26}O_4Si_2$ | 30.594 | 2.304 |
| Glucofuranoside, methyl 2,3,5,6-tetrakis-O-(trimethylsilyl)-, α-D- | $C_{19}H_{46}O_6Si_4$ | 31.542 | 0.875 |
| D-(+)-Turanose, octakis(trimethylsilyl)ether | $C_{36}H_{86}O_{11}Si_8$ | 31.747 | 7.487 |
| 9,10-Anthracenedione, 2-methyl-1,6-bis[(trimethylsilyl)oxy]- | $C_{21}H_{26}O_4Si_2$ | 32.25 | 1.137 |
| D-(-)-Ribofuranose, tetrakis(trimethylsilyl)ether(isomer 1) | $C_{17}H_{42}O_5Si_4$ | 32.758 | 8.519 |
| D-(-)-Ribofuranose, tetrakis(trimethylsilyl) ether (isomer 2) | $C_{17}H_{42}O_5Si_4$ | 33.889 | 1.727 |
| bis[2-Trimethylsiloxy]ethyl sulfone | $C_{10}H_{26}O_4SSi_2$ | 35.507 | 0.301 |

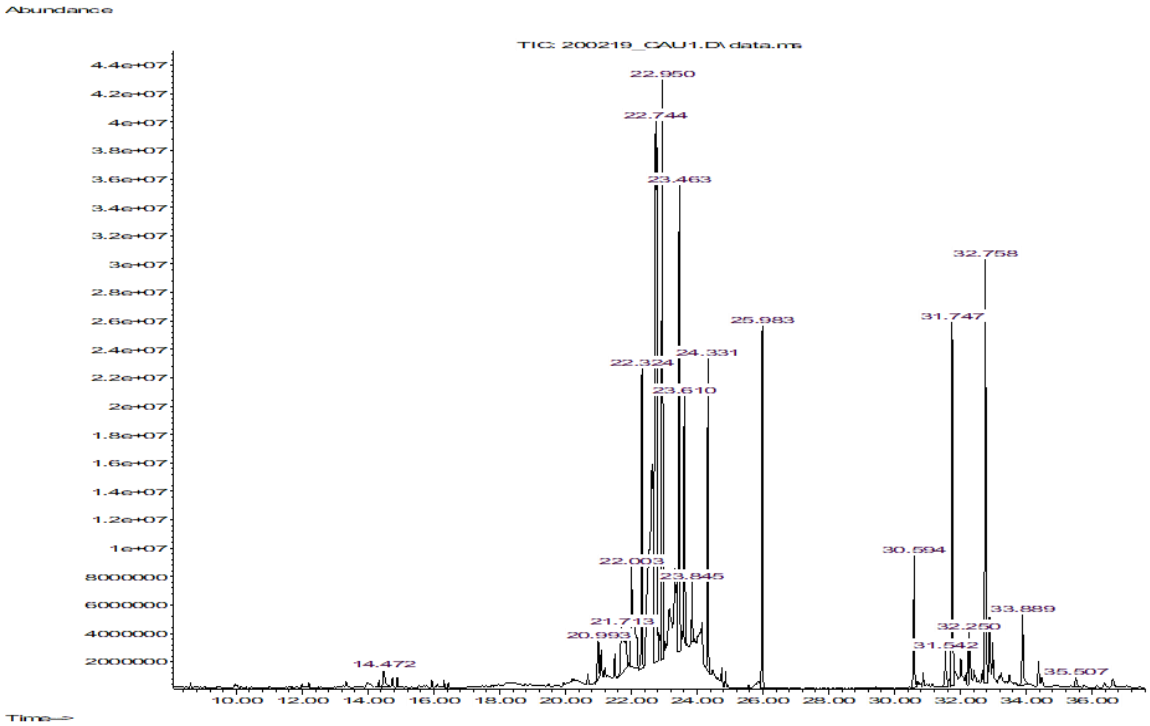


**Fig. 4.** *Chromatogram of ethanol extract of C. auriculata flowers.*

**Table 5.** Chemical constituents present in ethanol extract of *C. alata* flowers.

| Name of the Compound | Molecular formula | Retention Time (Min) | Total Percentage |
|---|---|---|---|
| β-Citronellol, trifluoroacetate | $C_{10}H_{20}O$ | 14.745 | 0.112 |
| 1,4-Diethoxy-2-nitrobenzene | $C_{10}H_{13}NO_4$ | 14.888 | 0.090 |
| 2(3H)-Furanone, dihydro-3,4-bis[(trimethylsilyl)oxy]-, trans- | $C_{10}H_{22}O_4Si_2$ | 15.941 | 0.084 |
| 1,5-Anhydro-D-sorbitol, tetrakis(trimethylsilyl) ether | $C_{18}H_{44}O_5Si_4$ | 22.007 | 6.379 |
| D-(-)- Erythrofuranose, tris(trimethylsilyl) ether (isomer 2) | $C_{13}H_{32}O_4Si_3$ | 22.33 | 6.238 |
| D-Pinitol, pentakis(trimethylsilyl) ether | $C_{22}H_{54}O_6Si_5$ | 22.744 | 23.094 |
| β-D-Mannopyranoside, methyl 2,3,4,6-tetrakis-O-(trimethylsilyl)- | $C_{19}H_{46}O_6Si_4$ | 22.954 | 9.539 |
| D-(-)-Tagatose, pentakis(trimethylsilyl) ether, methyloxime (syn) | $C_{22}H_{55}NO_6Si_5$ | 23.475 | 13.857 |
| D-(+)-Talose, pentakis(trimethylsilyl) ether, methyloxime (syn) | $C_{22}H_{55}NO_6Si_5$ | 23.619 | 9.062 |
| Sedoheptulose, o-methyloxime, hexakis-O-(trimethylsilyl)- | $C_{26}H_{65}NO_7Si_6$ | 23.852 | 2.508 |
| Glucopyranose, 2,3-di-O-methyl-1,4,6-tris-O-(trimethylsilyl)- | $C_{17}H_{40}O_6Si_3$ | 24.334 | 5.508 |
| Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)- | $C_{24}H_{60}O_6Si_6$ | 25.983 | 5.046 |
| Chrysin, bis(trimethylsilyl) ether | $C_{21}H_{26}O_4Si_2$ | 30.589 | 0.734 |
| D-(+)-Turanose, octakis(trimethylsilyl) ether | $C_{36}H_{86}O_{11}Si_8$ | 31.755 | 9.725 |
| D-(-)-Ribofuranose, tetrakis(trimethylsilyl) ether (isomer 1) | $C_{17}H_{42}O_5Si_4$ | 32.758 | 5.286 |
| 3-α-Mannobiose, octakis(trimethylsilyl) ether (isomer 2) | $C_{36}H_{86}O_{11}Si_8$ | 32.884 | 1.514 |
| D-Ribofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)- | $C_{17}H_{42}O_5Si_4$ | 33.887 | 0.702 |
| Silane, dimethyl(2-naphthoxy)heptadecyloxy- | $C_{29}H_{48}O_2Si$ | 34.375 | 0.495 |

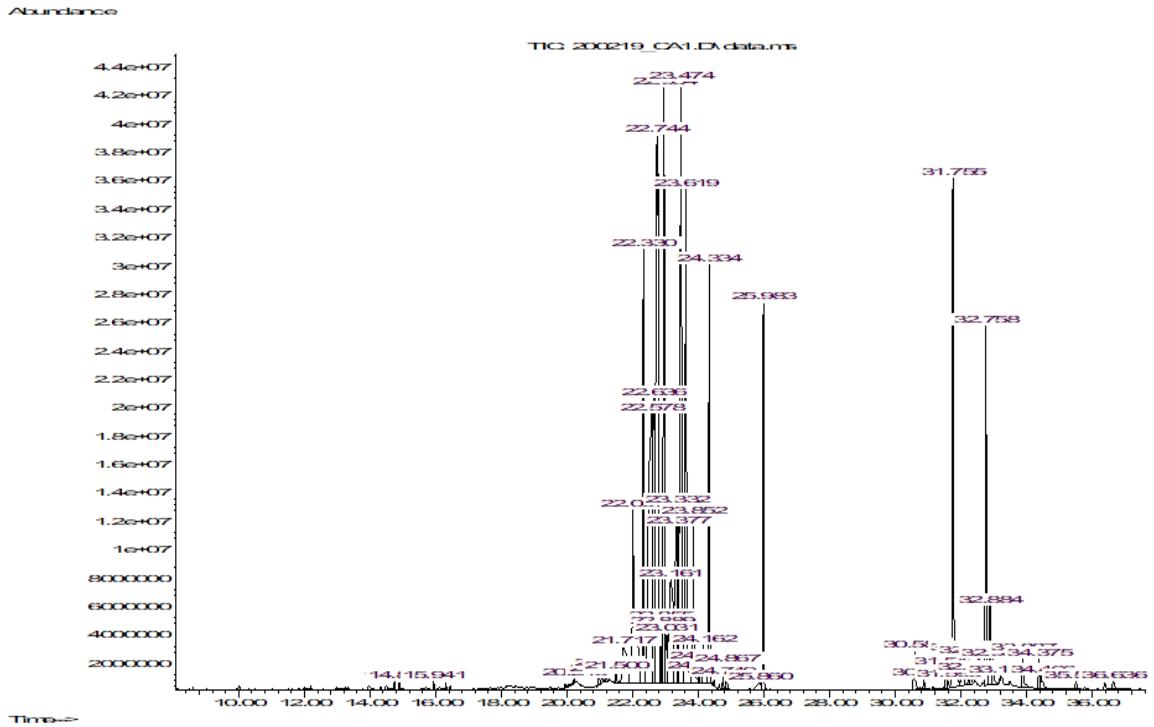


**Fig. 5.** *Chromatogram of ethanol extract of C. alata flowers.*

*rubrum* was reported by Pereira *et al*. (2014) and Shin and Lim (2004) indicating its anti-dermatophytic activity. *Malassezia* is a dermatophytic yeast and the presence of citronellol from ethanolic extract of *C. alata* could have attributed the anti-malassezial activity in this study. Similar work done by Kavitha *et al*., (2016) reported the susceptibility of *Malassezia japonica* to citronellol. Chrysin from beewax is reported to protect the skin against pathogenic microorganisms (Gorecka *et al*., 2020) and in this the study the presence of chrysin is noted in the ethanolic extract of *C. alata*. Furanones are naturally produced by plants and their antimicrobial activities are widely reported (Sharafutdinov *et al*., 2019; Rathore *et al*., 2015; He *et al*., 2015). The anti-malassezial activity of ethanolic extract of *C. auriculata* and *C. alata* is due to one or other bioactive compounds identified through the GC-MS analysis. Further, studies are needed for the purification of the individual compounds and their activity against *Malassezia* to formulate drugs from *Cassia*.

Species distribution in the etiology of dandruff is reported (Sharma and Sibi, 2017) and among the *Malassezia*, different species require different lipids for their growth (Mayser *et al*., 1997). It is reported that medium-chain triglycerides and medium-chain free fatty acids are toxic for *Malassezia* species (Papavassilis *et al*., 1999) and fatty acid monoesters could treat *Malassezia* associated diseases (Mayser, 2015). Identifying a bioactive compound from plants to control the growth of *Malassezia* is a way to develop plant-based drugs but different the susceptibility varies between *Malassezia* species (Sibi *et al*., 2014).

## Conclusion

Both chloroform and ethanol extracts from *C. auriculata* and *C. alata* flowers had the ability to inhibit *M. furfur* with MIC values of 50 µM, 88 µM, 75 µM and 70 µM thereby could serve as source of potential anti-dandruff compounds. The presence of fatty acids in chloroform extract and the antifungal compounds from ethanolic extract supported the potential application of *Cassia* species as potent natural sources for anti-dandruff activity. Thus, inhibition of *M. furfur* through fatty acids from *Cassia* flowers is a novel strategy to control dandruff and purification of antidandruff compounds and their mode of inhibition is needed to formulate effective treatment of *Malassezia* associated diseases.

## Conflict of interest

The authors declare that they have no conflict of interest.

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